WITec User Manual

WITec Suite 6.2

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About this manual

Warnings are marked with a red bar. Please read these warnings carefully to avoid problems that may otherwise occur.

> Throughout the manual, you will find text blocks like this one. These text blocks contain additional, useful information.

Text marked with a shaded rectangle, such as Menu Item Name, refer to a menu item in the software.

Text marked with a blue rectangle, such as Button Name, refer to a button or checkbox in the software.

Keyboard keys are highlighted as Key.

Disclaimer of Responsibility

WITec reserves the right to change the product specifications and the functionality, or the manual itself, at any time without prior notice.

Furthermore, WITec assumes no responsibility or liability for any misinformation, errors, or general inaccuracies that may appear in this manual.

Welcome to the WITec Suite

Welcome to the WITec Suite Software Help.

Operation Guide	Explanation of all measurement modes
WITec Control	Microscope Control Software
WITec Project	Data Evaluation Software
WITec TrueMatch	Raman Database Search Software
WITec ParticleScout	Particle Analysis Software
WITec User Manager	Access Rights Management Software

Press the **F1 key** anywhere in the software to open the context help or browse the Help Menu to open the help contents

Last update: 2024-01-12 (WITec Suite 6.2)

Operation Guide

Operation Guide Overview

Welcome to the WITec Operation Guide

This guide explains how to perform measurements with a WITec microscope using the WITec Suite, especially WITec Control.

The WITec product portfolio includes imaging systems for Raman, AFM and SNOM analysis as single technique solutions as well as correlative imaging configurations. All WITec microscopes are high-quality modular systems. Therefore each system will be an individual solution to match the needs of each customer. Maybe not all of the described techniques will be available on your system. If you are interested in extending the capabilities of your system, please contact us.

WITec Control is equipped with predefined Configurations matching the setup of your system. A configuration determines which hardware should be used and which data channels are recorded during the measurement. This guide explains the differences between the configurations and how to use them.

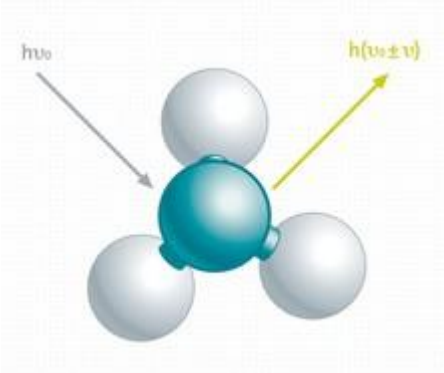
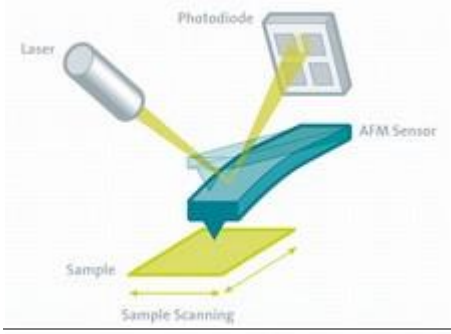
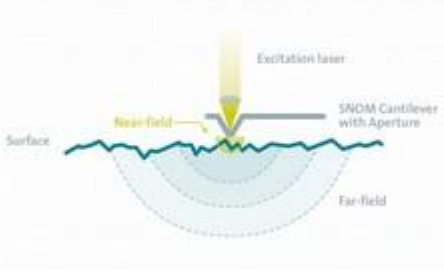
General

- Modularity
- Confocality
- Objectives

Common procedures:

- Power-up/-down the system
- Focus on sample
- Focus on sample from below

Techniques

	**Raman**	Raman spectroscopy, Photoluminescence spectroscopy
	**AFM**	Atomic force microscopy
	**SNOM**	Scanning near-field optical microscopy
	Confocal	Confocal microscopy and StrobeLock (time-resolved microscopy)
	Photocurrent	Analyze photosensitive devices
	SHG	Observe second-harmonic generation
	Lithography	DaVinci nanolithography package
	Profilometer	TrueSurface sensor as profilometer

Modularity

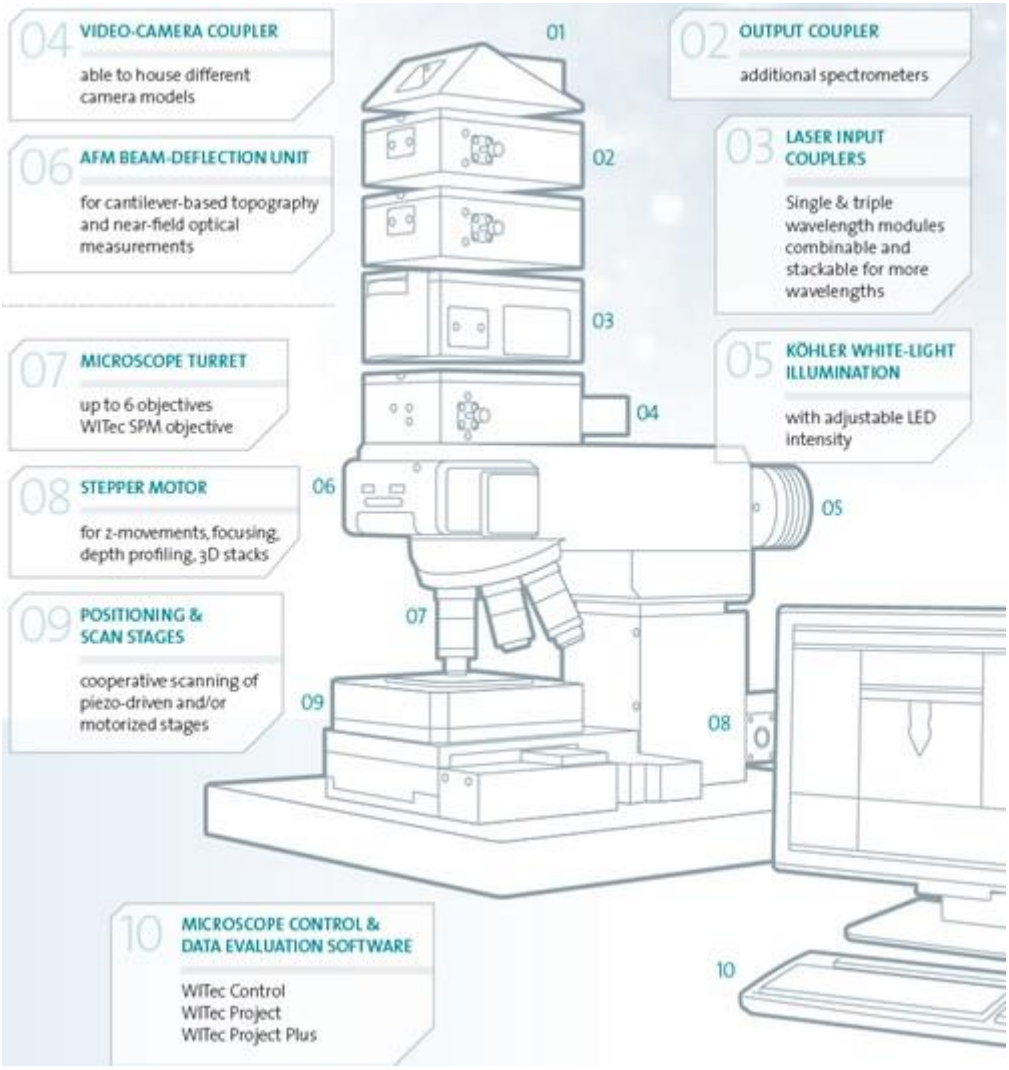


Figure 1: Sketch of the WITec alpha300 modules

All WITec microscopes are high-quality modular systems with exceptional optical throughput, unparalleled signal sensitivity and outstanding imaging capabilities. The common thread throughout is that all systems are based on the same hardware architecture. Whenever required it is possible to simply upgrade any system, even the most basic, with additional features and equipment, allowing our customers to keep pace with future challenges.

The WITec system contains several components common to various configurations of the instrument (such as the AFM and the scanning near-field optical microscope configurations). Therefore, the system can be fully upgraded to include AFM or SNOM capabilities at any time. In particular, the combination of Raman microscopy with AFM allows the chemical information gained by confocal Raman microscopy to be linked directly with the ultra-high lateral and topographical resolution of an Atomic Force Microscope at the same sample position with just a rotation of the turret. SNOM allows for optical investigation of the sample beyond the diffraction limit.

For Raman systems it is always possible to add further excitation laser wavelengths, detectors, spectrometers, or advanced modules like TrueSurface for profilometric Raman measurements.

Contact WITec for more details.

Confocality

Confocal microscopy requires a point source (usually a laser), which is focused onto the sample. The

reflected light (also Raman scattering or fluorescence) is collected with the same objective and focused through a pinhole in front of the detector (Fig. 1). This ensures that only light from the image focal plane can reach the detector, which greatly increases image contrast and with the proper selection of pinhole size, slightly increases resolution (max. gain in resolution: factor √2).

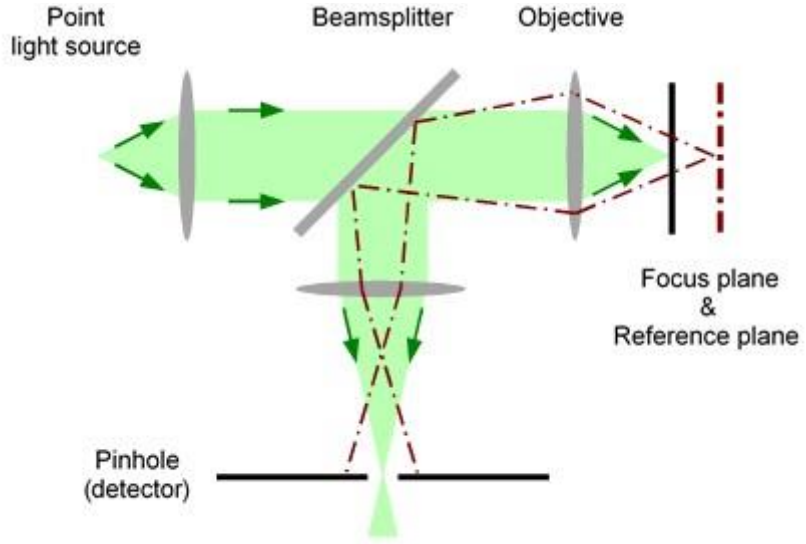


Fig. 1: Principal setup of a confocal microscope

In WITec systems, the laser light is delivered through a single-mode optical fiber. This type of fiber supports only a single transversal mode (LP01, Gaussian beam) which can be focused to a diffraction-limited spot. The light reflected by the sample is collected by the objective and is directed as a parallel beam toward the top of the microscope. Here, the light is focused onto a optical fiber. The core of this optical fiber acts as a pinhole for confocal microscopy or confocal Raman microscopy.

The fiber directs the beam to a photon counting device (for confocal microscopy) or a spectrometer equipped with a CCD camera (for confocal Raman microscopy). Using fibers for beam delivery and signal pick-up is very convenient because the excitation laser, the spectrometer, and the detectors do not need to be mounted on the microscope itself. They can be placed anywhere, far away from the microscope body.

Objectives

Objectives are among the most important components in a microscope. In WITec systems they define the magnification of the video image and the resolution of, for example, the Raman image. The objectives are infinitely corrected, meaning that the beam is parallel inside the microscope. Please ensure that the microscope objectives are used in the proper way. Most objectives are only intended for use in air. Some objectives are corrected for use with a cover slip and require an immersion medium to function properly.

Figure 1: Labeling of Zeiss objectives (Source: https://www.zeiss.com/microscopy/int/products/microscope-components/objectives.html)

The magnification is given for the image plane (at the position of the video camera or the collection fiber). The numerical aperture (NA = n · sin a) describes the resolving power of the objective. Additionally, the working distance (in mm) is printed on the objectives.

For instructions regarding the cleaning of objectives, please refer to the brochure, "The Clean Microscope" by ZEISS or to their website.

Power-up/-down

Power-up

1. Switch on the alphaControl and peripheral devices. This is usually done using the switch on the multi-plugs.
2. Power up the computer and wait until the pink H (service monitor) appears in the taskbar.
3. Start WITec Control.

There is no mandatory order for switching on devices. Even switching on or off the alphaControl while the computer is switched on is no problem as long as WITec Control is not running.

Power-down

1. Close WITec Control.
2. Shut down the computer.
3. If you do not use the system for several days, switch off everything using the switch on the multi-plugs.

Lasers

- Only switch on lasers if you want to use them, because the lifetime is limited.
- Switch off the lasers if you do not use them for more than one hour.
- Please refer to the lasers manual for specific information.

CCD cameras

- Switching off the CCD camera even when cooled down, does not harm the camera.

Focus on sample

The following steps explain how to easily focus on a sample and are common for all configurations. This applies not for use with the inverted objective (look here).

Procedure

1. Mount the sample on the microscope stage. Make sure it is fixed (i.e. with clamps) to avoid movements during the measurements.
2. If possible: Select an objective with low magnification (i.e. 10x).
3. For non-automated systems: Configure the beampath for video mode in brightfield illumination.
4. Use reflected light (top illumination) and make sure that the top camera is selected.
5. Close the field stop. For non-automated systems: Field stop is located on the right side of the microscope body marked with a "F".
6. Adjust the brightness to see at least some light, i.e. use Auto brightness.
7. Focus in the direction in which the image becomes brighter:
    - Use the Microscope-Z stage.
    - For RISE: Use the scan table z.
8. If the edges of the field stop are in focus, also the sample is in focus.
9. Open the field stop.
10. If necessary: Select an objective of higher magnification and repeat the steps starting with step 4.

Hints

- During focusing features can appear that are not the sample surface (originating from surfaces within the optical brightfield beampath). The edges of the field stop will not be in focus in that case. Slightly move the microscope stage, to see whether the features do move.
- For transparent samples there could be more than one focus plane.
- If you have difficulties to focus, it is also possible to focus using the laser spot at low laser power.
- For rough samples, maybe only parts of the field stop will be in focus.

The field stop is focusing in the focus plane because it is positioned at the back focal plane of the objective.

Focus on sample from below

The following steps explain how to focus on a transparent sample using the inverted objective. This applies not to the alpha300 R*i* (look here).

If you have a non-transparent sample, do the focusing on a glass slide and change back to your sample afterwards.

If you want to do measurements in transmission setup (e.g. Confocal or SNOM), it is necessary to align the inverted objective for collection under the laser from above. Please follow also the steps marked with "for transmission" below.

Procedure

1. Focus on the sample using the upright microscope with an objective not smaller than 20x and follow the steps until step 8.
2. Select the bottom camera and if necessary set the inverted beampath accordingly.
3. Use the coarse adjustment knob of the inverted objective to bring it close to the sample. (Take the working distance of the used objective into account.)
4. Focus on the sample using the control of the inverted objective.
5. For Transmission: Select the laser you want to use and configure the beampath accordingly to see the laser in the video image. Adjust it to low laser power (about 1 mW).
6. For Transmission: Move the inverted objective in x-y direction until the laser spot is centered in the green circle in the video image. Close the laser shutter.

Hints

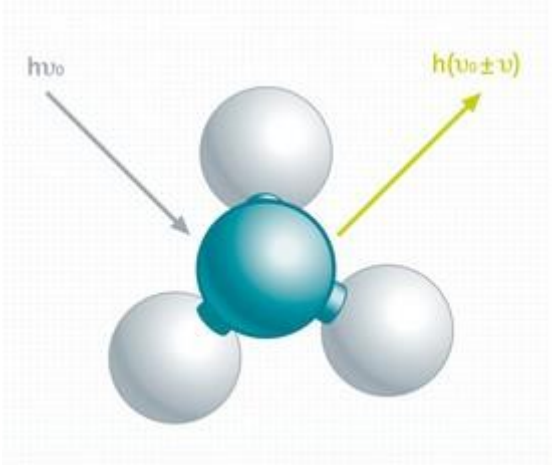
- For transparent samples there could be more than one focus plane, make sure you focused on the intended one.
- If the sample has no visible features, focus on the field stop of the upright microscope. (It is not visible, if the magnification of the upright objective is too low.)
- If you have difficulties to focus, it is also possible to focus using the laser spot. If the laser is coming from above, make sure it is focused on the sample surface in the Top view.
- For Transmission: If you don't see the laser spot at step 5 and the top camera is mounted

above the laser coupler in your system, check that the edge filter is removed.

- **For 6.:** Observe the laser using the bottom camera if the laser comes from above and the top camera if the laser comes from below.

Raman

Raman Overview



Quick Start:

- Signal optimization (Pinhole alignment)
- Setting up a Large Area Scan
- Change of Laser Wavelength

Raman spectroscopy is a method to observe vibrational modes of molecules which provides chemical and structural information about the sample. The needed excitation light source in the range from UV to NIR combined with the confocal light collection of the WITec microscope provides high spatial resolution. Furthermore the WITec UHTS spectrometers equipped with an optimized CCD camera allow Raman Imaging in high speeds and excellent spectral quality.

Topics:

- Introduction
- Theoretical Background
- Raman Configurations
- Spectrometer calibration
- Signal optimization (Pinhole alignment)
- Change of Laser Wavelength
- Setting up a Large Area Scan
- Example measurement

Raman modes:

- Raman – standard Raman, refer to the Configurations section for more information
- Raman inverted – Raman using the inverted objective
- Raman-AFM – for simultaneous Raman and AFM refer to Raman-AFM

All Raman configurations can also be used for just doing normal spectroscopy like for Photoluminescence (PL) by changing the unit in the spectrograph settings to nm.

Measurement modes:

- Oscilloscope: Continuous readout of spectra e.g. for focusing.
- Single Spectrum: Acquisition of a spectrum at the current position.
- Spectral Stitching: Acquisition of a spectrum with extended spectral range by stitching spectra from different spectral positions.
- Fast Time Series: Continuous acquisition of spectra over time. (Can be used as alternative to single spectrum for saving each accumulation.)
- Slow Series: Intermittent time series, laser power series or polarizer series.
- Line Scan: Acquisition of spectra along a line in three-dimensional space.
- Large Area Scan: 2D or 3D Raman Imaging using the motorized stage up to centimeter scale.
- Image Scan: 2D or 3D Raman Imaging using the piezo stage for highest resolution.
- Sample Raster: Automated single spectra or image scans on predefined points.

Advanced features:

- Manual Topography Correction: Enables to correct the tilt of the sample or a simple surface.
- TrueSurface Mk1 and Mk2: Learning the topography by a CCS and follow during the scan.
- TrueSurface Mk3: Live topography correction during the scan.
- Signal Stabilization: Compensates slow focus changes due to thermal drift.
- ParticleScout: Automated Raman analysis of optically identified particles.
- Polarization: Raman polarization studies
- EMCCD camera: Ultra Fast Raman imaging
- InGaAs camera: Raman Imaging in the NIR

System requirements:

- Raman (M, R, RA, RAS and RS systems)
- access and RISE systems

Introduction

The Raman effect describes the interaction of electromagnetic waves (light) with matter in which a vibrational quantum is excited (Stokes Raman scattering) or annihilated (Anti-Stokes Raman scattering).

When light interacts with a molecule, most photons are elastically scattered and therefore retain the same energy as the incident photons. This is Rayleigh scattering, which is visible in the blue of the sky that results from sunlight scattered by water molecules.

However, a very small fraction (approximately 1 in 10^6 to 10^7 photons) is inelastically scattered, which means that the energy of the scattered photon is different (usually lower) than the energy of the incident photon. This is called the Raman effect and it was discovered by Chandrasekhara Venkata Raman in 1928. He used a filtered beam of sunlight for the excitation source and his eye to detect the frequency-shifted light as this was long before the development of the first laser by Maiman in 1960. Raman was awarded the Nobel Prize in Physics in 1932 for this discovery. The theory underlying the Raman effect had been published five years earlier by A. Smekal (1923).

The great utility of the Raman effect lies in the fact that the energy shift between the incident and the Raman-scattered photon is caused by the excitation (or annihilation) of a molecular vibration. Each molecule has several vibrational modes with defined energy shifts that are visible in its characteristic Raman spectrum. This serves as a fingerprint for the type and coordination of the

molecule involved in the scattering process.

In material research Raman spectra provide qualitative and quantitative information about:

- chemical nature: structural units, type and degree of branching, end groups, additives
- conformational order: physical arrangement of molecular chains
- state of order: crystalline, mesomorphous, and amorphous phases
- orientation: type and degree of molecular chain and side group alignment in anisotropic materials.

Recording Raman spectra at a high spatial resolution enables the generation of clear and informative 2D and 3D Raman images. WITec systems provide exceptional Raman imaging capability by combining spatial resolution down to the sub-micrometer regime with unrivalled sensitivity, simultaneously.

Knowledge Base

Answers to (almost) every question about Raman microscopy can be found in Knowledge Base.

The book for the system...

For further information, please refer to Confocal Raman Microscopy, edited by WITec scientists Olaf Hollricher, Thomas Dieing and Jan Toporski, It includes a comprehensive overview of the theoretical background, practical considerations and real-world applications of Raman microscopy along with sub-sections on instrument technology, novel materials, geosciences, life and pharmaceutical sciences, materials science and many other topics. It can be purchased in print or e-book formats directly from Springer or through online shops.

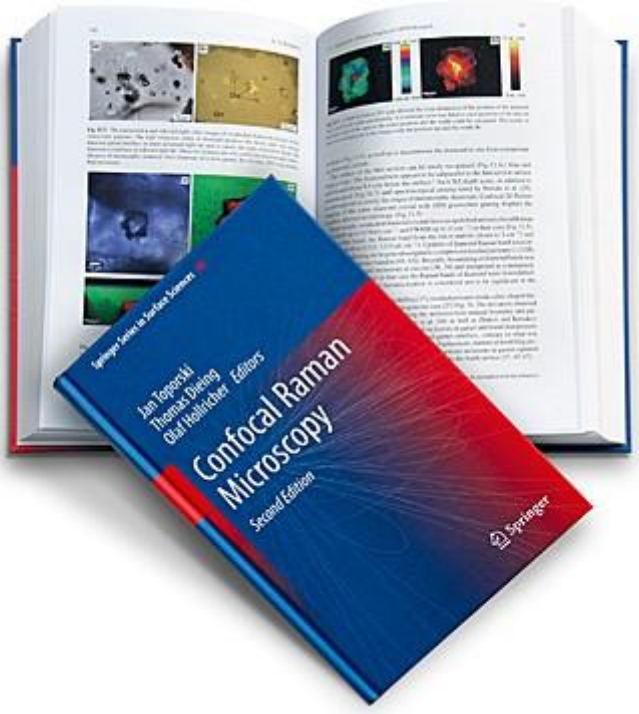


Figure 1: Confocal Raman Microscopy, 2nd edition, Editors: Jan Toporski, Thomas Dieing, Olaf Hollricher, ISBN: 978-3-319-75380-5

Further literature

1. Modern Raman Spectroscopy: A Practical Approach, Ewen Smith, Geoffrey Dent, ISBN: 9780471496687, DOI:10.1002/0470011831
2. Raman Spectroscopy for Chemical Analysis, Richard L. McCreery, ISBN: 9780471252870, DOI:10.1002/0471721646

Theory

In quantum mechanics, the scattering process between a photon and a molecule is described as an excitation of a molecule to a virtual state lower in energy than a real electronic state and the (nearly immediate) de-excitation.

The lifetime of the virtual state is extremely short and can be calculated by the Heisenberg uncertainty relation:

$$\Delta t \cdot \Delta E \geq \frac{\hbar}{2}$$

With typical photon energies of 1–2 eV, the lifetime of the excited state is only about 10^{-15} s. After this extremely short time, the molecule falls back either to the vibrational ground state or to an excited state (Fig. 1). When the initial and final states are identical, the process is called Rayleigh scattering.

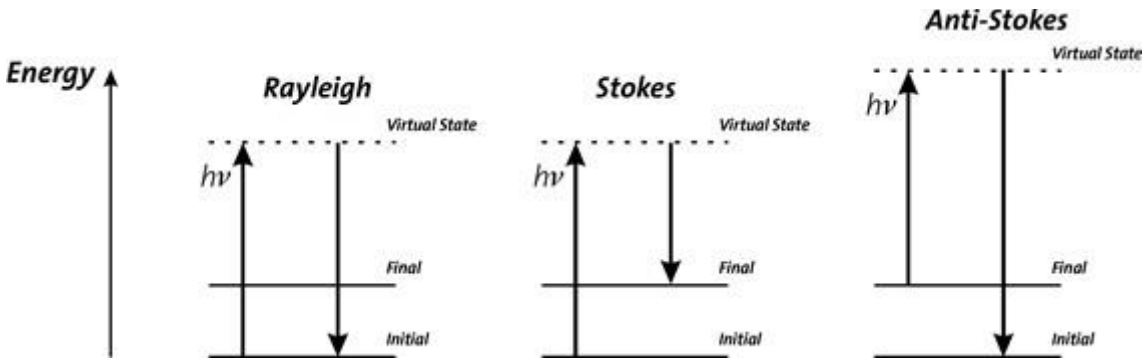


Fig. 1: Energy level diagram for Raman scattering

If the initial state is the ground and the final state a higher vibrational level, one refers to Stokes scattering, if the initial state is energetically higher than the final state, to Anti-Stokes scattering.

The difference in energy between the incident and the Raman scattered photon is equal to the energy of a vibration quantum of the scattering molecule. A plot of intensity of scattered light versus energy difference is called a Raman spectrum.

When a photon interacts with a molecule, the electrical field $\vec{E}$ induces a dipole moment $\vec{P}$ in the molecule:

$$\vec{P} = \vec{\vec{\alpha}} \cdot \vec{E}$$

The proportionality constant $\vec{\vec{\alpha}}$ is the polarizability tensor of the molecule and is a measure of the ease with which the electron cloud around a molecule can be distorted. In the case of an isotropic molecule, α reduces to a scalar.

The time dependence of the electromagnetic field is

$$\vec{E} = \vec{E}_0 \cos(2\pi\nu t)$$

If one takes a vibrating diatomic molecule as a model system, assuming a simple harmonic motion,

its internuclear distance can be written in the form

$$q_v = q_0 \cdot \cos(2\pi\nu_v t)$$

The polarizability a is a function of internuclear distance. For an isotropic molecule, α can be expanded in a Taylor series

$$\alpha = \alpha_0 + \left(\frac{d\alpha}{dq_v}\right)_0 q_v + \cdots$$

$$\approx \alpha_0 + \left(\frac{d\alpha}{dq_v}\right)_0 q_0 \cos(2\pi\nu_v t) := \alpha_0 + \alpha_1 q_v$$

where higher than linear terms are neglected for small interatomic displacements.

If we now look at the molecule in the external electrical field, one finds

$$\vec{P} = \alpha \cdot \vec{E} = (\alpha_0 + \alpha_1 q_v)\vec{E}_0 \cos(2\pi\nu t) = (\alpha_0 + \alpha_1 q_0 \cos(2\pi\nu_v t))\vec{E}_0 \cos(2\pi\nu t)$$

$$= \alpha_0 \vec{E}_0 \cos(2\pi\nu t) + \alpha_1 q_0 \vec{E}_0 \cos(2\pi\nu_v t)\cos(2\pi\nu t)$$

$$= \underbrace{\alpha_0 \vec{E}_0 \cos(2\pi\nu t)}_{Rayleigh} + \underbrace{\frac{1}{2}\alpha_1 q_0 \vec{E}_0 \cos(2\pi(\nu+\nu_v)t)}_{Anti-Stokes} + \underbrace{\frac{1}{2}\alpha_1 q_0 \vec{E}_0 \cos(2\pi(\nu-\nu_v)t)}_{Stokes}$$

As one can see, besides the elastically scattered Rayleigh line, additional lines appear in the spectrum which are shifted $\pm\nu_v$ relative to the excitation light.

The position of a Raman line is usually given in wavenumbers (1/cm), which is the energy shift, relative to the excitation line:

$$\bar{\nu} = \frac{1}{\lambda_{incident}} - \frac{1}{\lambda_{scattered}}$$

$\lambda_{incident}$ and $\lambda_{scattered}$ are the wavelengths (in cm) of the incident and Raman scattered photons, respectively.

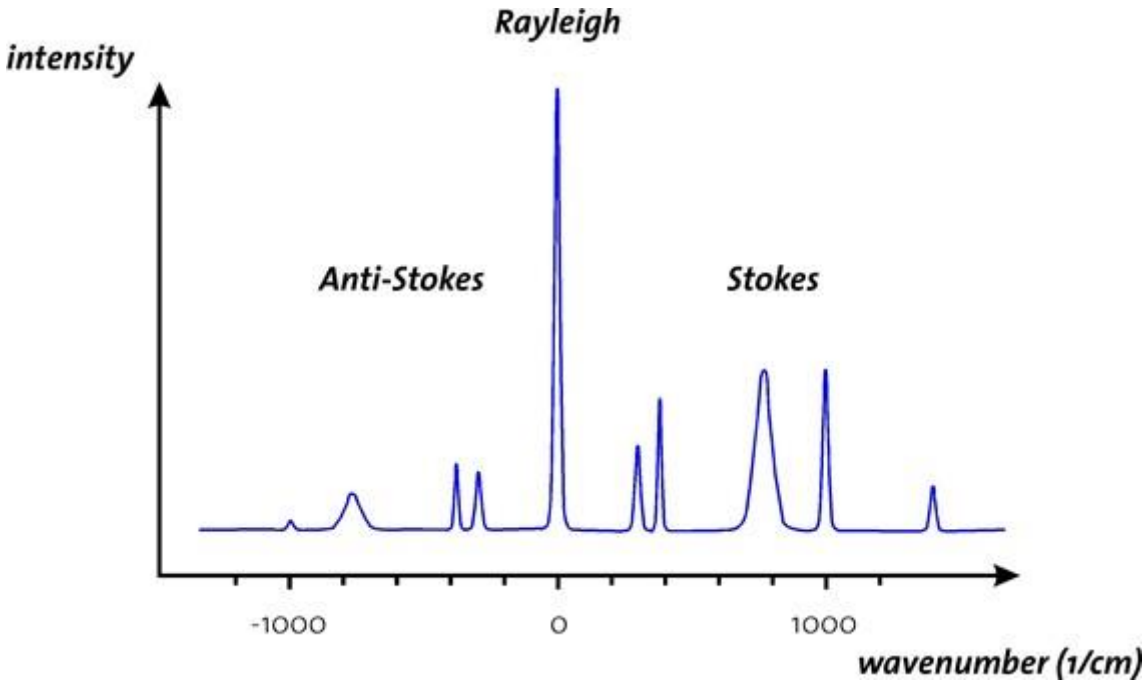


Fig. 2: Typical Raman spectrum

As can be seen in Fig. 2, a typical Raman spectrum is symmetric to the Rayleigh line and the Anti-Stokes lines are smaller than the Stokes shifted lines.

From classical scattering theory, one finds that the intensity I of scattered light is proportional to the 4th power of the excitation frequency ν.

$$I \sim \nu^4$$

Exciting a sample with blue light of 400 nm would therefore give a 16 times higher Raman signal than using red light of 800 nm.

The problem of using blue (or UV) excitation light is fluorescence. Most samples show fluorescence when they are excited with blue light. The Raman effect is extremely weak compared to fluorescence. If a sample shows fluorescence, obtaining a Raman spectrum is usually very difficult because of the strong fluorescence background. In the red (or even IR) part of the spectrum fluorescence is usually not a problem anymore, but the excitation intensity has to be much higher ($I \sim \nu_4$!). Another problem is, that Silicon detectors cannot be used above 1100 nm (bandgap energy of Si: 1.12 eV). Other IR detectors (like InGaAs) are extremely expensive, show much more thermal noise than silicon and photon counting detectors with a reasonable dark count rate were not available up to now. In real experiments one must always find a compromise between detection efficiency and excitation power. From the above equation one would assume that

$$I_{Stokes} \sim (\nu - \nu_v)^4, \text{ and } I_{Anti-Stokes} \sim (\nu + \nu_v)^4$$

and therefore $I_{Anti\text{-}Stokes} > I_{Stokes}$. Experimentally, one finds the opposite: $I_{Anti\text{-}Stokes} < I_{Stokes}$.

Here, quantum mechanics comes into play. For Anti-Stokes scattering, the molecule must already be in an excited vibrational state.

The Boltzmann distribution defines which portion of N molecules are thermally excited to an energy level E_j

$$N_j = N \cdot e^{-\frac{E_j}{k_B T}}$$

Using the energy of a harmonic oscillator, one gets

$$N_j = N \cdot e^{-\frac{\left(j+\frac{1}{2}\right)h\nu}{k_B T}}$$

The probability of finding a molecule in the ground state is much higher than finding it in an excited state. At room temperature, Stokes scattering is therefore much more effective than Anti-Stokes scattering. To calculate the relative intensity, the exponential function must be taken into account

$$\frac{I_{Anti-Stokes}}{I_{Stokes}} \sim e^{-\frac{h\nu_v}{k_B T}} \cdot \left(\frac{\nu + \nu_v}{\nu - \nu_v}\right)^4$$

Usually, the e-function will dominate this term, so that

$$I_{Stokes} > I_{Anti-Stokes}$$

If one measures the intensity ratio between Stokes and Anti-Stokes lines, one can determine the sample temperature.

Configurations

Different configurations are available for Raman measurements. The configuration defines the used CCD camera 1, 2 or 3 and thus the respective spectrometer that should be used for the measurement. The following list describes the purpose of each configuration. Only Configurations suitable for your system are installed by default.

Raman CCDX	Standard Raman configuration which uses microscope z
Raman without z-stepper CCDX	Is used for systems without microscope z e.g. RISE, z movement during measurement by piezo stage, Topography correction and signal stabilization are not available
Raman inverted CCDX	Is used for Raman from below for systems with additional inverted objective, z movement during measurement by piezo stage if equipped, otherwise no z movement possible, Topography correction and signal stabilization are not available
attoRaman CCDX	Raman Imaging in an attocube cryostat, no z movement during measurement

Calibration

Introduction

Any spectrograph used for spectroscopy experiments needs to be calibrated in order to obtain reliable measurement results. The calibration of any WITec system will have been performed at WITec before delivery of the system. However it may be desirable to recalibrate the spectrograph from time to time especially if it was subject to strong temperature changes.

In order to calibrate the spectrometer an Argon/Mercury calibration lamp is used. For an UHTS400S NIR InGaAs with an InGaAs camera a Xenon lamp is needed.

Once the calibration is started, the system will drive the selected grating to various positions and determine the position of certain Ar and Hg (or Xe) lines both on- and off-axis in order to determine the calibration parameters.

> In order to check the calibration, just a verification can be performed without changing the current calibration.

Procedure

1. For non-automated systems: Configure the beampath for Calibration.
2. Click on Spectrograph Calibration under Additional Devices.
3. Select the Spectrograph you want to calibrate.
4. Optional: Activate Verify only, if you just want to check the calibration.
5. Click on Calibrate all or Calibrate for the respective grating.

Further information:

Spectrograph Calibration

Alignment

The entrance of the output fiber acts as a confocal pinhole. To optimize the intensity of the Raman spectrum and the spatial resolution the fiber has to be aligned on the laser spot position. If the system has more than one spectrometer, the alignment has to be done with the output coupler that connects to spectrometer used for the measurements.

> This procedure is recommended after changing the laser wavelength or if you observe lower spectral intensity than expected.

Required sample:

Silicon sample (preferably the one delivered with the system)

General steps

We recommend to perform the alignment of the pinhole on a silicon sample.

1. Focus on the surface of the silicon. Make sure that your laser position is on a clean and uniform area of your silicon sample.
2. For non-automated systems configure the beam path for Raman (choose the right laser wavelength and the output coupler accordingly, camera coupler and white light slider should be out).
3. Adjust the laser to full power.
4. Start the oscilloscope to see the spectrum currently recorded by the spectrometer. Use a short integration time < 0.25 s to be able to see the effect of the subsequent alignment steps spontaneously.

> If everything is configured correctly there should be at least a small peak at 520 cm^{-1} (the first order of silicon). This is the basis for further adjustment. If you have no signal please refer to Hints and Troubleshooting at the bottom.

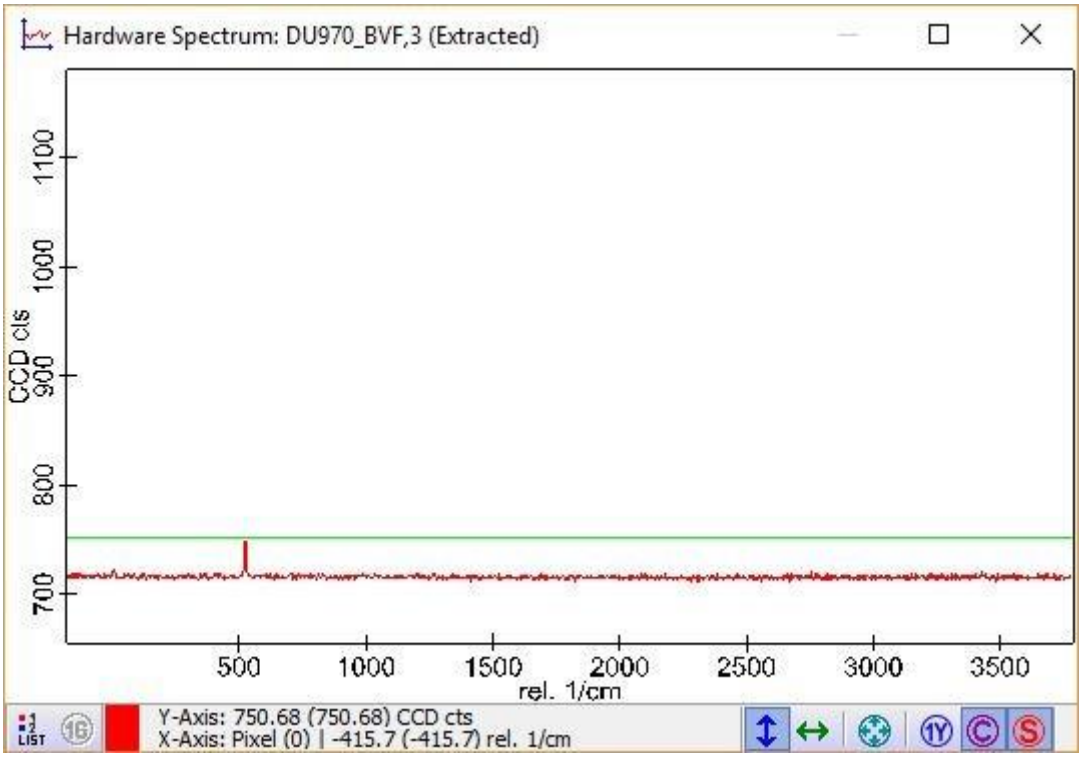


Figure 1: Low intensity silicon spectrum

5. If a spectrum comparable to the one in Figure 1 is visible maximize its intensity by optimizing the focus with the z-stage, piezo stage or manual focus drive (depending on system). (Go carefully in one direction using single steps and check the intensity. Keep the direction if it increases or change the direction if it decreases. Stop at the maximum.)

Automated output coupler

The alignment of automated output couplers could be done using the software. Click Output Adjustment  to open the dialog.

For Autobeam:

- The alignment is fully automated. The silicon is not needed. Click on Start Automated Adjustment
- For some wavelengths a manual optimization is necessary using the Manual Adjustment section and following the steps below under Adjustment.

For non-Autobeam:

- The alignment can be done using the EasyLink controller or the mouse following the steps below under Adjustment.
- If the Silicon peak is not visible, you could try a Video alignment.

Manual output coupler

On the backside of each output coupler (on the upper section of the microscope tower) or on top of the binoc, you have two screws for the alignment in the x- and y-direction (Figure 2).

Never turn any screw without observing the silicon spectrum in the oscilloscope!

If you turn the knobs, try to avoid applying force on the microscope tower, as this will lead to defocusing and a lower signal. After you turned a screw, remove your fingers before you check the signal strength.

If you have more than one output coupler, make sure you are touching the right screws. Stop turning the screws, if you see no change in the intensity and check again that you turn the right screws.

Figure 2: Fiber alignment screws (left: starting 2015, right: before 2015)

Adjustment

- The goal is to get the maximum intensity.
- By default, the graph viewer zooms out automatically. Just observe the change of the y-axis.
- Concentrate on the first order of silicon as long as the intensity of the spectrum is still weak.

Try to increase the spectral intensity by changing the alignment in the x and y direction:

1. Change the position in x-direction and check the intensity. If it decreases, turn in the other direction. Otherwise, increase it to the maximum.
2. Use the y-direction to do the same. And try again with each of the directions, until you cannot increase the intensity anymore.
3. Optimize the focus using the z-stage, piezo stage or manual focus drive (depending on

system) like before, to get the highest possible intensity.

4. If the signal is high enough, use the second order Raman line (around 950 cm^{-1}) for further adjustment.
5. Start from step 1 again and repeat all the steps until you have found the absolute maximum of the signal.

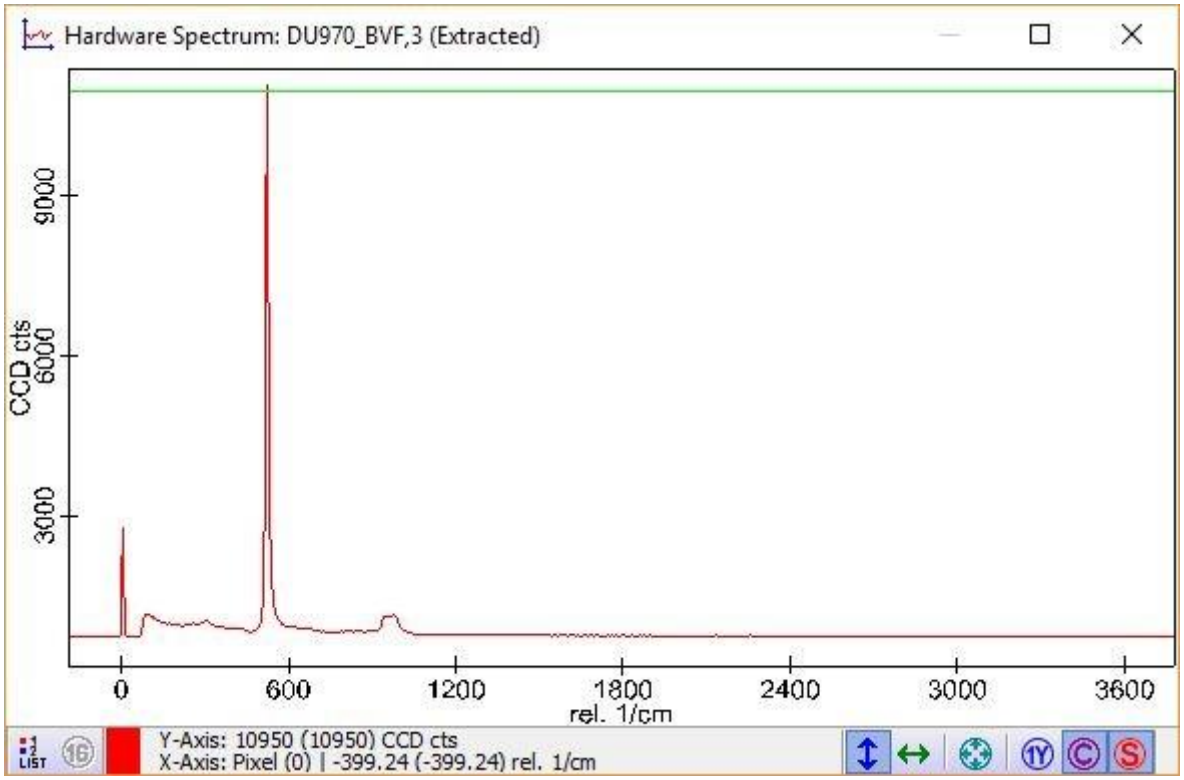


Figure 3: Silicon spectrum with optimized fiber position

Hints and Troubleshooting

If it is not possible to get any Raman signal, check the following points:

1. For non-automated systems, check if the beam path is set up correctly for Raman measurements and if you have selected the correct configuration in the software for the spectrometer you want to use.
2. Check if the right grating is chosen and if the center wavelength is configured correctly: A smaller grating is recommended with a spectral range to cover the Rayleigh peak at 0 cm^{-1}, the 1st order silicon band at about 520 rel. cm^{-1} and also the 2nd order at about 950 cm^{-1}.
3. Check the laser power if possible.
4. Increase the integration time to several seconds.

If all other things are excluded, the fiber might be at a completely wrong position. **Please ask WITec support for your further advice.**

Video Alignment

The non-Autobeam automated output coupler is equipped with an adjustment camera, showing the laser beam position.

Activate it by clicking on Video in the Output Alignment window. You should see the laser spot and the probe position (red circle). Decrease the laser power until you can just clearly see a small spot.

The goal is to get back the laser beam position to the center of the red circle (compare with Figure 4). You can move the laser spot like described above with the EasyLink controller or the software buttons. Once the laser beam is back in this circle, it should be possible to get at least a small signal in Raman mode. This will be the base for further adjustment like described above.

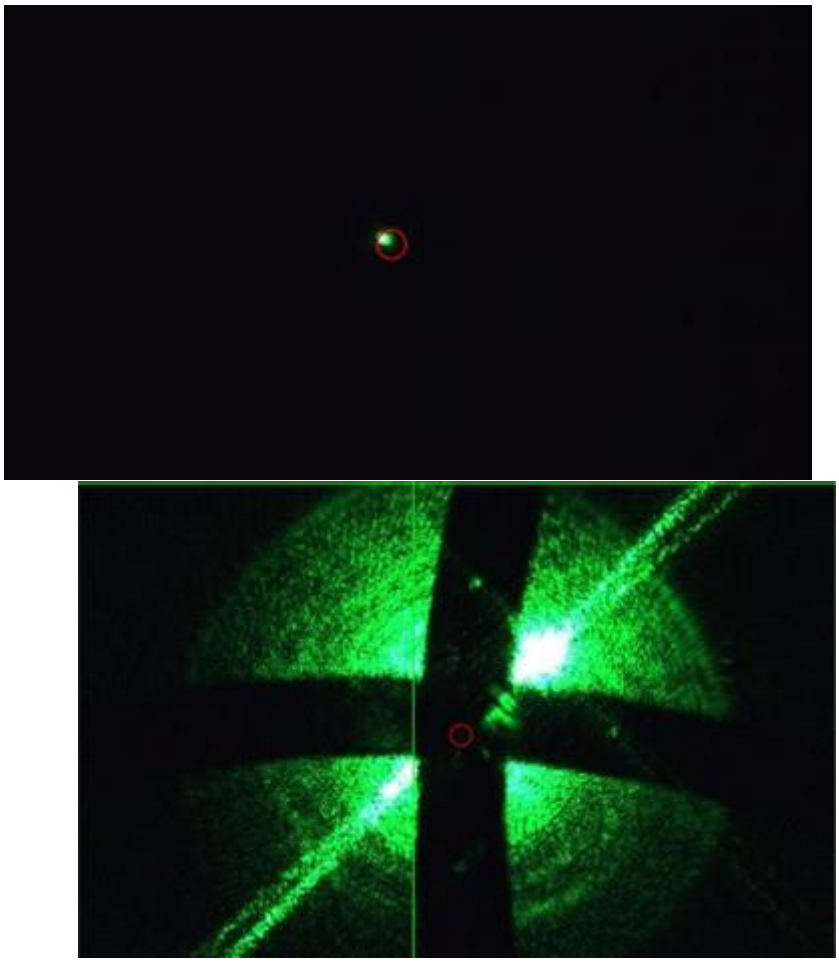


Figure 4: View of the adjustment camera (left) and in combination with a Rayshield coupler (right).

Change Laser Wavelength

If the laser you want use implies a spectrometer change, refer to the first section. If not, you can jump to the second section.

Change the spectrometer

1. Select the appropriate configuration for the spectrometer you want to use.
2. For non-automated systems: Configure the beampath for the use of this spectrometer.
3. The laser used with the spectrometer the last time is automatically selected.
4. For non-automated systems: Configure the beampath for this laser.
5. If you want to use a different laser, refer to the next section.

Change the laser

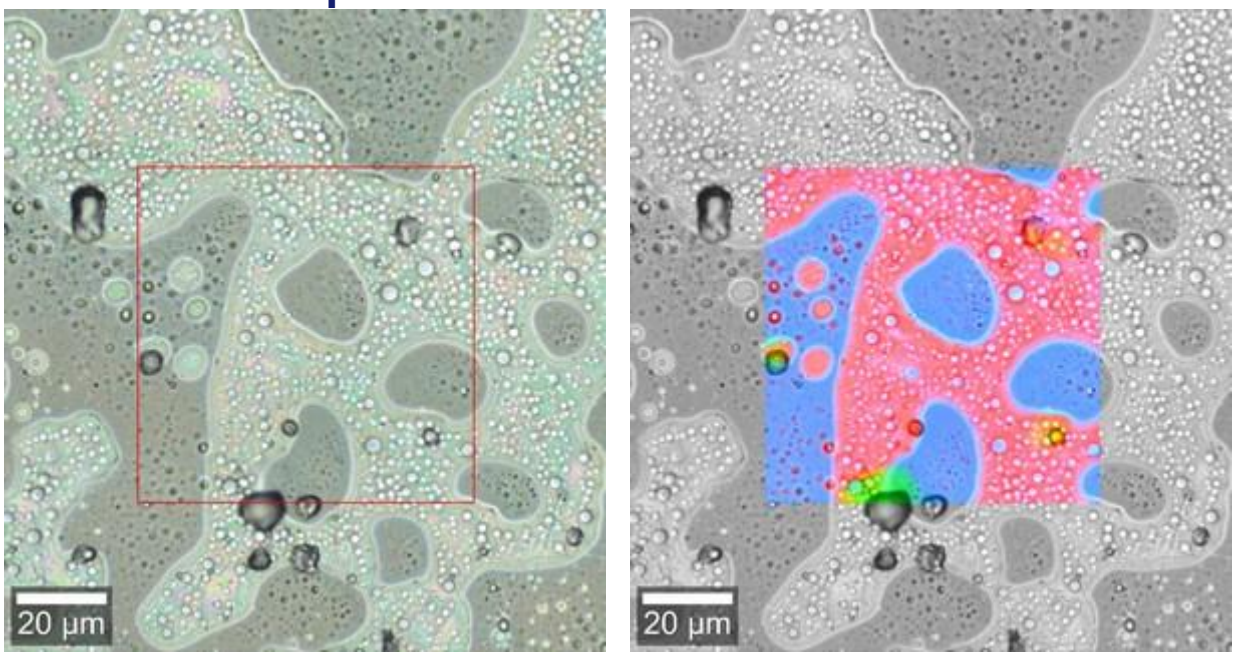
1. Select the laser you want to use.
2. For non-automated systems: Configure the beampath for the laser.
3. If necessary: Select an appropriate grating (in the spectrograph section).
4. Adjust the spectral center (in the spectrograph section).

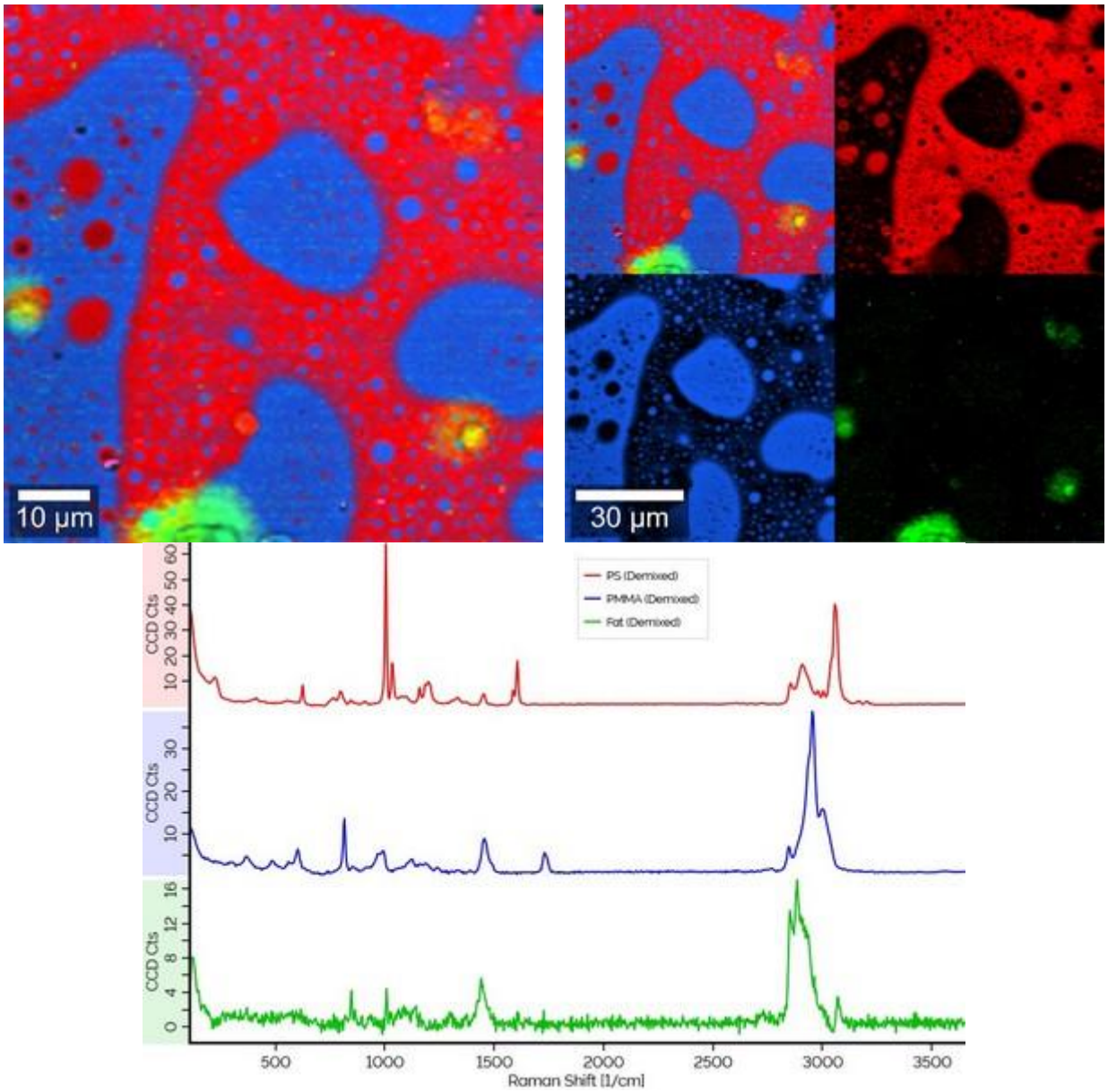
Change the laser (using a predefined state)

1. Select the state for the laser you want to use.
2. For non-automated systems: Configure the beampath for the laser.

After changing the spectrometer or laser, check the alignment.

Reference sample





Results of a 2D Raman scan on a dip-coated PS-PMMA polymer blend on glass: Video image with red rectangle showing the measurement area (top left), Video image with overlayed Raman image (top right), Color coded Raman image (middle left), Raman image and single component images (middle right) and corresponding spectra (bottom) from TrueComponent analysis

The dataset was evaluated using the Raman wizard with CRR, Shape background subtraction and TrueComponent analysis.

Table 1: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	75 x 75
Pixel	150 x 150
Integration time [s]	0.03
Excitation wavelength [nm]	488
Objective	100x/0.9

Spectrometer	UHTS 300 S
Grating [g/mm]	600
CCD camera	front-illuminated
Duration [min]	23

Large Area Scan

The Large Area Scan is using the motorized stage for Raman Imaging. Even large regions from tens of microns to centimeters can be measured.

System requirements:

- motorized stage (+ system)
- For access+ only Stepwise Raster is possible (and Area if licensed)
- Not possible with RISE and access

License requirements:

- CrossTableScanning for continuous measurement modes

Procedure

1. Select the Scan Method:
    a. Stepwise Raster (no continuous movement, slow mode, single spectra)
    b. Area (Imaging in the x-y-plane)
    c. Depth (Imaging in z-direction)
    d. Stack (3D-Imaging)
2. Define the Geometry of your measurement (the center position defines the center of your measurement also in z-direction.)
    a. By mouse with the Listen Position/Area function (consider to correct Width and Height to round values). Define the Depth for Depth Scan or Stack Scan.
    b. By directly typing in the values for Width, Height and/or Depth. The Center Position could be set by directly typing in the position or by pressing Center at Current Pos. to use the current position.
3. Optional: Change the Gamma value to adjust the angle of the measurement in the x-y-plane. (Select 90° to make the y-axis the first moving direction, if Area is the selected Scan Method.)
4. Define the number of pixels for the Image with Points per Line and Lines per Image (and Layers per Scan for Stack Scan) (This defines the resolution of your image in conjunction with the Width, Height and/or Depth parameter.)
5. Set the integration time:
    a. For Stepwise Raster define it in the Single Spectrum section.
    b. For all continuous measurement modes use the Integration Time parameter in the Large Area Scan section.
6. For non-automated systems: Change the beam path for Raman measurements.
7. Adjust the focus for your measurement using the applicable option:

   a. For Stepwise Raster and Area: Optimize the focus for your measurement by using the Oscilloscope. (Best position is probably the center of your measurement area.)
   b. For Depth and Stack use one of these options:
      i. Focus in the middle between highest and lowest point of your measurement.
      ii. Focus on the sample surface (Software-z should be zero) and change the Center (z) parameter to negative values to shift the measurement downwards or to positive values to shift the measurement upwards (i.e. for a depth of 10 µm use -4 µm as Center (z) value to measure from 1 µm above the surface to -9 µm below the surface).
8. Click at Start Large Area Scan to start the measurement.

Further information:

Large Area Scan, 3D Data Analysis

Hints

- The smallest step for the motorized stage is:
  - 100 nm for alphaControl with a serial number 120-1050-XXX (Marvin 4b) or lower
  - 25 nm for alphaControl with a serial number 120-1060-XXX (Marvin 5) or higher
- Try to use multiples of the minimum step size as resolution for the x-y-plane. Lower values can lead to artifacts in the image.
- Use the oscilloscope mode to check the necessary integration time.
- Consider to use a lower laser power for focusing to prevent damage from your sample. For scanning a higher laser power is possible in most cases.
- Make sure that User-z is selected while adjusting the focus, otherwise changing the focus has no effect on the measurement position.
- Consider using the Signal Stabilization function for measurements of several hours.

Image Scan

The Image Scan is using the piezo stage for Raman Imaging within its range.

System requirements:

- Piezo stage

Procedure

1. Select the Scan Method:
   a. Area (Imaging in the x-y-plane)
   b. Depth (Imaging in z-direction)
   c. Stack (3D-Imaging)
2. Define the Geometry of your measurement (the center position defines the center of your measurement also in z-direction.)
   a. By mouse with the Listen Position/Area function (consider to correct Width and Height

to round values). Define the Depth for Depth Scan or Stack Scan.

b. By directly typing in the values for Width, Height and/or Depth. The Center Position could be set by directly typing in the position or by pressing Center at Current Pos. to use the current position.

3. Optional: Change the Gamma value to adjust the angle of the measurement in the x-y-plane. (Select 90° to make the y-axis the first moving direction, if Area is the selected Scan Method.) (Even rotating the measurement area along the other axes is possible by the hidden parameters Alpha and Beta.)
4. Define the number of pixels for the Image with Points per Line and Lines per Image (and Layers per Scan for Stack Scan) (This defines the resolution of your image in conjunction with the Width, Height and/or Depth parameter.)
5. Set the integration time.
6. For non-automated systems: Change the beam path for Raman measurements.
7. Adjust the focus for your measurement using the applicable option:
   a. For Area: Optimize the focus for your measurement by using the Oscilloscope. (Best position is probably the center of your measurement area.)
   b. For Depth and Stack use one of these options:
      i. Focus in the middle between highest and lowest point of your measurement.
      ii. Focus on the sample surface (Software-z should be zero) and change the Center (z) parameter to negative values to shift the measurement downwards or to positive values to shift the measurement upwards (i.e. for a depth of 10 µm use -4 µm as Center (z) value to measure from 1 µm above the surface to -9 µm below the surface).
8. Click at Start Scan to start the measurement.

Further information:

Image Scan, 3D Data Analysis

Hints

- Consider to use a lower laser power for focusing to prevent damage of your sample. For scanning a higher laser power is possible in most cases.
- Image Scan is using the microscope z for moving in z direction for Depth and Stack scan. Exception: If the configuration is using piezo z.
- Use the oscilloscope mode to check the necessary integration time.
- Make sure that User-z is selected while adjusting the focus, otherwise changing the focus has no effect on the measurement position.
- Consider using the Signal Stabilization function for measurements of several hours.

Slow Series

The slow series offers several options:

- Slow time series – Intermittent time series with fixed time interval
- Laser power series – spectra at linearly varying laser power (only for TruePower laser)

- Polarizer series – spectra at linearly varying polarizer angle (only with automated polarizer)
- Analyzer series – spectra at linearly varying analyzer angle (only with automated analyzer)
- External triggered series – spectra on external triggered events (with COM Automation interface and e.g. LabView)

Slow time series

Advanced options in the default tree:

- It is possible to close the laser shutter in between the measurements using Microscope State after Single measurement.
- It is possible to do an autofocus before each measurement using the subsequencer.
- In combination with the COM Automation it is possible to add user data channels to each datapoint or to trigger the next measurement by software.

All needed parameters are described in the Slow Series section.

Laser power series

Keep Dose constant option:

- Keep Dose constant reduces the integration in the amount the laser power is raised.
- The integration time defines the minimum integration time..
- Even with keep dose constant a small effect on the intensity will be visible especially at very short integration times, because the integration cannot be determined 100 % exactly from the CCD camera.

Polarizer/Analyzer series

If you perform a Polarizer or Analyzer series the angle between polarizer and analyzer can be fixed with the synchronize angle option.

Data evaluation

You will get several data objects as result (at least two). One contains the spectra, others contain the elapsed time, laser power etc. All have their data points plotted against P, the number of the data point. Also if you use e.g. the filter view to get a intensity distribution from the Raman spectra, it will be plotted against P. Refer to the parametric view to create graphs e.g. intensity against time or intensity against laser power.

Further information:

Series Slow, Polarization

Sample Raster

Sample Raster enables processing a script at a predefined list of up to several thousand points.

The following tasks are possible for Raman:

- Auto Focus
- Single spectrum
- Image scan

It is not possible to use TrueSuface or other Topography correction for Sample Raster.

For automatic measurements of optical visible particles please refer to the ParticleScout.
For more sophisticated automation projects please refer to our COM Automation interface and e.g. LabView.

System requirements:

- motorized stage (+ system)
- Piezo stage for Image scans
- Not possible with RISE and access

Procedure

The following steps describe how to set up a Sample Raster measurement in an easy way doing an autofocus and taking a single spectrum.

1. Focus on your sample.
2. Click on Point List Editor.
3. Activate the Move Sample to Mouse Position.
4. Select a point by clicking in the video image or in a recorded image.
5. Click on Take current point as new point in the Point viewer.
6. Repeat points 4 and 5 until all desired points are in the list.
7. Enter *autofocus; singlespectrum* in the Command line parameter.
8. Define the parameters in the Autofocus and Single spectrum section.
9. For non-automated systems: Configure the beampath for Raman and open the laser shutter.
10. Optional: Click on Start Script to test the parameters at the current position.
11. Click on Start Raster to start the measurement.

Further information:

Sample Raster, Single spectrum, Image scan, Auto Focus

Manual Topography Correction

The purpose of the Manual Learned Topography Correction is to measure along simple sample surfaces without having the TrueSurface hardware. It is possible to compensate the tilt of the sample by using the 3-point tilt correction or even more complex surfaces by the 5 x 5 option.

System requirements:

- motorized xy- and z-stage (emulation for RISE)
- Not possible with R, RISE and access

Possible for Large Area Scan (including Depth and Stack scan) and Line Scan.

Procedure

1. Define your Geometry parameters for the Large Area Scan.
2. Go to Manual Learning in the Topography Correction section and select either Learn Plane (3 Pts) or Learn Surface (5x5 Pts) by clicking on the respective button.
3. The software now starts the oscilloscope to enable focusing using the Raman spectrum (For non-apyron systems: Configure the beam path for Raman).
   Alternative: It is also possible to switch back to video modus and use the video image for focusing.
4. Optional: It is possible to slightly change the current position, when it is not suitable for focusing.
5. If the height difference of the sample is more than 200 µm, the Software-Z travel range needs to be extended. Refer to the microscope-z section.
6. Click on Next Step.
7. Repeat the steps 3 to 5 until all points are focused.
8. Optional: Switch to Software-z by clicking S: in the Microscope Z area of the Video Control Window. (Prevents changing z-position of the surfaces relative to the sample by moving Microscope Z.)
9. Optional: Move to a point on your sample within the measurement area and click Goto Surface. Now it is possible to refocus the surface on the Raman spectrum by using the Oscilloscope (For non-apyron systems: Configure the beam path for Raman). (Make sure Software-z is switched to U:)
10. Switch Topography Correction to On in Large Area Scan.
11. For non-automated systems: Configure the beam path for Raman (insert Output and Laser coupler, remove video and brightfield coupler).
12. Click on Start Large Area Scan.

Further information:

Topography Correction

TrueSurface Mk1 and Mk2

TrueSurface Mk1/2 Overview

The TrueSurface Microscopy option enables confocal Raman imaging following the surface topography. Therefore, the topography is recorded in a first step with a Confocal Chromatic Sensor (CCS).

TrueSurface Mark I:

The first generation of TrueSurface was only available for the alpha500. The CCS is installed close to the turret and has an offset of several centimeter.

TrueSurface Mark II:

In the second generation of TrueSurface the CCS is integrated as objective in the turret. The first version of this objective has a longer black body, the second version has a shorter metallic body.

Topics:

- Calibration
- Setting up a measurement

System requirements:

- TrueSurface Mark I or Mark II
- Not possible with RISE and access

Possible for following measurement modes:

- Large Area Scans (including Depth and Stack scan)
- Line Scans

Calibration

The precondition to get good results with TrueSurface is that the offset between the objective and the Confocal Chromatic Sensor (CCS) has to be corrected. This is accomplished by the objective compensation.

If you have TrueSurface Mark I please refer to Mk1 Calibration. If you have TrueSurface Mark II please follow the next steps.

Sample for calibration:

- silicon (i.e. silicon with cross)

1. The CSS needs to be calibrated like any other objective (only necessary if not calibrated before):
    - use the piece of silicon with cross (metallic CCS)
    - if contrast is to weak, use the edge of silicon (black CCS)
2. Start the Objective Compensation Wizard (located in the Menu in the Video Control Window).
3. Follow the instructions for TrueSurface Mk2 found by clicking the question mark.

Hints

- Check that the appropriate TSMO2 objective is installed in the slot marked with TS.
- Make sure that the objective you want to use for the measurement is also compensated. Check in the Objective Compensation Wizard (located in the Menu in the Video Control Window).

Calibration Mk1

The following instructions apply only to alpha500 systems with TrueSurface Mark I.

Sample for calibration:

- pinhole probe with 50 µm hole

Recording a profilometer image of the pinhole

1. Switch to the Profilometer configuration (in the section Confocal).
2. Mount the pinhole probe (with adhesive tape) to the TrueSurface area of the cross table. (The dashed line is marking the allowed area.)
3. Locate the pinhole in the video image using a small magnification objective and switch to the 50x objective. Put the pinhole into the center of the video image.
4. Optional: Click the Set Zero button of the Sample positioner, to easily find back to this position.
5. Save a video image of the pinhole.

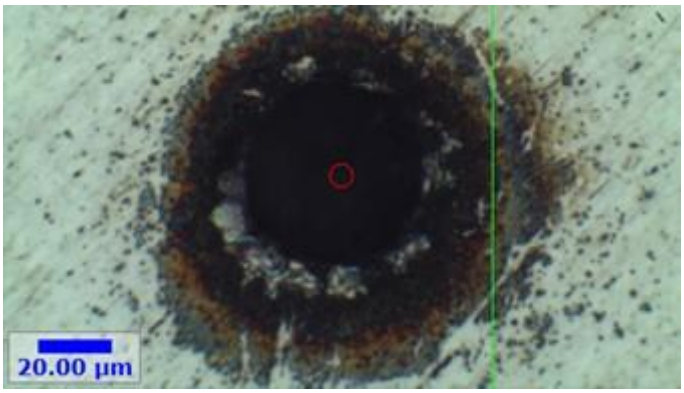


Figure 1: Video image of the pinhole

6. Switch to the TSMO1 objective (has to be Slot 7).
7. Use the remote control to drive with the pinhole probe to the Confocal Chromatic Sensor (CCS) (roughly [-90 mm; -1 mm] from the video center). Try to get the light beam as close as possible to the middle of the pinhole probe.
8. Define a Large Area Scan of about 5000 µm x 5000 µm to find the pinhole. Click on Center at current position and start the scan.
9. You should see the pinhole as a black dot in the resulting images. Decrease the area of the Large Area Scan (e.g. select with Listen Area) until you get pictures like in Figure 2. Keep both images open.

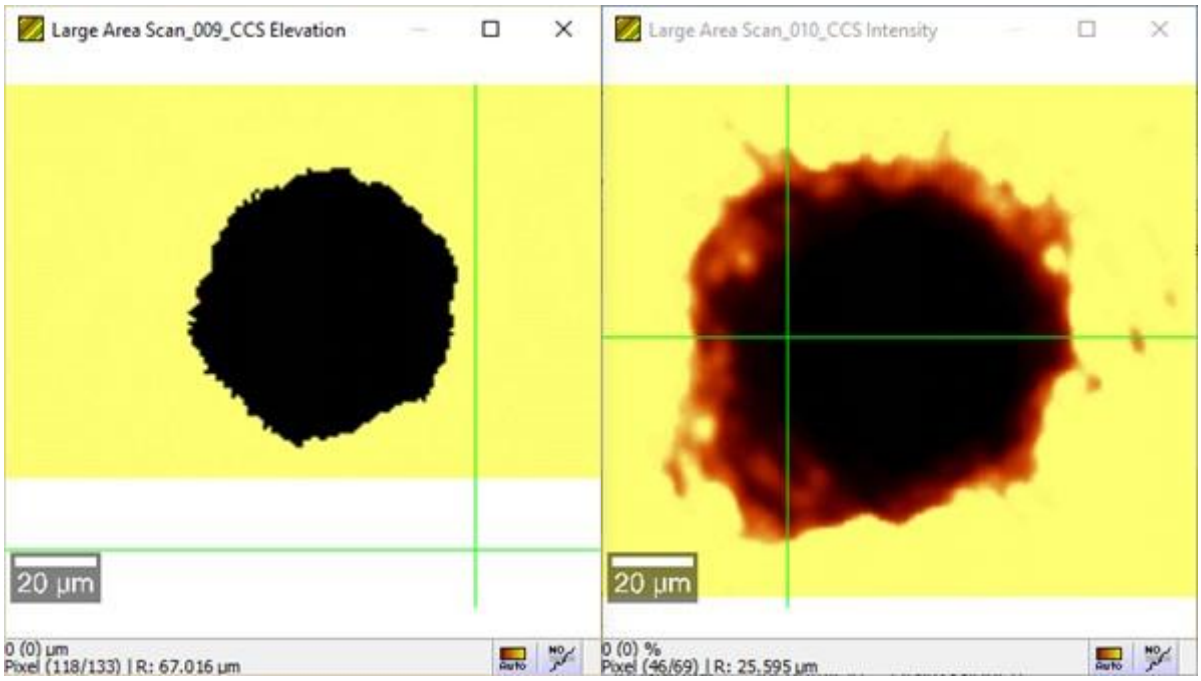


Figure 2: Pinhole with CCS sensor

10. Switch to the 50x objective, activate the Move Sample to Mouse Position and click in the previously recorded video image to move the pinhole back under the 50x objective.

Objective Compensation

1. Start the Objective Compensation Wizard (located in the Menu in the Video Control Window).
2. Move the center of the pinhole under the cross. Make sure the flat area around the pinhole is in focus.
3. Click on Start Calibration.
4. Select the TSMO1 objective and acknowledge the appearing window.
5. Click on the flat part around the pinhole in one of the two profilometer images.
6. Adjust the focus until the CCS Elevation [µm] in the status is half of the sensor range (i.e. 1500 µm for 3 mm).
7. Click in the center of the pinhole in one of the two profilometer images as exact as possible.
8. Click on Stop Calibration and close the wizard.

Check the compensation

1. Put the pinhole in the center of the video image using the 50x objective and save an image.
2. Select the TSMO1 objective.
3. Start a Large Area Scan with 200 µm x 200 µm and Center at current position using the Profilometer configuration.
4. Select the 50x objective after the measurement.
5. Profilometer image and video image should match. (Check by moving through the image with mouse button pressed or use the Overlay tool.)
6. If you are not satisfied, check the hints section and redo the objective compensation using

the new images.

Hints

- Check that the appropriate TSMO1 objective is installed in Slot 7.
- Make sure that the objective you want to use for the measurement is also compensated. Check in the Objective Compensation Wizard (located in the Menu in the Video Control Window).
- Fix the pinhole as good as possible, any shift of the probe will cause an offset in between the surface and the Raman map later on.
- Delete the compensation of the TSMO1 objective, if the stage goes to a completely wrong position, when you select the objective.

Procedure

1. Define your Geometry parameters for the Large Area Scan.
2. Go to a point on your sample within the measurement area, change to the CCS objective.
3. Only Mk2: Click on TS in the Video Control Window and for non-automated systems: Configure the beam path for TrueSurface (insert TrueSurface coupler, remove video and brightfield coupler).
4. Set the parameters of the Optical Distance Sensor. (Reduce Sampling Rate, if the signal is too weak. Move arround on the sample to check at different positions.)
5. Set Image SizeX [Pixels] and Image SizeY [Pixels] under Learn By CCS LA-Scan in the Topography Correction section.
6. Click on Learn By CCS LA-Scan.
7. After the scan click on Edit Surface Scan.

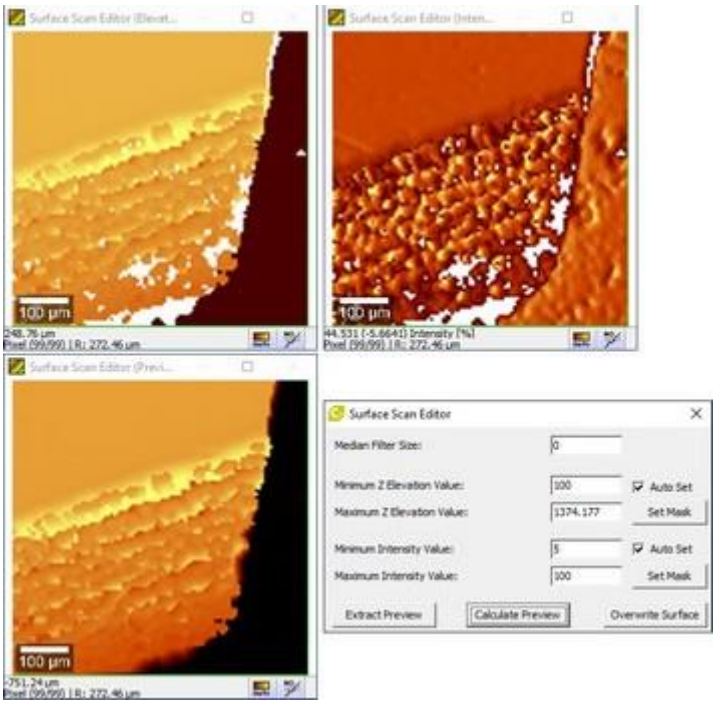


Figure 1: Surface Scan Editor

8. Surface points with a Z Elevation below zero or too low intensity have to be removed. Change the filter settings and click on Calculate Preview. (Figure 1)
9. Click on Overwrite Surface (Figure 1)
10. Optional: Click on Extract Preview (Figure 1) (Image could be used for navigation in 14.)
11. Optional: Switch to Software-z by clicking S: in the Microscope Z area of the Video Control Window. (Prevents accidentally changing z-position of the surfaces relative to the sample by moving Microscope Z.)
12. If the roughness of the sample is greater than 200 µm, the Z Travel has to be adjusted. Extend the Software-z travel range that is fitting the roughness of your sample.

If the Extended Z Travel is to small the Geometry parameters in the Large Area Scan become red and the measurement will not start.

13. Switch to the objective, which should be used for the measurement.
14. Optional: Move to a point on your sample within the measurement area and click Goto Surface in Topography Correction. Now it is possible to refocus the surface on the Raman spectrum by using the Oscilloscope (For non-automated systems: Configure the beam path for Raman). (Make sure Microscope Z is switched to U:.)
15. Switch Topography Correction to On in Large Area Scan.
16. For non-automated systems: Configure the beam path for Raman (insert Output and Laser coupler, remove video and brightfield coupler).
17. Click on Start Large Area Scan.

Further information:

Topography Correction, Optical Distance Sensor, Large Area Scan

Hints

- Less pixels can be used for the CCS scan compared to the Large Area Scan.

TrueSurface Mk3

TrueSurface Mk3 Overview

WITec's TrueSurface Microscopy option enables confocal Raman imaging guided by surface topography. In its 3rd generation, topographic Raman imaging uses an advanced optical profilometer integrated within the instrument to provide one-pass simultaneous operation.

Topics:

- Choice of Objective
- Adjustment and Hints

System requirements:

- alphaControl with a serial number 120-1050-XXX (Marvin 4b) or higher
- Not possible with RISE and access
- Objective with magnification of 20x or higher

Possible for all measurement modes besides:

- Sample Raster

Introduction

TrueSurface (TS) enables real-time large area topographic and Raman imaging within the scan range of the motorized positioning device even for rough or inclined samples. The effect is demonstrated in Figure 1 on a silicon lens. The image on the left shows the topography of the sample surface. Without TS, only one plane is in focus during a measurement, resulting in a ring of strong Raman signal (middle). The image on the right shows the same area measured with TS active, which allows the acquisition of a Raman map with a consistently strong signal.

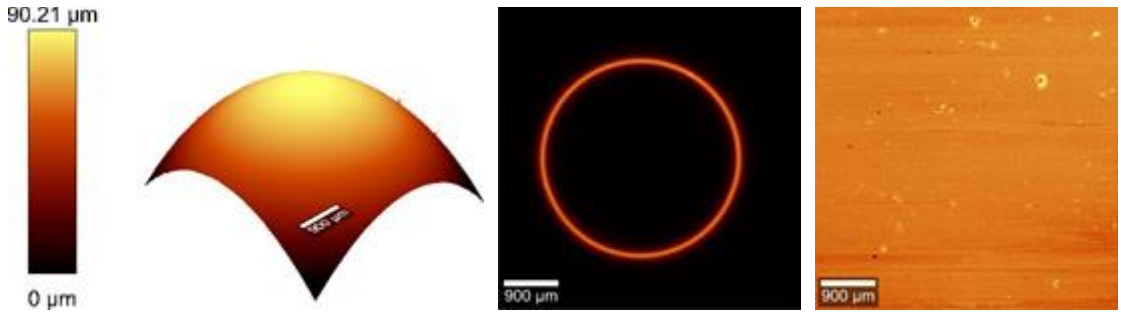


Figure 1: Si lens. Left: Topography. Middle: Only one depth level is in focus with the standard confocal Raman microscope. Right: The same image as in the middle, but with TrueSurface activated the Si signal is constant from all depths as the sample surface is always kept in focus.

Technique Principles

TrueSurface focuses light onto the same spot on the sample at which the excitation laser for Raman measurements is focused. This establishes the distance between the objective and the sample. As

reflected light is measured, a different index of refraction at the sample surface is required. A feedback loop allows the system to keep the distance constant and thus the sample surface always stays within the Raman measurement's focus. This enables simultaneous measurement of the sample's topography and Raman signal.

Choice of Objective

Many objectives are suitable for TS measurements, but they differ in their properties and not all objectives are recommended in combination with all samples. Several objectives have already been tested and recommended for operation with TS. A warning message indicates if any untested objective is selected, nevertheless it may be possible to use it. Objectives with a magnification of less than 20x are not suitable.

For a proper choice of objective, the following aspects should be considered:

- The objective's **transmission** properties for TS differ. Some objectives thus give a low TS signal.
- The TS principle requires reflected light from the sample surface to be measured. Edges or strongly inclined areas can scatter or reflect the light beyond the collecting angle of the of the objective, which results in lower TS signal, especially for objectives of low **numerical aperture** (NA). Figure 1 shows video images of a silicon step sample. In the image on the left, acquired with a 20x/0.5 NA objective, the edges appear black because less light from there is collected by the objective. In the image on the right, the 100x/0.9 NA objective could collect light reflected from the edges due to the higher NA and the respective larger collecting angle.

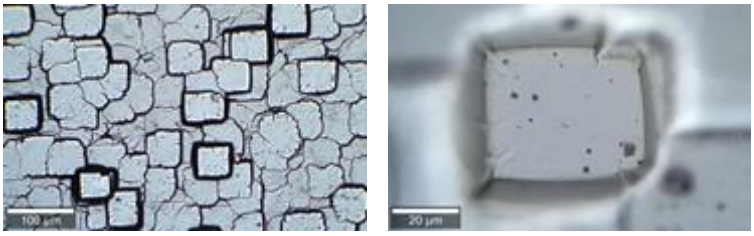


Figure 1: Video image with 20x/0.5 NA objective (left) and video image with 100x/0.9 NA objective (right)

- The roughness of the **sample surface** is important, as the reflected light captured from out-of-focus areas after a large step might not be strong enough for a continuous feedback. Measurements on samples with large height variations may be easier with low magnification objectives because a small magnification is accompanied by a large focal range, while high magnification correlates to a small focal range.

The following step heights are typically manageable for standard objectives:

100x objektive:	+/-4 µm
50x objektive:	+/-16 µm
20x objektive:	+/-100 µm

Objectives with a small magnification are therefore better suited, if the sample features high steps, while a high NA is required to measure sharp edges.

Adjustment

Procedure

1. Focus on your sample with the objective that should be used for the measurement.
2. Move the microscope focus position in the middle between highest and lowest point within your measurement area.
3. Set the Software-z to zero.

4. If necessary: Extend the Software-z travel range so that the highest and lowest point could be reached. Please also mind the objective's working distance.
5. For non-automated system: Configure the beampath for TrueSurface.
6. Turn on TS in the TrueSurface section and click on Start.
7. Adjust the signal intensity to about 50 %.
8. Move around within measurement area and check whether TS follows the topography. If not please refer to the hints section. Check the signal intensity during the movement.
9. Start the Oscilloscope mode.
10. For non-automated systems: Configure the beampath for Raman.
11. Use the Focus Shift value to maximize the Raman signal or to adjust a certain z offset. (The value is not in µm. Please refer to the software-z to observe the change in µm.)
12. Now the Raman measurement could be set up in the same way like without TS.

Parameters and Hints

If the feedback does not work well, the following parameters should be reconsidered:

1. If the topography has **too steep edges**:
   - If the height difference of the edge exceeds the catching range of the used objective, consider to use an Objective with lower magnification.
   - or try to use a different position on the sample.
   - If you don't want TrueSurface to follow the edge (hole in the sample surface), make sure the Min. Value (%) is high enough, to stop on the surface.
2. The **signal level is too high**:
   - Changing the beampath from camera to Raman causes a higher signal level. Try to lower the TS Light intensity. A too high signal results in less precision.
3. The **signal level is below minimum**:
   - A signal level below minimum stops the z-movement until the signal is higher. Try to raise the TS Light intensity. It should be a good compromise between high and low value areas.
4. TrueSurface **goes to the upper z-limit** and stays there:
   - Most likely TrueSurface acts on noise. Try to raise the Min. Value (%). This causes TrueSurface to stop instead of moving away from the surface.
5. The topography looks **smeared**:
   - TrueSurface is not able to follow the surface fast enough (i.e. on rough surfaces). Raise the P-gain or reduce the scan speed by raising the integration time or the points per line.
6. The topography looks **noisy or oscillating**:
   - The microscope starts to vibrate maybe in combination with a shrill sound. Reduce the P-gain until the oscillation is gone.
7. If there are **problems at the beginning of line** in a slow scan (one line takes several seconds or minutes):
   - The backward movement of the scan is too fast. Use a higher Min. Time for Retrace [s] for the Large Area or Image Scan.
8. For flat samples with **continuously changing shape**:
   - Maybe an offset of the optimum focus can be observed. Use a small I-gain (i.e. 0.02). In normal case the I-gain should be 0.
9. The **sample does not resist** the TrueSurface light source:

- Limit the intensity or fix the gain to only control the light intensity with the slider by clicking on options.

Signal Stabilization

Signal Stabilization Overview

The purpose of the Signal Stabilization is to compensate focus drifts in long term (several hours) measurements due to changes of the ambient temperature.

Topics:

- Setting it up
- Hints and Troubleshooting

System requirements:

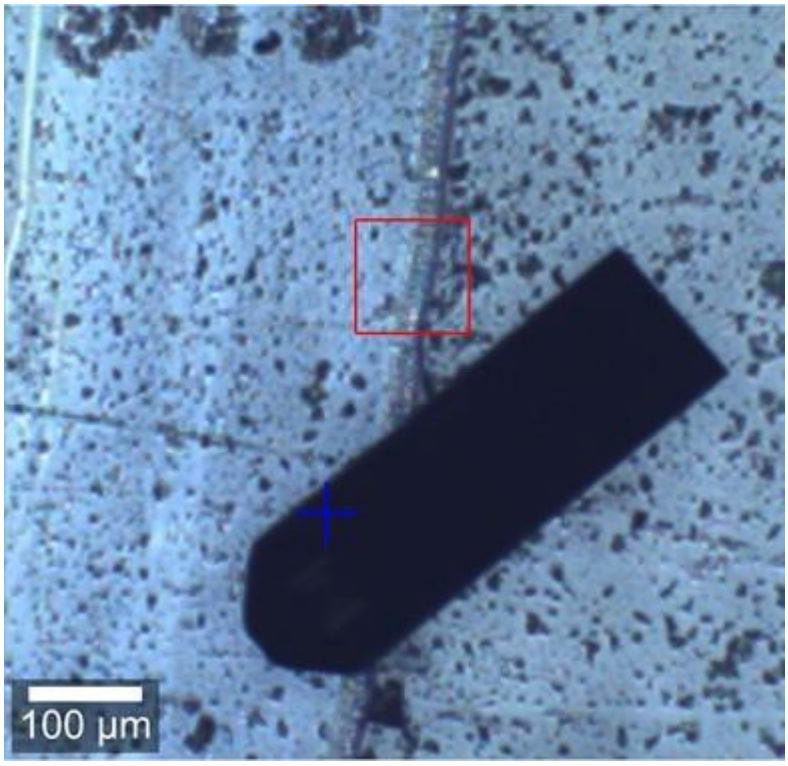
- Not possible with RISE (works only with emulation) and access
- High magnification objective > 10x

Possible for all continuous measurement modes of Image Scan and Large Area Scan:

- Images
- Depth Scans
- Stacks
- including Topography Correction for the Large Area Scan

A reference point at the sample is needed i.e.:

- sample itself (sufficient Raman signal needed)
- silicon (i.e. Cantilever (Figure 1))
- adhesive strip



Procedure

1. Define your measurement parameters for the Image Scan or Large Area Scan (at least the geometry and integration time).
2. Choose the appropriate Stabilization Mode (Peak for samples giving just a signal at the surface or Positive Edge for sample giving a signal also under the surface (Figure 1))

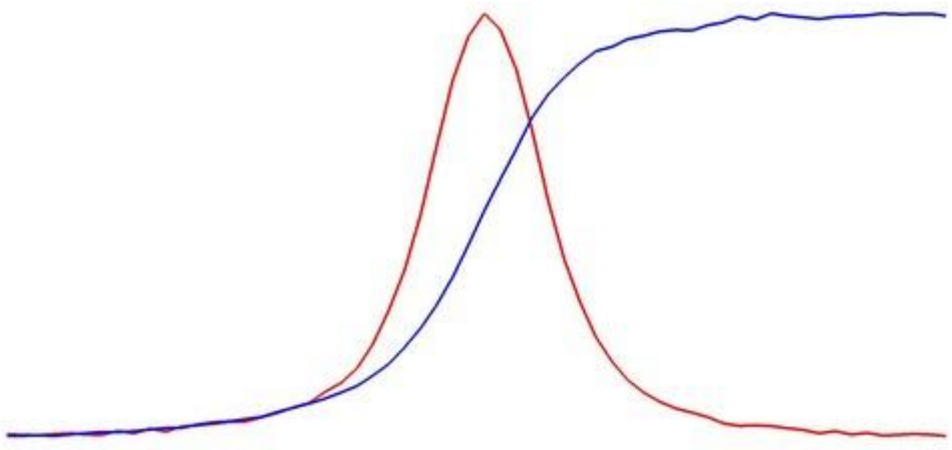


Figure 1: Depth profile of silicon (red) and adhesive strip (blue)

3. Choose the Actuator for Compensation. If possible use the Scan Table for the best results because of the better linearity for very small steps.
4. Define your reference point using Listen Stabilization or typing the coordinates. (Refer to Hints points a to f)
5. For Listen Mask select Multiple and mark the spectral regions in the Hardware Spectrum window. (If necessary, start the Oscilloscope.) (Switch back to Never afterwards.)
6. Click Start Stabilization to check the performance and success in the Messages window. (Change the beam path to Raman before.)
7. If the stabilization failed, focus on maximum spectral intensity for peak mode or on half of maximum for edge mode using the Oscilloscope. (User-z (U:) has to be selected.)
8. Repeat clicking on Start Stabilization several times until the stabilization height shown in the Messages window is nearly zero (Figure 2).

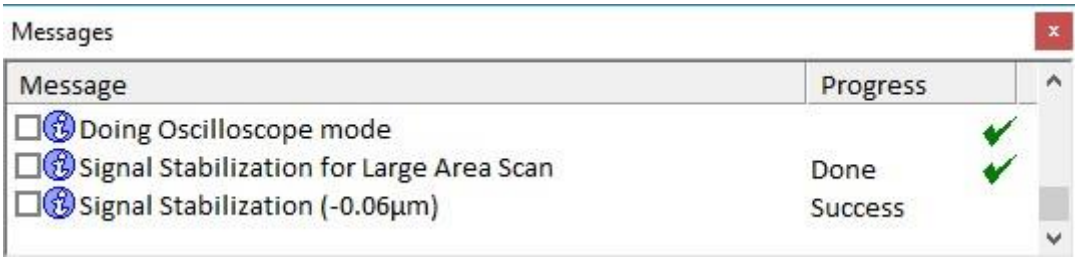


Figure 2: Messages window

9. Adjust the focus for your measurement using one of the two following options:
   a. Optimize focus within the measurement area and change the Center (Z) value to the current Software-z value **or** optimize focus in the center of the measurement area and click on Center at Current Pos.
   b. Use topography correction (Manual or with CCS-Scanner). After the topography was

read in, choose the appropriate of the two following options:

i. If you are doing manual topography correction by Raman spectrum, you do not need to do something additionally.
ii. If you are doing manual topography correction by Video image or using the CCS-Scanner, go to a point within your measurement, click on Goto Surface, keep the current Software-z in mind, optimize the focus, calculate the difference between the current Software-z and the value before optimizing the focus, enter this value at Z-Shift and finally check this value at several points.

10. Click at Start Large Area Scan or Start Image Scan to start the measurement.
11. You can observe the progress of the Signal Stabilization in the messages window. (Figure 3) If it fails at one time, it will try again at each following stabilization step. (Pointing with the mouse at the message gets you a hint, why it did not work.)

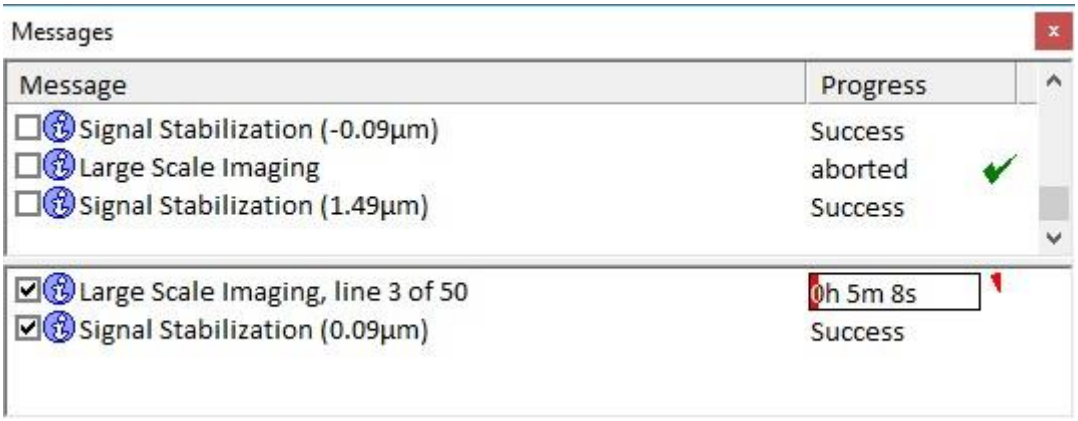


Figure 3: Messages window

Further information:

Signal Stabilization, Large Area Scan, Image Scan

Hints and Troubleshooting

Preconditions for choosing a reference point:

a. Enough Raman intensity under measurement conditions (Stabilization uses the same integration time as the measurement)
b. The area around the reference point should be flat (otherwise slight drifts in x-y-plane could affect the focus)
c. The reference point should be stable for the selected laser power.
d. The focus at the reference point should drift in the same way like the sample.
e. It is possible to choose a reference point within your measurement area, but this could cause a modification of the sample at this point, i.e. photobleaching.
f. Multilayer material could disturb the stabilization. Select a spectral range which is unique for the layer you want to use for stabilization. Maybe check it by doing a depth scan at the reference point.

If you followed the procedure, but the signal stabilization is still not working, check the following points:

1. Move the mouse to the message in the message window and read the hint.
2. Did you select the right objective?
3. Did you select an appropriate spectral range?
4. Have you burned your reference point?
5. Maybe the spectral quality is too bad. Check the signal to noise ratio while testing the stabilization. Try a higher Number of Accumulations, a longer integration time for the measurement or use a better Raman scatterer as reference point.
6. Are there unique bands for the layer you want to use for stabilization, in case of a multilayer material?
7. Have you focused to the maximum signal at the reference point for peak mode or to the half of the maximum for edge mode with User-z (U:) selected?
8. Make a depth scan at the reference point and check the profile whether you selected the right Stabilization Mode. (compare with this figure)
9. Have a look at the signal intensity while you are testing the stabilization. For peak mode it should go down to the half of the intensity. If the signal does not reduce that much due to a bad z-resolution (i.e. laser with high wavelength or 100 µm fiber), try a higher Step Size Multiplier i.e. 1.2. If the signal reduces more than this (i.e. 355 nm laser), try a lower Step Size Multiplier i.e. 0.9.
10. The Signal Stabilization could only work for slow drifts, if the sample surface is moving too fast, it will not work. TrueSurface Mark 3 could be a solution in this case.

If you want to stop the Signal Stabilization during the measurement change Stabilization Enable to No.

Polarization

In most cases Raman spectra can be measured without paying specific attention to the polarization direction of the incident laser light or an additional analysis of the scattered light. However if it comes to selection rules, the polarization of light in Raman spectroscopy gains in importance. With polarized light, Raman spectra will only have some Raman active modes and by rotating the polarization, other modes become accessible.

Symmetry of the vibrational modes are very important in vibrational spectroscopy. In case of Raman spectroscopy measurements, one can differentiate the symmetric modes from others by using polarized light. In this way, information about the symmetries within samples/molecules can be obtained.

System requirements:

- Polarization kit

Raman polarization studies

Polarized Raman spectroscopy uses a polarized laser excitation and an analyzer that typically is used to acquire spectra, either parallel or perpendicular to the excitation laser. The resulting spectral information allows an insight into molecular orientation and vibrational symmetry. In essence, it allows the user to obtain valuable information relating to molecular shape, for example in synthetic chemistry or polymorph analysis and is most often used to understand the orientation of molecules in organized environments such as crystal lattices, liquid crystals and polymer samples.

The ratio of the peak intensity of the parallel and perpendicular component is known as the depolarization ratio and can be obtained as shown in the following equation, where ⍰ signifies the

peak intensity with excitation and Raman analysis polarizer orientated perpendicular to one another, and // signifies parallel orientation.

$$\rho = I_{\perp}/I_{//} \qquad \text{or} \qquad I_{depolarized}/I_{polarized}$$

If ρ for a particular peak is less than 0.75, this peak has arisen from a totally symmetric vibrational mode. This peak/vibration is called a polarized band. Non-symmetric modes have a depolarization ratio of more than 0.75 and these are called depolarized bands.

Fig. 1 and 2 demonstrate the parallel and perpendicular spectra obtained for uniaxial isotactic polypropylene. It is apparent that certain peaks are highly polarized and show significant differences in peak intensity depending on the polarizer orientations.

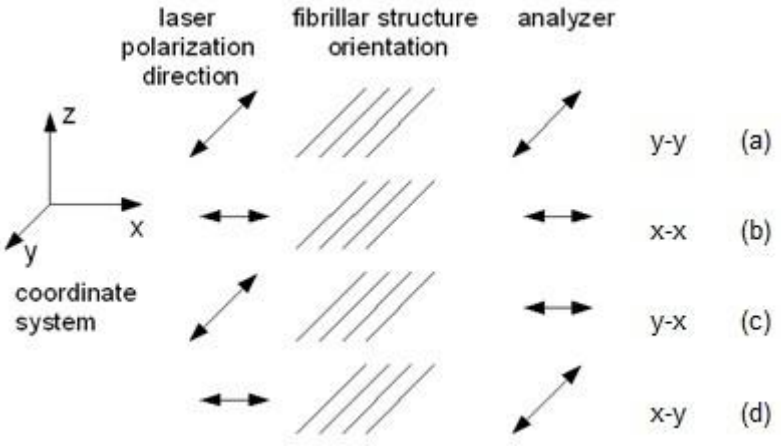


Fig. 1: Schematics of laser polarization with respect to an oriented structure and the corresponding analyzer positions.

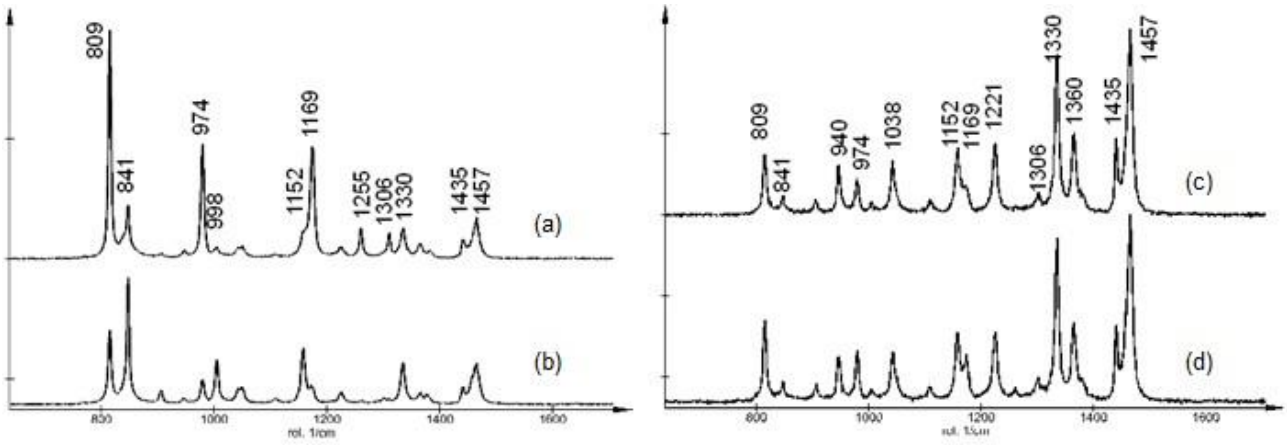


Fig. 2: Polarization dependent Raman spectra acquired from isotactic polypropylene in the four possible configurations.

For rotating the polarized light of the excitation laser a lambda half plate can be used. A lambda quarter plate is capable of changing the linear polarized light to circular polarized light. These plates need to fit the used excitation wavelength.

EMCCD Camera

This section gives a brief introduction and some background information spectroscopic electron multiplying CCD (EMCCD) cameras. The key advantage of using an electron multiplying CCD

(EMCCD) camera is the improvement in the signal to noise ratio for fast measurements. Therefore, the sources of noise within the CCD camera are analyzed first in the section below, before the EMCCD technology and its advantages in the signal-to-noise ratio (S/N) are introduced. Following this, the examination of a contaminated PMMA sample on a glass slide will be presented as an example to illustrate the performance of the system in combination with the EMCCD camera.

System requirements:

- EMCCD camera (Newton)

Background: Sources of noise within a CCD camera

The back-illuminated CCD cameras typically used in confocal Raman setups have a quantum efficiency of more than 90 %. Therefore, most of the photons arriving at the detector are converted into electrons. This means that when optimizing the S/N, the signal can hardly be improved further and thus the noise needs to be reduced in order to produce an improvement.

The photons detected by the CCD camera underlie the Poisson statistic. For any given signal, the noise associated with it will be the square root of the signal itself. This noise is called the photon shot noise. For a signal of 100 electrons, the detection uncertainty is 10 electrons and therefore the maximum S/N is 10. Other sources of noise are mainly thermal noise (dark noise) and readout noise. As the shot noise sets the upper S/N limit, the goal must be to minimize all other sources of noise.

Thermally generated carriers within the CCD chip are responsible for the dark noise. These can be reduced through efficient cooling and high quality CCD cameras will have a thermal dark current below 0.01 electrons/pixel/s at -60 °C. This cooling is sufficient for integration times of several seconds and becomes irrelevant in confocal Raman imaging where typical integration times are below 100 ms.

The electrons generated through the photons are converted to digital counts by the readout amplifier. This conversion process is the source of the readout noise. It is directly linked to the quality of the readout amplifier and the readout speed used. Typical values are a noise of 5-10 electrons for a 50 kHz readout rate and about 30 electrons at 2.5 MHz.

The aim of any spectroscopic experiment is to achieve a shot noise-limited signal. This is the case if the shot noise is the dominant source of noise. If single spectra are recorded, this can simply be achieved by increasing the integration time. However, as stated earlier, in confocal Raman imaging, short integration times and thus fast readout of the detected data are essential. Unfortunately, the faster the readout of the detector is, the higher the readout noise of the amplifier becomes. A 1024x128 pixel CCD equipped with a 50 kHz readout amplifier can be read out in about 22 ms, which is also the shortest possible integration time. If a readout noise of 10 electrons is assumed, every signal below 100 electrons/pixel will be readout limited (Poisson noise ≥ readout noise). If a fast readout amplifier is used which displays a readout noise of 30 electrons, even a signal of 900 electrons (1000 photons/pixel on the detector) will be readout limited.

S/N improvements using an EMCCD camera

An EMCCD is a normal CCD with an additional readout register which is driven with a much higher clock voltage than a normal CCD readout register. Due to this high clock voltage, an electron multiplication through impact ionization is achieved with an adjustable total amplification of the signal of up to 1000 times. Since this amplification occurs before the readout amplifier, it is always possible to amplify the signal above the readout noise. The S/N ratio is then always limited by the Poisson noise of the signal, even if a very fast readout amplifier is used. As an example, a 1600×200 pixel EMCCD with a 2.5 MHz readout amplifier can be read out in about 2 ms.

The following calculations show the improvement in S/N that can be expected for different signals. It is assumed that the quantum efficiency (QE) of the CCD is 90 % and that a 2.5 MHz readout amplifier is used. This amplifier has an associated readout noise of 30 electrons. The A/D conversion is generally set by the manufacturer so that this noise corresponds to 1 A/D count (30 electrons = 1 A/D count).

If 100 photons reach the detector in standard CCD mode, 90 will be registered which equals 3 A/D counts. The Poisson noise will be 9.5 electrons or 0.3 A/D counts and the readout noise 1 A/D count. Therefore the S/N will be about 2.9.

For an EMCCD, the signal is amplified before it reaches the A/D converter. While this amplification is variable, the maximum amplification of 1000 is used for illustration in this example. In this case, the 90 electrons detected will be amplified to 90,000 electrons, resulting in 3000 A/D counts. The Poisson noise will be amplified to 9500 electrons, which translates to 317 A/D counts. The electron multiplication process adds another noise factor of 1.4 (the so-called excess noise factor). Thus, the total noise including this factor and the 1 A/D count due to the A/D conversion is 443 A/D counts. Therefore, the S/N for the EMCCD in this mode is 6.8, which is an improvement of a factor of 2.4 compared to the "normal" CCD camera.

This improvement depends strongly on the amount of photons hitting the CCD camera and is much more prominent for small signals than for large signals. For higher signals, in which the signal intensity is no longer readout limited, the excess noise factor of the EM process reduces the S/N ratio of an EMCCD to below that of a normal CCD. In this case, the EM register can be switched off and then the "normal" readout register is used. Thus, the EMCCD behaves just as a standard back-illuminated CCD.

Figure 1 shows the dependence of the S/N of a "normal" CCD camera as well as that of the EMCCD camera on the signal reaching the detector. Additionally, the ratio of these two S/N ratios is shown.

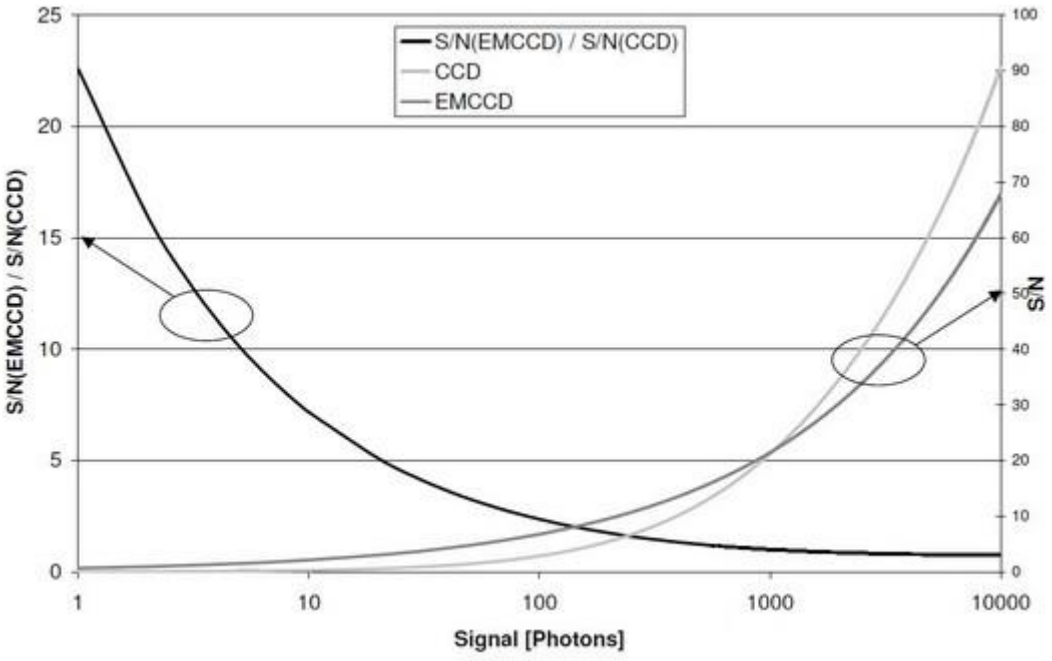


Figure 1: The theoretical S/N for a "normal" CCD camera and an EMCCD camera as a function of the signal reaching the detector. The ratio of the two S/N ratios is shown as well.

It can be seen that the advantage of the EMCCD camera clearly lies in the region where only a faint signal can be detected. Above approximately 1100 photons reaching the detector, the "normal" CCD camera mode is preferable. Due to the fact that the EMCCD camera with a gain of 1000 would be saturated at about 1900 photons, its operation up to about 1000 photons is not a problem. For small signals, as are present in ultra fast confocal Raman imaging measurements, the S/N of the

EMCCD camera can be as much as 20 times higher than a "normal" CCD camera.

Experimental Setup and Results

The sample for the tests presented in the following was an ultra-thin poly(methyl methacylate) (PMMA) film spin-coated onto a glass substrate. The layer was scratched and the PMMA removed in parts of the sample (in the center of the figures below). Here the height of the sample was determined by AFM to be 7.1 nm. Additionally, a needle-shaped contamination was found on the surface with a thickness of 4.2 nm. The origin and material composition of this contamination layer was not known initially, but could be determined by the confocal measurements.

The results presented in Figure 2 were obtained using a WITec system equipped with a UHTS 300 spectrometer and an EMCCD detector. The images were obtained by acquiring 200×200 Raman spectra in a 50×50 µm scan range and integrating the intensity of the CH_2 stretching band of PMMA at around 3000 cm^{-1}. Excitation power was 20 mW at 532 nm using a 100x, NA=0.9 objective.

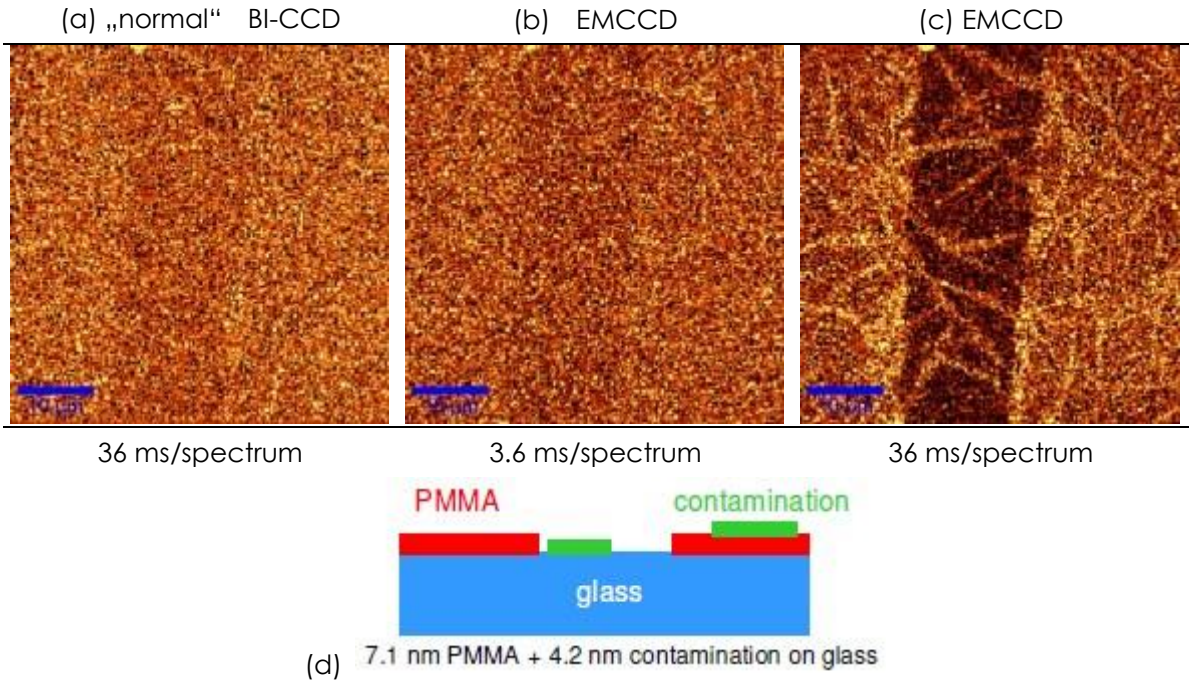


Figure 2: Confocal Raman Images of a 7.1 nm PMMA layer on glass obtained in the CH_2 stretching band around 2950 cm^{-1}: (a) back-illuminated CCD, (b,c) EMCCD. Scale bar: 10µm. (d) Schematic of the sample.

Figure 2 (a) was obtained with a standard back-illuminated (BI) CCD using a 62 kHz readout amplifier and 36 ms integration time per spectrum. With a little imagination, the scratch in the center of the image is just visible, but the S/N ratio is much smaller than 1. Figure 2 (b) shows the same part of the sample imaged with an EMCCD with a gain of about 250. The image shows nearly the same S/N, but now the integration time was only 3.6 ms, 10 times faster than in Figure 2 (a). The complete image acquisition took 25 minutes for Figure 2 (a), but only 3.4 minutes for Figure 2 (b). Figure 2 (c) was taken with the EMCCD, though now with the same integration time as in Figure 2 (a). One can not only clearly see the scratch, but also the contamination in the form of a needle-like structure across the PMMA and glass surface. Figure 2 (d) shows a sketch of the sample.

Further information:

Spec Camera

Further Reading

1. T. Dieing, O. Hollricher, High-resolution, high-speed confocal Raman imaging. *Vibrational Spectroscopy* **48**, 22-27 (2008). doi:10.1016/j.vibspec.2008.03.004

InGaAs Camera

For recording spectra in the NIR, InGaAs array detectors are the best choice. Dependent on the ratio of the elements the bandgap of the material can be shifted. Usually sensors are sensitive up to 1.7 µm or with extended range up to 2.2 µm. Silicon-based sensors have a bad sensitivity in the NIR region especially above 1000 nm.

A significant difference between a silicon-based CCD and an InGaAs array is how the readout is accomplished. In a CCD the electric charge of each line is shifted to a readout register. From there it is shifted pixel by pixel to an amplifier and an A/D converter. The resulting data has a high homogeneity because each pixel is treated in the same way. InGaAs arrays are a kind of active-pixel sensor (APS) like CMOS sensors where each pixel has its own electronics. Some arrays also treat even and odd pixels with different electronics. This results in some effects that has to be taken into account:

1. Each pixel has a different offset.
2. Each pixel has a different gain.
3. The gain changes with intensity and is also dependent on the position where the light hits the pixel.
4. The dark current is very high.
5. Each pixel has a different dark current rate.
6. Gain, offset and dark current depend on detector and scene (spectrograph) temperature.

Therefore a correction of the raw data is necessary. Three routines are available in WITec Control.

At least offset and dark current correction are highly recommended.

Offset Calibration

The offset of each pixel is determined using shortest integration time and without light falling on the detector. The recorded average spectrum is then subtracted from all measured spectra and an offset of 2000 is added (see Fig. 1).

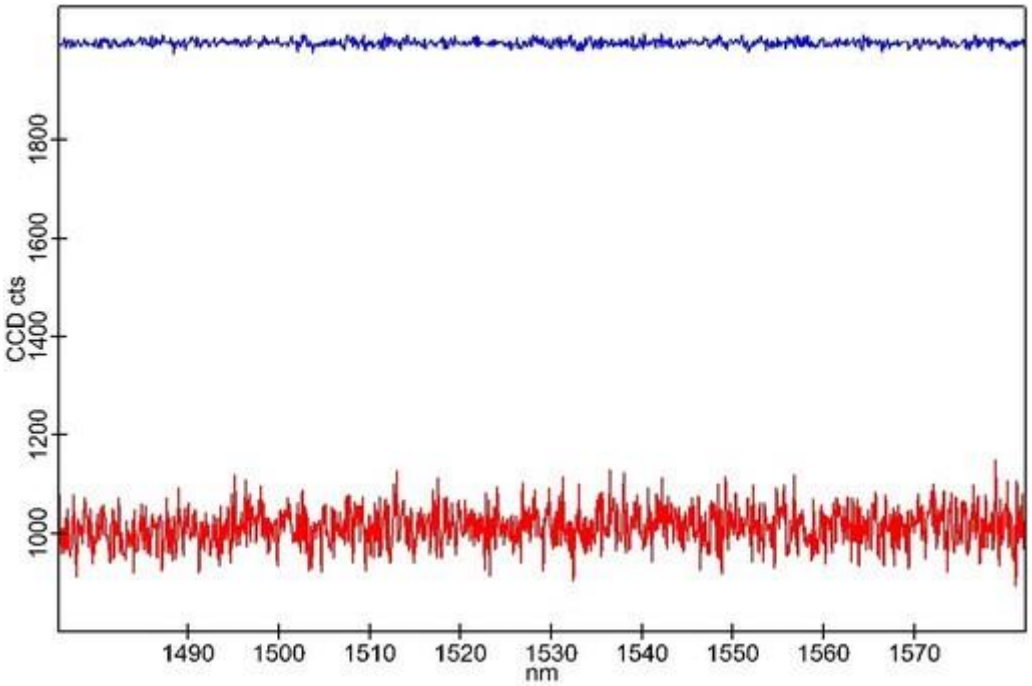


Fig. 1: Single spectra without (red) and with (blue) offset correction.

Intensity Correction

In order to calibrate the gain of each pixel, a spectrum with a smooth distribution is needed (see Fig. 2).

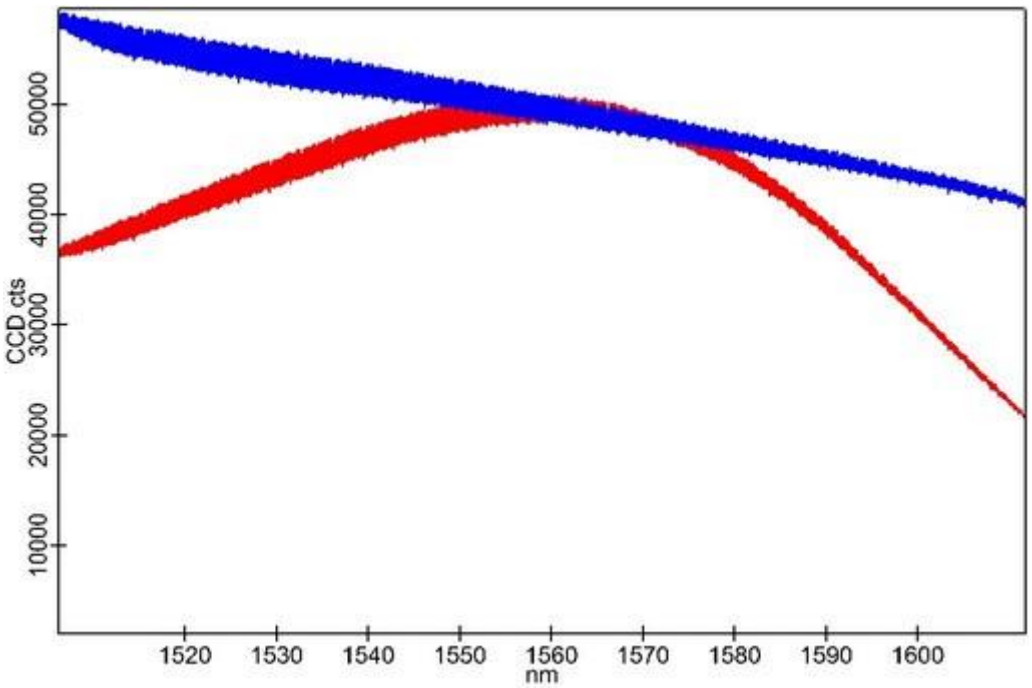


Fig. 2: Spectra of a LED (red) and tungsten halogen lamp (blue) with 600 g/mm@1560nm grating.

For the intensity correction a grating with a high groove density (e.g. 600 g/mm@1560nm) in combination with a tungsten halogen lamp with big filament is recommended to get a smooth spectrum. Using a broader spectral range the spectrum is not smooth due to light absorption of

water and other gases and cannot be used for the gain intensity calibration. Using a lamp with a small tungsten filament can produce oscillations in the spectrum.

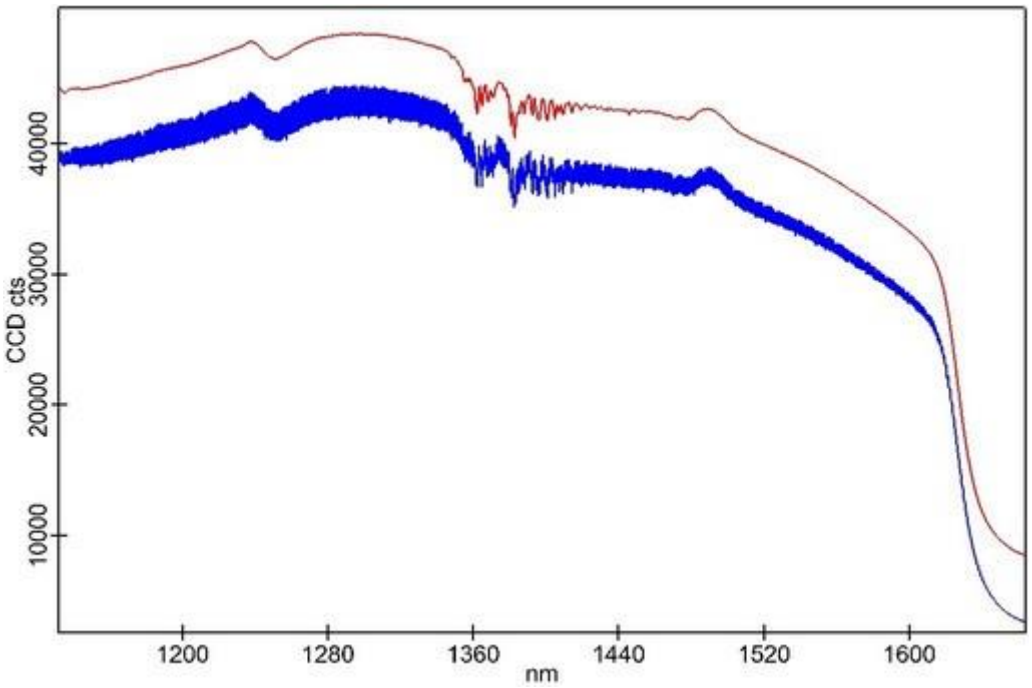


Fig. 3: Spectrum of a tungsten halogen lamp with (red) and without (blue) intensity calibration

Dark Current Correction

Background signal is coming from dark current of the detector as well as from the temperature radiation of the surrounding (scene). If the detector and the scene temperature is constant, each pixel has an individual but constant dark signal rate. The measured signal is proportional to the integration time.

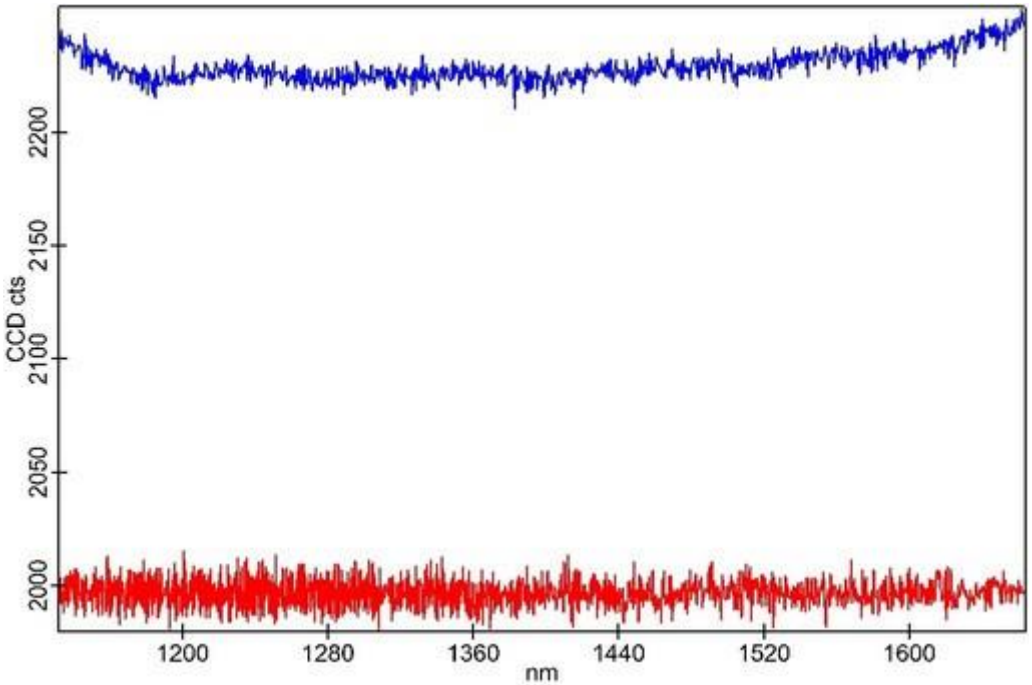


Fig. 4: Single spectra without (blue) and with (red) dark current correction (0.5 s integration time).

Spectral Stitching

Spectral stitching can be done, but artifacts in the stitched spectrum are possible.

Further information:

Spec Camera

Confocal

Confocal Overview

Confocal Microscopy is using a pinhole to reject out-of-focus light to improve the optical resolution and forms an image by scanning with a point detector.

Topics:

- Theoretical Background
- Setting up a measurement
- Example measurement

Confocal modes:

Depending on the system configuration the following confocal modes are possible with a WITec system:

- Confocal microscopy in reflection and transmission
- Confocal fluorescence microscopy in reflection and transmission
- StrobeLock – Time-resolved microscopy

Configurations:

The following configurations are available depending on the equipped photon counting device:

- Confocal PMT/APD
- Confocal Transmission PMT/APD (z movement during measurement by piezo stage)

Measurement modes:

- Oscilloscope: displays the output channel of a photon counting device as a function of time similar to the display of an oscilloscope.
- Image Scan: acquisition of confocal images using the piezo scanner
- Large Area Scan: acquisition of confocal images using the cross-table

System requirements:

- systems equipped with a photon counting device
- inverted objective for measurements in transmission

Theory

The principles of confocal microscopy can be found in the general section.

To take full advantage of the lateral and depth resolution possible with confocal microscopy, the size of the pinhole (core diameter of the multi-mode fiber for detection) must be properly chosen.

The optimum pinhole diameter depends on the optical properties of the microscope objective along with the wavelength employed and can be calculated using the following formula:

$$D \leq \frac{\lambda \cdot v \cdot M}{NA \cdot \pi}$$

where λ is the wavelength of the laser, M is the magnification and NA is the numerical aperture of the microscope objective.

The property ν is given in optical coordinates and should be 2.5 for the best depth resolution and 0.5 for maximum lateral resolution. If $\nu < 0.5$ is chosen, the lateral resolution will be $\sqrt{2} \approx 1.4$ times better than for conventional microscopy. However, in this case most of the light reflected from the sample does not reach the detector, so one sacrifices efficiency.

Procedure

Remarks

- For confocal fluorescence microscopy additional filters are needed to block the light of the laser.
- Reflection mode: A coupler with 50:50 beamsplitter or removeable edge filter is necessary. (For systems built after 2017 the edge filter is maybe not removable, so the signal will be very low, if there is no fluorescence from the sample.)
- Check that you are use an appropriate fiber (refer to Theory)
- Align the pinhole before measuring using the oscilloscope.

Before starting the oscilloscope ensure that the laser power is adjusted to very low power. Although the count rate of the photon counting devices (APD or PMT) is constantly monitored to avoid overexposure, a strong and abrupt rise of it, can destroy the unit.

Procedure

Choose an appropriate Confocal configuration.

Alignment for reflection mode

1. Focus on a piece of silicon.
2. For non-automated systems: configure the beampath for confocal.
3. Adjust the laser to very low power.
4. Click on Start Oscilloscope.
5. Rise the laser power until you see a signal.
6. Optimize the signal (compare to Raman).

Alignment for transmission mode

1. Focus on a cover glass using the inverted objective including the steps marked with "For transmission".
2. For non-automated systems: configure the upright and inverted beampath for measurement.
3. Adjust the laser to very low power.
4. Click on Start Oscilloscope.
5. Optimize the signal using the inverted objective.

Measurement

1. If the system is aligned, insert your sample focus on it (for transmission mode from both sides).
2. Click on Start Oscilloscope.

3. Adjust the laser power.
4. Click on Stop.
5. Start your measurement.

Hints

- Make sure the detector is not overloaded. Refer to Detection.

StrobeLock

StrobeLock Overview

StrobeLock is an extension for WITec microscope systems that enables fluorescence lifetime measurements. The available measuring modes include Fluorescence Lifetime Spectroscopy, Fluorescence Lifetime Imaging as well as Time-Resolved Luminescence Microscopy.

Topics:

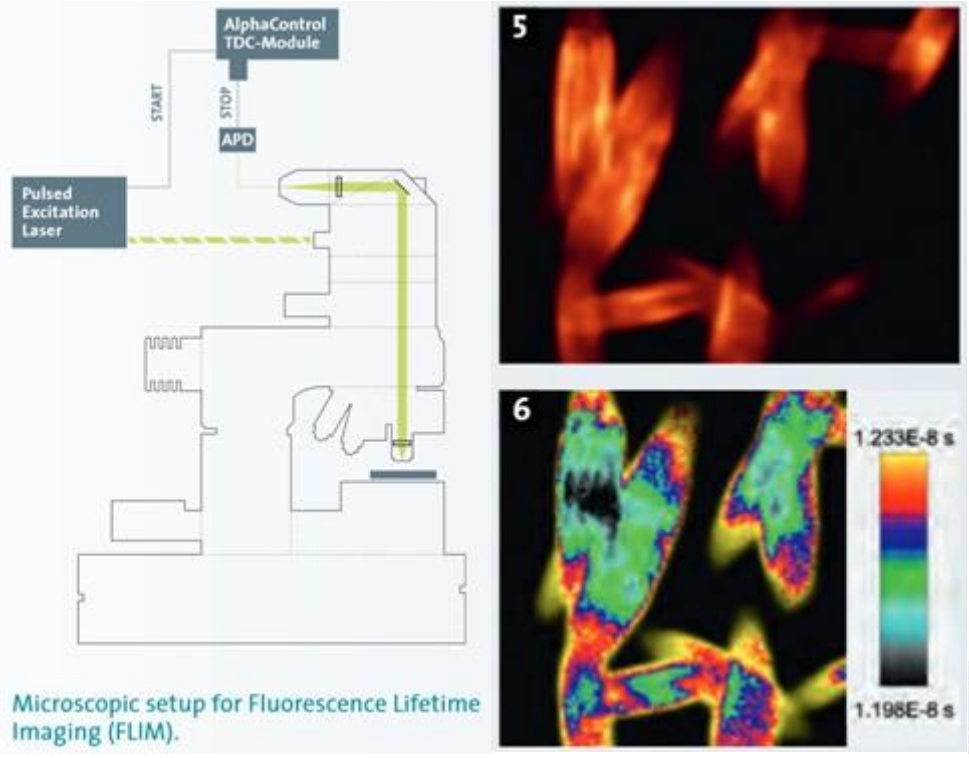
- Theoretical Background
- Laser settings – for PicoQuant LDH Laser only
- Setting up a measurement
- Example measurement
- Data evaluation

Measurement modes:

- Oscilloscope: Displays the output channel of a APD as a function of time.
- Single Spectrum: Acquisition of a time-spectrum at the current position.
- Fast Time Series: Continuous acquisition of time-spectra over time.
- Slow Series: Intermittent time series.
- Line Scan: Acquisition of time-spectra along a line in three dimensional space.
- Large Area Scan: 2D or 3D Imaging using the motorized stage up to centimeter scale.
- Image Scan: 2D or 3D Imaging using the piezo stage for highest resolution.

System requirements:

- a single-photon avalanche photo diode (SPAD)
- an appropriate pulsed laser for the optical excitation of the sample
- a Time-Correlated Single-Photon Counting electronic module (TCSPC) included in the alphaControl controller



Microscopic setup for Fluorescence Lifetime Imaging (FLIM).

5 Total fluorescence intensity
6 FLIM

Theory

To understand the principle of fluorescence lifetime measurements some theoretical considerations are helpful.

A fluorescent sample can be regarded as an ensemble of single fluorescing objects. An experiment on such an ensemble shows the same characteristics as a measurement on a single one of these objects performed very often.

The simplest model for describing the fluorescence behavior of such an emitter is a two-energy-level system:

- It exhibits a ground state and an excited state.
- It can be brought from the ground state to the excited state by energy absorption.
- The relaxation from the excited state back to the ground state happens spontaneously and the energy difference is emitted in form of a fluorescence photon.

If the emitter was brought to the excited state at $t = 0$, the fluorescence intensity at a later time t can be described by

$$I(t) = I_0 \cdot e^{-\frac{t}{\tau}},$$

where τ is the characteristic lifetime of the excited state and I_0 is a constant factor.

If the emitter exhibits not only one but n possible paths for absorption and subsequent emission of a fluorescence photon, this intensity becomes:

$$I(t) = \sum_{i=0}^{n} A_i \cdot e^{-\frac{t}{\tau_i}},$$

where A_i describe the relative probabilities of the different paths and τ_i represent the characteristic lifetimes of the different excited states.

In Fluorescence Lifetime measurements this characteristic temporal behavior of a sample's emitted fluorescence is determined:

- The sample is excited in a pulsed way. This can happen e.g. by a pulsed laser or electrically.
- A detector that is sensitive for very small amounts of light emitted by the sample.
- An electronic device that records the time differences between excitation events and fluorescence photon emission.
- By measuring these time-differences very often a histogram is recorded – in WITec Control it is called Time-Spectrum.
- Such a time-spectrum can be described by the equations above and the characteristic parameters like the fluorescence-lifetime *t* can be determined by exponential fits.

Laser settings

The following section is only relevant if a pulsed laser of the type **PicoQuant LDH** is used for excitation of the sample.

1. Start the laser by the power switch on the backside and the key switch on the frontside of the laser driving electronics (figure 1).
2. Choose the desired laser pulse repetition rate by using the TRIGGER and REP. FREQUENCY adjustment knobs:
    a. Check the manual of the PicoQuant LDH laser for the appropriate combination of both settings.
    b. For **20 MHz**, which is a good starting point, use:
        i. **TRIGGER: INT 1**
        ii. **REP. FREQUENCY: 4**
    c. For continuous wave operation set TRIGGER to CW.

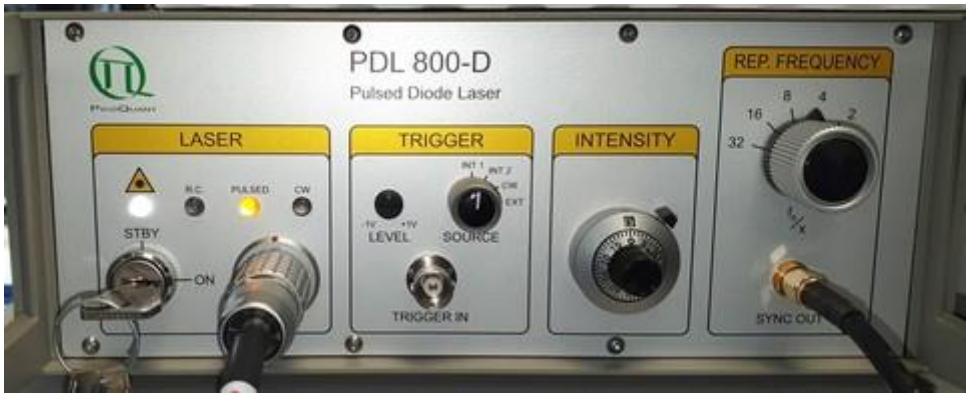


Figure 1: PDL 800-D driving electronics for PicoQuant LDH laser

3. Find out the appropriate diode current setting:
    a. Observe the emitted laser power by one of the following possibilities:
        i. using a power meter
        ii. observing the laser spot on the video image
        iii. measuring the count rate from a fluorescing sample
    b. Open the manual laser power adjustment screw at the laser head (Figure 2).
    c. Increase the diode current using the INTENSITY potentiometer screw (Figure 1). As long as the laser threshold is not reached, the emitted laser power will not increase at all or only slowly. As soon as the laser threshold is reached, the increase will be significantly faster. Note the corresponding screw position.

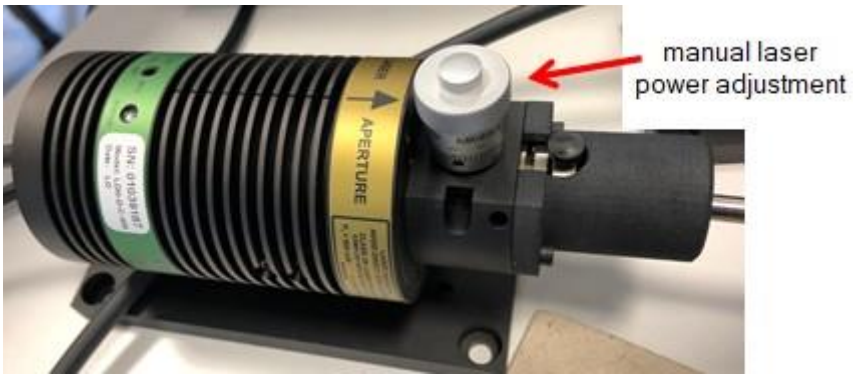


Figure 2: PicoQuant LDH series laser head

d. For the best performance in time resolved measurements (like FLIM), the excitation laser pulses should be as short as possible in general.
In case of the PicoQuant LDH this can be achieved, if the **diode current is as small as possible** (see also the PicoQuant laser's data sheets) – **but still above the laser threshold**! A potentiometer setting of 10 % above the threshold value determined in the step before is a good starting point (adjust e.g. 7.7, if the laser threshold screw position was 7.0).

4. The diode current setting can be kept constant from now on – **change the emitted laser power by the micrometer screw at the laser head only**! Touching the INTENSITY screw is only necessary if a higher maximum laser emission power is required.

Parameters

Mount your sample or use a reference sample for the adjustment and follow the steps in Focus on sample.

The appropriate excitation laser power

1. Select the configuration Time Resolved Microscopy.
2. Close the manual laser attenuator at the laser head completely.
3. Start the oscilloscope and have a look on the graph showing the count rate; at least the dark count rate of the APD should be measured with small fluctuations.
4. Switch the laser to pulsed operation – 20 MHz is recommended.
5. Start the oscilloscope and increase the laser power slowly until a significant count rate is measured (status window or graph showing the fluorescence count rate).
6. Increase the laser power until the appropriate fluorescence count rate is reached:
To prevent over-representing short-time photon-counting events, the fluorescence count rate should not exceed 1 % of the laser repetition rate, e.g. 200 kHz count rate for a laser repetition rate of 20 MHz.
7. In the Time Spectrum window a decreasing exponential decay curve similar to the one shown in Figure 2 should be visible now.

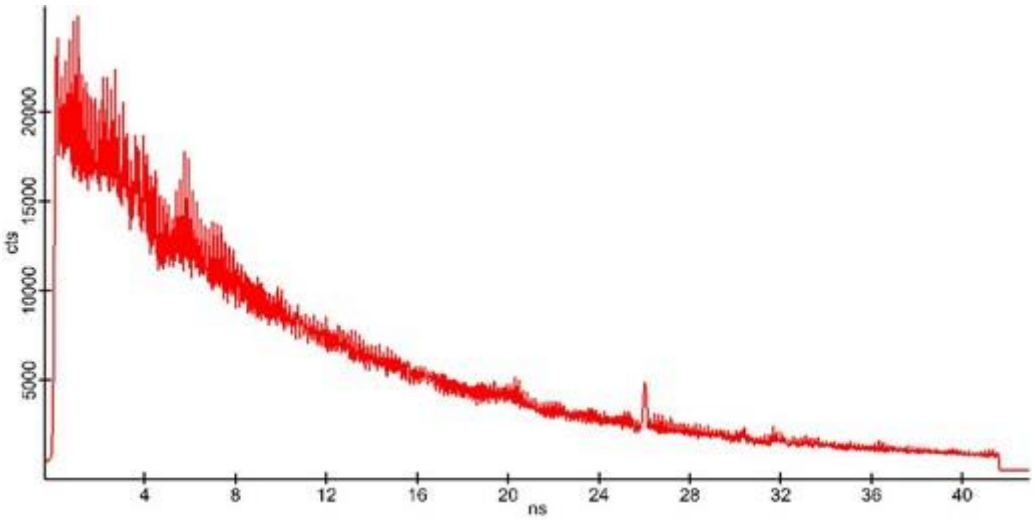


Figure 1: Example for "uncorrected" Time Spectrum from reference sample

Time Spectrograph Parameters

All parameters relevant for the recording of time spectra are stored in the corresponding WITec Control configuration. If they are not set correctly, it might happen that:

1. only a part of the curve above can be seen
2. the time scale is shifted
3. there are short-time oscillations in the spectrum (as visible in the spectrum above)
4. there is a small peak-artefact (in the spectrum above at around 26 ns)

If you see one of the things above, you have to optimize the parameters in the Time Spectrograph parameter group:

1. Laser Repetition Rate has to correspond to the repetition rate of the excitation laser.
2. With the parameters Time Bins and Time Binning the recording time and the timing resolution of the time-spectra can be defined. Recommended starting values for 20 MHz laser repetition rate:

   Time Bins: 1800

   Time Binning: 1
3. Most probably the visible time spectrum will be shifted to the right compared to the one shown above. This difference can be corrected with the following two parameters:
   a. Adjust Time Offset until the zero of the x-axis corresponds to the the steep increase of the time-spectrum.
   b. If desired: Shift the whole time-spectrum to the left edge by changing Start Time.
4. Record a single spectrum e.g. with 1 s integration time and 10 accumulations. Probably, two artefacts will be visible (compare to the spectrum above):
   a. A small peak (in Figure 1 at 26 ns): Such a peak occurs if the specified laser repetition rate is not 100 % correct. To correct for that:
      i. Change Laser Repetition Rate slightly (e.g. from 20 MHz to 19.95 MHz).
      ii. Record a single spectrum again and check if the peak changes.
      iii. Proceed optimizing Laser Repetition Rate until the peak disappears (almost) completely.
   b. Oscillations on a sub-ns scale: They are caused by the non-linearity of the TDC board

time bins. To remove them, the readout of the TDC board must be calibrated:

i. Close the manual attenuator of the laser completely.
ii. Change the microscope to get white light on the sample.
iii. Minimize the intensity of the white light source.
iv. Start Oscilloscope and switch on the white light source.
v. Adjust the illumination intensity to achieve a count rate corresponding to 1 % of the laser repetition rate.
vi. Stop the oscilloscope.
vii. Press Calibrate.
viii. Observe the recorded time spectrum using the y-axis auto-scale.
ix. If the spectrum is not changing qualitatively anymore, stop the measurement. The calibration is finished now.
x. Save the calibration using Save Calibration.

5. If a single spectrum is recorded again, all anomalies should be removed (as in the spectrum in Figure 2).

If one of the parameters in the WITec Control section Time Spectrograph is changed, it might be necessary to repeat the calibration of the previous step completely.

It makes sense to store dedicated configurations and calibrations for all the different settings that are used frequently (e.g. for different relevant repetition rates).

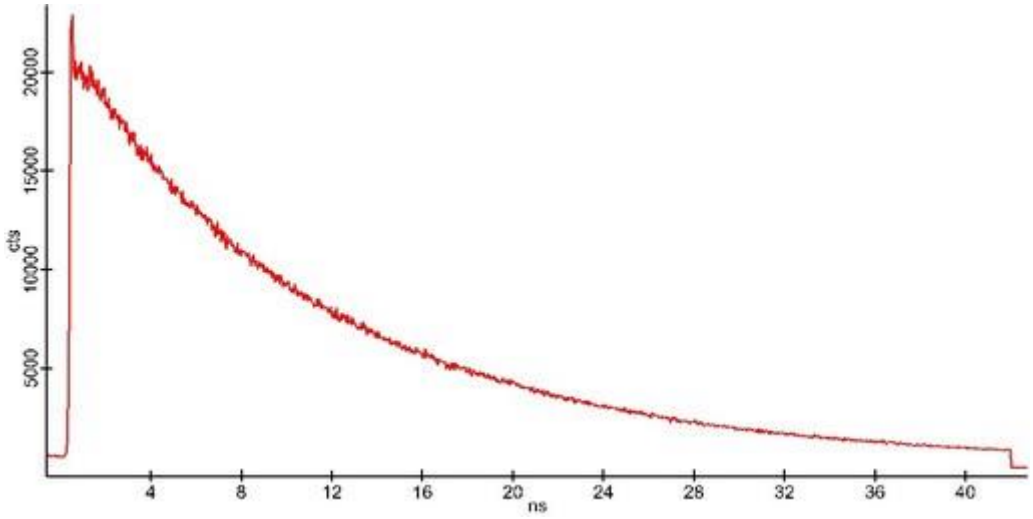


Figure 2: Corrected Time spectrum

Further information:

Time Spectrograph, Detection

Reference sample

Appropriate reference samples:

- For excitation wavelengths between 450 and 630 nm: The diamond reference sample can be used.
- For other wavelengths: A marker pen on a flat surface (e.g. glass) is feasible; yellow and red colored pens work the best.

It is recommended to check the fluorescence spectrum of the reference sample:

1. Choose an appropriate Raman configuration. Use a small grating and set the Units to nm in the Spectrograph parameter group. Record a fluorescence spectrum.
2. If possible: Switch the laser to continuous wave operation.
3. Focus the laser on the reference sample and check if a reasonable fluorescence spectrum can be detected.
   In Figure 1, the fluorescence spectrum of the diamond reference sample recorded with a Time-Bandwidth Lynx laser is shown. Using a PicoQuant LDH the Raman lines would not be visible.

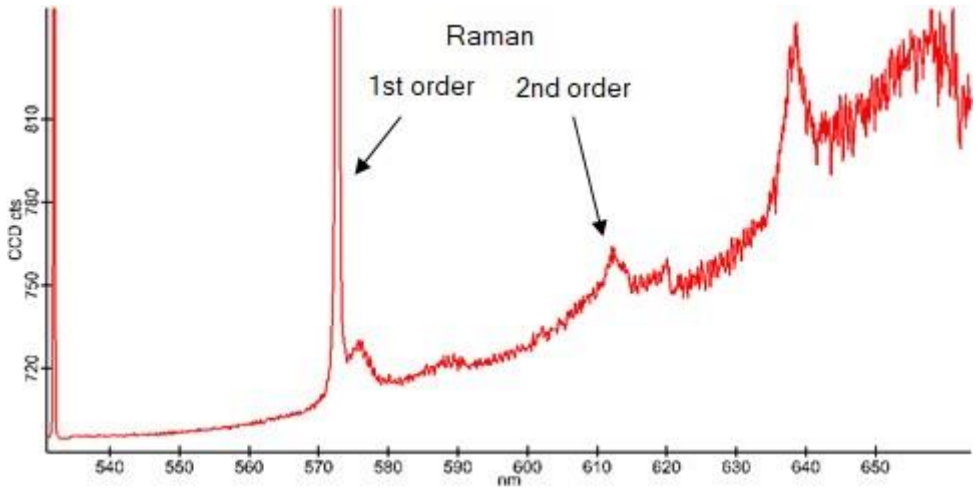


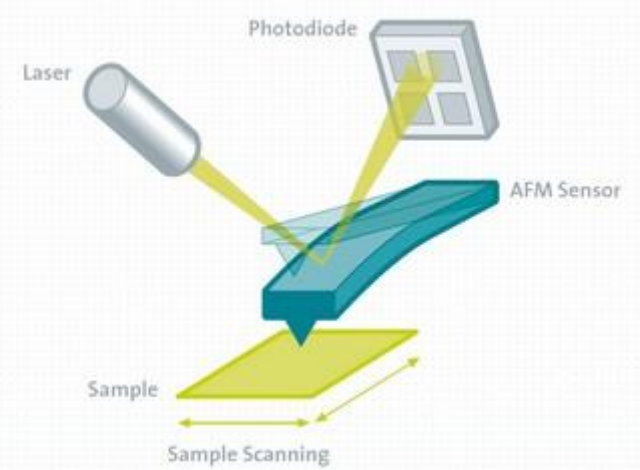
Figure 1: Fluorescence spectrum of diamond reference sample recorded with Time-Bandwidth Lynx laser.

Data evaluation

- The fluorescence lifetime measurements basically work the same as all other spectral measurements with the WITec instrument. The only difference: time-spectra are recorded instead of e.g. Raman or fluorescence spectra.
- A FLIM-measurement is a 2D- or even 3D-scan where the fluorescence lifetime(s) is/are displayed instead of Raman intensities or similar.
- Even all other routines that are known from confocal Raman spectroscopy and imaging are also possible in FLIM: Single Spectra as well as Line Scans, Image Scans and Large Area Scans also in 3D.
- As mentioned above the parameter visualized in FLIM images is/are the decay time(s) from the equations in the Theory section. This/these time(s) can be yielded by using the Advanced Fitting tool and choosing a single- (or multi-) exponential function to describe the measured time spectra.

AFM

AFM Overview



Atomic force microscopy (AFM) is a type of scanning probe microscopy (SPM), where the sample is scanned mechanically on the nanometer scale. Besides the sample topography additional properties can be determined with special AFM modes.

Topics:

- Introduction
- Setting up a measurement

AFM modes:

- Contact Mode – standard AFM mode with constant force
- AC Mode (Tapping Mode) – for soft samples, material contrast by phase image
- DPFM (Digital Pulsed Force Mode) – provides information about adhesion and stiffness
- AC Lift Mode – for MFM or EFM, magnetic or electric forces can be observed
- C-AFM (Conductive AFM) – measure local conductivity
- KPFM (Kelvin Probe Force Microscopy) – work function is observed (surface potential)
- PRFM (Piezoresponse Force Microscopy) – image domains of piezoelectric or ferroelectric materials
- Raman-AFM – Contact or AC Mode combined with Raman

System requirements:

- AFM (A, RA, RAS and AS systems)

Introduction

Since the invention of atomic force microscopy (AFM) in 1986 by Binning, Quate and Gerber [G.Binning, C.F.Quate and Ch.Gerber; Phys.Rev.Lett. 56, 930 (1986)], AFM has rapidly developed into a powerful and invaluable surface analysis technique on micro- and nanoscales and even on atomic and molecular scales. Using AFM, it is possible to image surfaces in real-space with a resolution down to the level of molecular structures. In addition to the imaging of small topographic features on surfaces, AFM is also used to image additional surface properties such as adhesion, stiffness, magnetic properties, conductivity and many more.

Over the past decades, a large number of additional AFM imaging modes were developed for a variety of applications. AFM can be used on any kind of sample. Therefore the number of publications in materials science, life science, and related industries has increased tremendously since its invention.

PI Controller

The feedback settings allow access to the parameters used in conjunction with the PI control of the scan table Z axis.

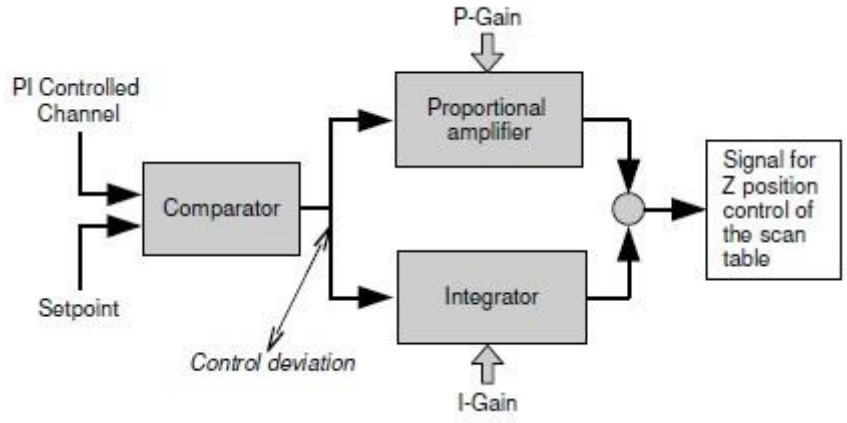


Figure 1: Schematic illustration of the PI controller

Figure 1 illustrates schematically the functional principle of this digital controller. An adjustable setpoint is first compared to a selectable PI controlled channel. The resulting control deviation is used in conjunction with a proportional amplifier and an integrator to calculate the control signal for the Z axis of the scan table in order to minimize the control deviation.

More information can be found on wikipedia or in AFM literature.

Setting up a measurement

Sample mounting and focusing

1. Select the appropriate AFM configuration.
2. Make sure the magnetically fixed cantilever arm is removed.
3. Focus on the sample using the AFM objective and search for the region of interest.
4. Optional: Set the Microscope-Z user position to zero for your reference.

Cantilever mounting

5. Move the microscope up at least 1500 µm.
6. Attach a cantilever (glued on a washer) to the cantilever arm and mount it to the inertial drive. (If necessary: Connect the cable of the cantilever arm.)
7. Position the cantilever manually by gently moving the bottom part of the inertial drive in X, Y and Z until the cantilever is somewhat visible in the video image.
8. Move the microscope down not closer than 100 µm above the sample surface. (The cantilever must be within 2000 µm above the sample for the automatic tip-approach.)

Adjustment

9. Click on Start Adjustment in the section Adjustment. Follow the steps displayed in the Message window (press Next Step after completing one). Attention – Useful hints are included for most steps:
    a. Position the cantilever under the beam like in Fig. 1:
        - The Cantilever Control is opened automatically.
        - Activate Show Cantilever Position and put the cross to the assumed tip position by selecting Set Probe position in the Menu. Acknowledge the compensation.
        - If the beam is not visible the blue circle marks its position. Maybe the illumination needs to be reduced in order to see the beam.
        - if the cantilever is tilted more than approximately 10°, the cantilever should be re-mounted.
    b. Center the beam spot on the four quadrant diode:
        - Use the T-B and L-R adjustment screws at the beam deflection unit.
        - Observe the changes on the green spot in the AFM Status.
        - If the spot is on one edge, observe the Sum signal (bar on the right). It will increase if the screw is turned in the correct direction.
    c. Further adjustment is explained for the respective AFM mode (Contact, AC, DPFM).
    d. Check the P-Gain and I-Gain. (5 is a good starting value for both.)

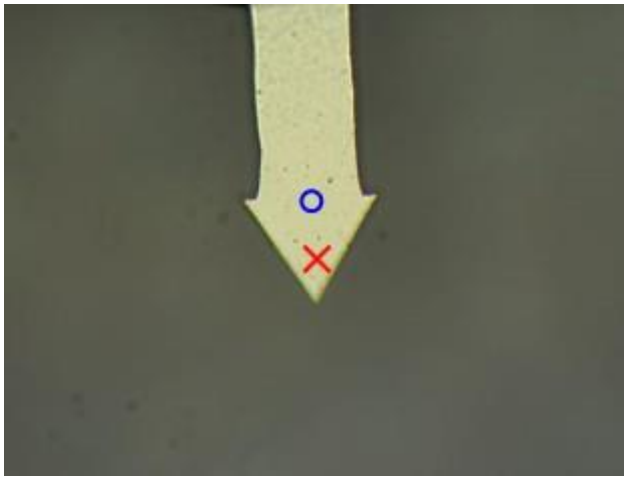


Fig. 1: Cantilever in the video image at appropriate position.

Approach and measurement

10. Click on Start Approach.
11. Start the measurement.

Further information:

Adjustment, Feedback settings, Tip approach

Hints

- If you hear a noise from the stage during contact, the feedback is too aggressive. Immediately reduce the I- and P-Gain until the noise stops.

- Too low values for P- and I-gain will result in slow reactions on topography changes.
- Too high values for P- and I-gain will result in an oscillation.
- Higher values of P- and I-gain result in a more exact topography recording.
- I-gain results in more aggressive reactions and is in most cases slightly lower than the P-gain.
- More information can be found under PI Controller.

AFM Contact

Contact mode Overview

In this imaging mode, the tip is always in contact with the surface under a constant force.

Topics:

- Theoretical Background
- Setting up a measurement
- Example measurement

Measurement modes:

- Image Scan: acquisition of AFM contact mode images
- Line Scan: acquisition of force-distance curves along a line
- Distance Curve: acquisition of force-distance curves at the current position

Recommended Cantilevers:

- Contact Mode cantilevers with a spring constant of k = 0.2 N/m (at least below 1 N/m) are recommended.

For samples that are either soft or weakly bound to the substrate, AC mode, which operates in the intermittent contact regime, provides better results. Because in contact mode lateral forces might lead to the dragging of particles weakly bound to the substrate, thus resulting in blurred images.

Theory

The operating principle of an AFM is rather simple. A probe is scanned over a sample and various interactions between tip and sample are used as feedback mechanisms to trace the surface topography (Fig. 1).

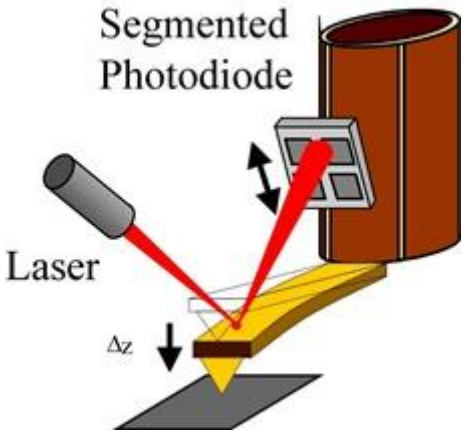


Fig. 1: Principle of AFM operation.

At the free end of a cantilever (typically 100 to 200 µm long) a sharp tip (less than 10 nm across) is mounted. This tip is brought into contact with the sample while the repulsive force between tip and sample bends the cantilever. The bending of the cantilever is measured using a highly focused

beam deflection system as shown in Fig. 1. By keeping the bending of the cantilever constant, a constant force is applied to the sample while scanning the tip across the surface. The WITec microscopes are sample scanning systems, where the cantilever remains at a constant position and the sample is scanned precisely underneath it. This setup has the advantage of fixed optical beam-paths, which eliminates the requirement of tracing the beam deflection system. The vertical movement of the scanner follows the surface profile and is recorded as the surface topography.

Several forces typically contribute to the bending of the AFM cantilever. The force most commonly associated with scanning force microscopy is the interatomic van der Waals force. The dependence of the van der Waals force upon the distance between tip and sample is described by a Lennard Jones potential V, which can be written as

$$V_{sample}(z) = Az^{-12} - Bz^{-6}$$

Here z denotes the tip sample distance and A and B are interaction parameters. As the tip is approaching the surface, attractive forces act between the tip and sample before repulsive forces start to dominate. In an AFM, the tip is attached to a flexible cantilever which is subject to Hook's law

$$V_{canitlever}(z) = k\frac{(z - z_0)^2}{2}$$

where k is the spring constant of the cantilever and z_0 is the tip-sample distance for an unbent cantilever. An example of a resulting force distance curve of this coupled system is shown in Fig. 2.

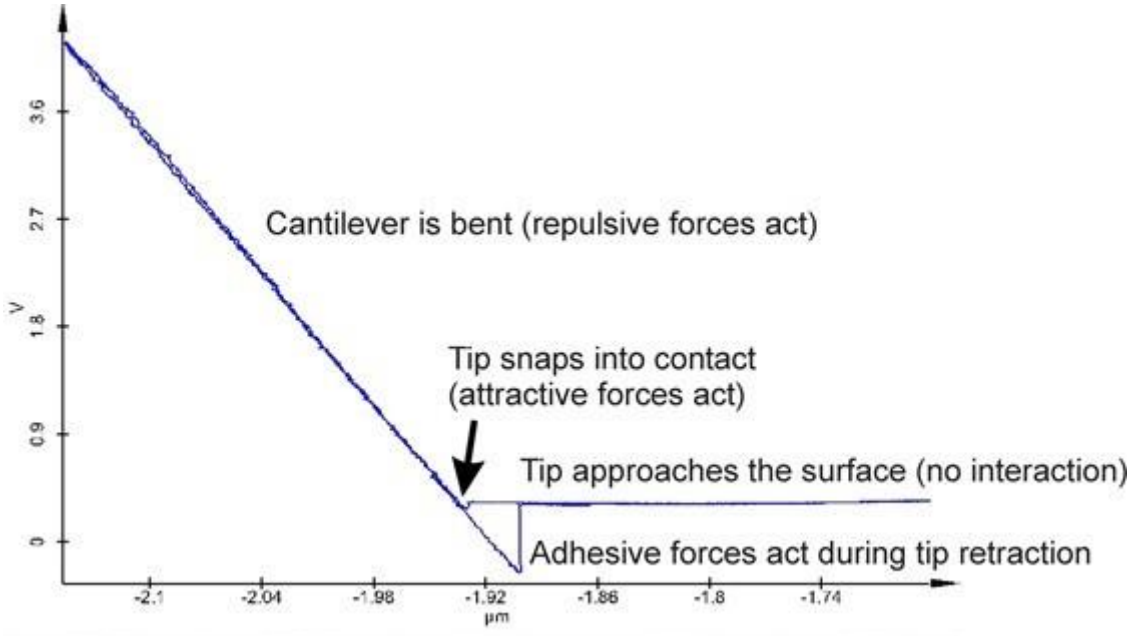


Fig. 2: Force distance curve in an AFM measurement.

These curves contain all tip-sample interactions, enabling the mapping of material properties on the nanometer scale.

Different mechanical properties of the surface can be evaluated from the friction images. In this case the torsion of the cantilever is recorded. The operating principle of friction mode is shown in Fig. 3.

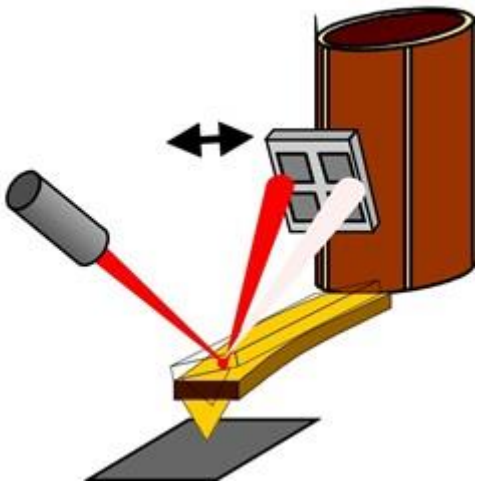


Fig. 3: Principle of AFM friction measurements.

Parameters

Follow the steps in the general procedure. Further adjustment (10. c) for Contact mode is explained in the following:

Setpoint

The recommended value for the Setpoint is 0.5 V.

The feedback parameter in AFM contact mode is the bending of the cantilever, which ensures a constant force between tip and sample. The up and down movement of the scan stage is recorded as surface topography. Any deviation from the constant bending of the cantilever, measured on the photo-detector, is represented in the deflection image. These images highlight the edges of various topographic levels.

High setpoint values correspond to high tip-sample interaction forces.

The force distance curve shown in Fig. 1 displays the initial T-B value and the range within which the setpoint should be selected for contact mode measurements. The feedback loop maintains a constant bending of the cantilever at the selected setpoint.

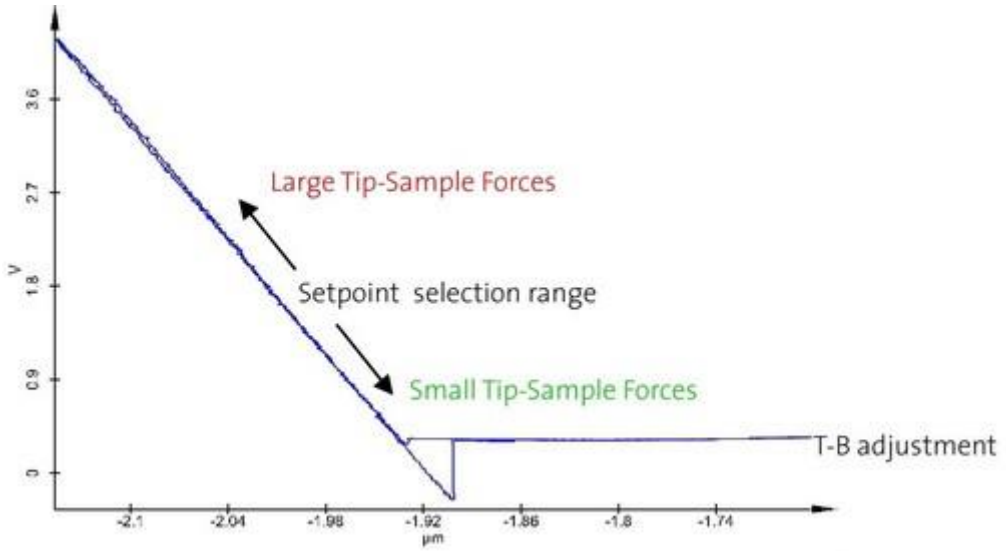


Fig. 1: Typical force-distance curve showing the setpoint selection for AFM contact mode measurements.

Recorded Channels

Image Scan

Each data object in a AFM image scan is created twice marked with [F] for data aquired during the forward movement and [B] for data aquired during the backward movement.

Distance Curve

Opening the recorded data channels will show them separately. To get the view shown when recorded use the parametric view.

Further information:

Feedback settings, Data channels (T-B, L-R, Topography, Feedback), Image scan, Distance curve

Reference sample

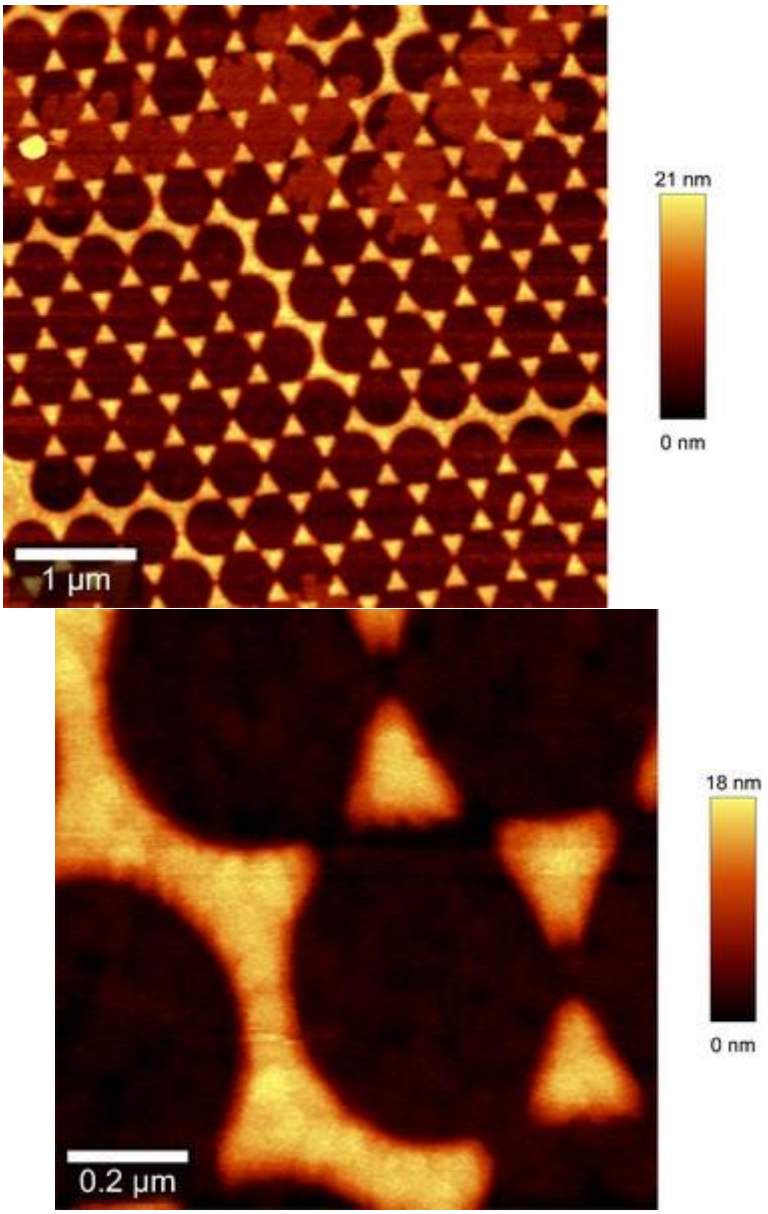


Figure 1: Topography of the Fischer Al-projection pattern (0.5 µm)

Table 2: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	5 x 5 (left), 1 x 1 (right)
Pixel	256 x 256
Time per Line [s]	0.5
P-Gain [%]	8
I-Gain [%]	6
Setpoint [V]	0.5
Cantilever	Contact (0.2 N/m)

AFM AC

AC mode Overview

In Acoustic (AC) mode, also known as Tapping mode, the cantilever is oscillated at its resonant frequency which results in an intermittent contact mode.

Topics:

- Theoretical Background
- Setting up a measurement

Measurement modes:

- Image Scan: acquisition of AFM AC mode images,
- Line Scan: acquisition of amplitude- & phase-distance curves along a line,
- Distance Curve: acquisition of amplitude- & phase-distance curves at the current position.

Recommended Cantilevers:

- Force Modulation (FM) cantilevers with resonance frequencies in the range of 65−85 kHz and a spring constant of k = 2.8 N/m. These cantilever are recommended for the imaging of delicate samples where small tip sample interaction forces are required.
- Non-Contact (NC) cantilevers with resonance frequencies of 230−300 kHz and spring constants of k = 42 N/m, are recommended for very soft samples (e.g. gels, plastic foils) and for samples in which the phase contrast within the material is the highest priority.

Theory

In Acoustic (AC) mode, also known as Tapping mode, the cantilever is oscillated at its resonant frequency with a free amplitude A_0. While the cantilever is approaching the surface, the oscillating amplitude is damped to a value A, which depends on the distance to the surface (see also blue curve in Fig. 1). The ratio

$$r = \frac{A}{A_0}$$

defines the damping of the amplitude while the tip is in contact with the surface and is proportional to the applied force. By keeping the damping of the amplitude constant, the surface topography can be imaged. The interaction between the tip and the sample is predominately vertical, though negligible lateral forces are encountered. Consequently, AC mode AFM does not suffer from the tip or sample degradation effects that are observed in contact mode AFM, and is therefore a suitable technique for imaging soft samples.

Phase images can be recorded simultaneously along with the surface topography. In this image, the phase shift between the free oscillation and the oscillation while the tip is in contact with the surface is recorded. Since the phase shift depends on the viscoelastic properties of the sample as well as on the adhesive potential between the sample and the tip, the phase image outlines domains of varying material properties without describing the nature of the properties themselves. Nevertheless, phase images are often used to characterize soft samples at high resolution.

Parameters

Follow the steps in the general procedure. Further adjustment (10. c) for AC mode is explained in the following:

1. Enter the driving amplitude (Driving Amp. pk-pk [V]) in the in the frequency sweep section.

A typical starting value is 0.05 V (FM) to 1 V (NC) depending on the type of the cantilever. For golden KPFM cantilever arms use 1 V for FM cantilevers.

2. Auto-Resonance is performed. Check if the automatically selected resonance frequency is within the cantilever range. If this is not the case check the hints section.
3. Adjust the free cantilever oscillation amplitude displayed in the status section by increasing or decreasing the Driving Amp. pk-pk [V].

Cantilever oscillation amplitude: FM cantilever 1–2 V and NC cantilever 1–1.5 V.

4. Optional: Lower the starting value for the setpoint (damped amplitude) to about 75 % of the free cantilever oscillation amplitude.

The setpoint is automatically reduced during approach.

Setpoint

The setpoint in this imaging mode is the damped amplitude A (equation) and should be selected at a lower value than the free amplitude A_0. The amplitude distance curve shown in Fig. 1 illustrates the correlation between the free amplitude and the range within which the setpoint should be selected in an AC mode measurement.

Low setpoint values correspond to high tip-sample interaction forces.

When cantilevers with relatively low spring constants are used for AC Mode imaging (e.g. 2.8 N/m), the free amplitude of the cantilever decreases slightly before the tip touches the surface due to the air compression between the lever and the surface as shown in Fig. 1.

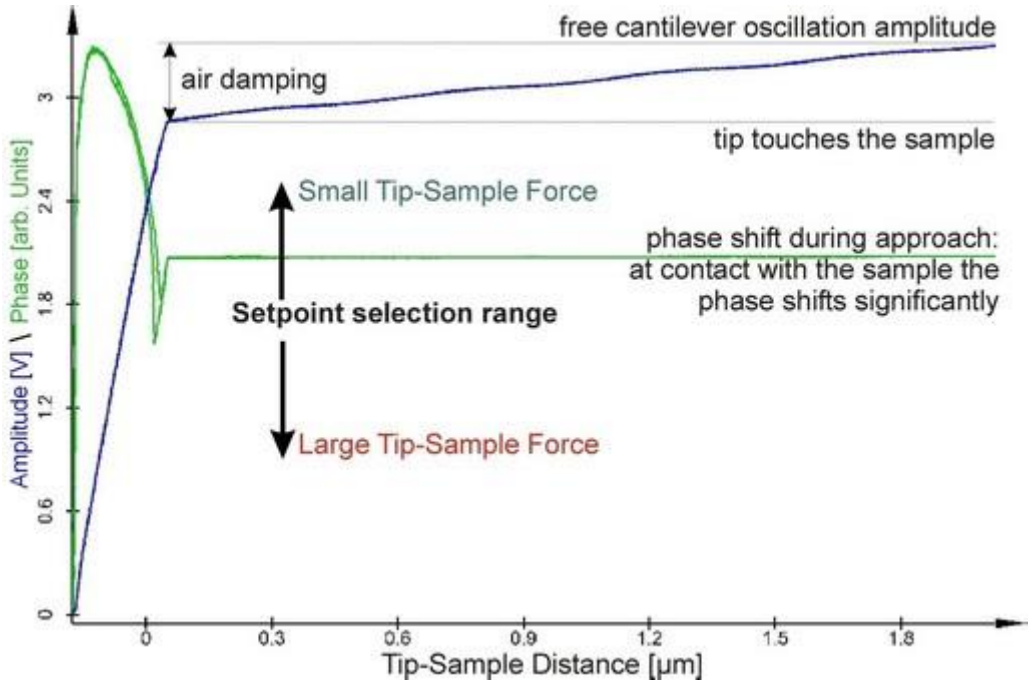


Fig. 1: Phase (green) and Amplitude (blue) as a function of tip-sample distance. The effect of the setpoint selection is also indicated.

Further information:

Feedback settings, Frequency sweep, Data channels (Lock-in R, Lock-in Phi, Topography, Feedback)

Hints

- A driving frequency slightly below the resonance frequency of the cantilever can improve the sensitivity of the measured phase. The damping lowers the resonance frequency when the tip is in contact. Therefore a lower driving frequency at about 30° depending on the sample using i.e. the Listen option after the Auto-Resonance.

If the resonance frequency of the cantilever is not found correctly during Auto-Resonance, check the following points:

- The cantilever is not properly fixed to the cantilever holder, frequencies other than the cantilever resonance frequency might then be more pronounced and therefore automatically selected. In this case, remove the cantilever from the holder, check for dirt particles on the cantilever holder or the cantilever-washer and repeat the step by step adjustment procedure.
- Perform a manual resonance sweep by selecting the start and end frequencies in the frequency sweep section of the Control Window. The selected range should include ±50 kHz of the cantilever resonance frequency. Once the sweep is completed and the resonance curve is displayed in the graph window, set the driving frequency to the resonance frequency found. (The numerical value of the resonance frequency can either be manually entered or the listen option can be used to select the value in the graph window by mouse.)

AFM DPFM

DPFM Overview

The Digital Pulsed Force Mode (DPFM) is a non-resonant, intermediate contact mode which extends the capabilities to aquire additional surface properties such as local stiffness and adhesion with high lateral resolution.

Topics:

- Theoretical Background
- Setting up a measurement
- Data evaluation

Measurement modes:

- Image Scan: acquisition of AFM DPFM mode images.

Required License feature:

- PFM Mode

Recommended Cantilevers:

- Contact Mode cantilevers with a low spring constant of k = 0.2 N/m are recommended for imaging soft polymers and biological samples which are not sticky. The contrast in the stiffness image is enhanced using these cantilevers though the user must ensure that the cantilever still snaps out of contact.
- Force Modulation (FM) cantilevers with a high spring constant of k = 2.8 N/m are recommended for the imaging of sticky samples. These cantilevers are stiff enough to overcome the higher adhesion forces of these samples at the cost of some of the contrast achievable in the stiffness image.

Chemically modified cantilever may improve the material contrast and enhance tip-sample interactions.

Theory

The Digital Pulsed Force Mode (DPFM) is a non-resonant, intermediate contact mode which extends the capabilities of an AFM beyond simply measuring topography. It allows the acquisition of additional surface properties such as local stiffness and adhesion with high lateral resolution at normal scan-speeds. It also avoids surface damage due to lateral shear forces and a controlled normal force is used as the feedback signal. In this imaging mode, the alphaControl introduces a sinusoidal modulation on the cantilever with an amplitude of between 10−500 nm at a user-selectable frequency. Typical frequencies range from 1−10000 Hz, which is usually well below the resonance frequency of the cantilever. The amplitude of the signal is adjusted so that the tip snaps in and out of contact during each period. Therefore, a complete pulsed force curve as shown in Fig. 1 is measured during each cycle.

The pulsed force curve shown in Fig. 1 displays the same tip-sample interaction features as a force-distance curve. (By using the distance information instead of time as the X-axis, the DPFM curve is actually transformed into a force-distance curve.) During the DPFM modulation cycle, the AFM tip is

initially well above the sample surface. Moving closer to the surface, the tip snaps into contact due to the attractive force between the tip and sample surface. As the piezo pushes the tip further toward the sample, the repulsive force reaches a maximum (Fmax). The piezo then pulls back and consequently the repulsive force decreases and the force signal changes sign from repulsive to attractive. Finally, the tip loses contact to the surface when the force on the cantilever is larger than the attractive force between tip and sample (adhesion peak). The subsequent free oscillation of the cantilever around the baseline is damped. The cycle is then restarted.

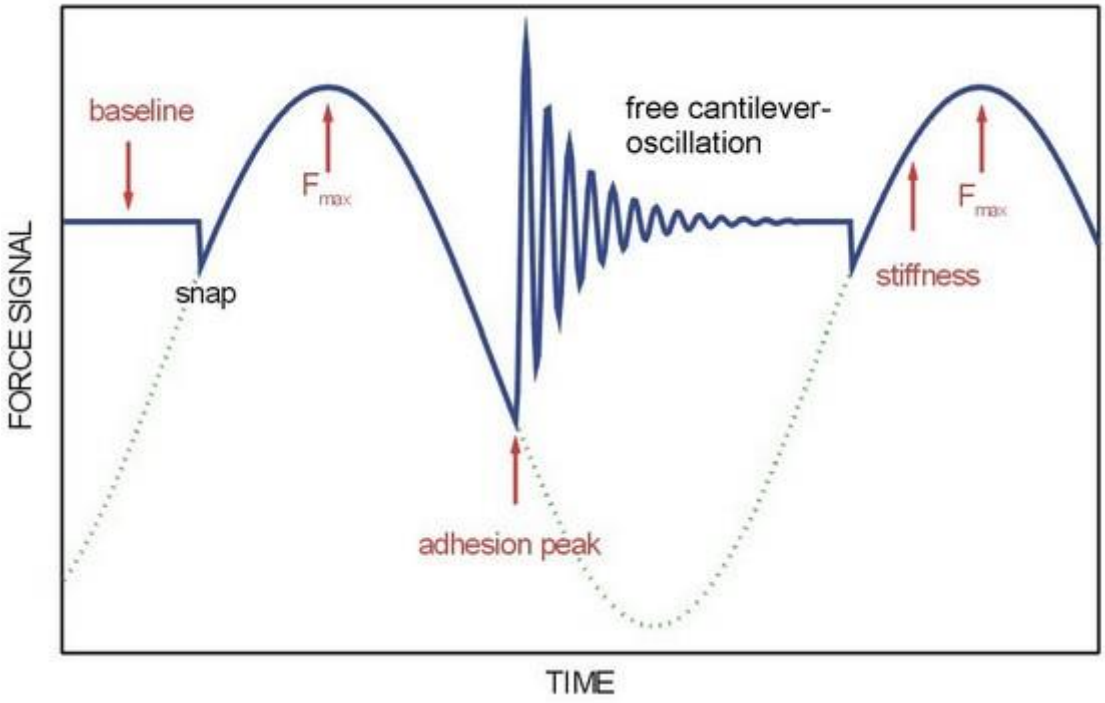


Fig. 1: Schematic DPFM curve with sinusoidal modulation. The parameters of interest are marked.

The high speed control electronics allow the digitization of each curve at a rate of 5 MHz. The force signal of the AFM is monitored in a graph window where it is presented in an oscilloscope-like mode. With this, it is possible to set evaluation areas, from which different features of the force signal are extracted and converted into an image. The online processable features are:

Maximum Force

This value is determined from each pulsed force curve and is used by the alphaControl as the PI controlled channel (see WITec Control manual Section 3.4.5). Therefore, in DPFM operation, the maximum repulsive force acting between tip and sample is kept constant. The value of Fmax is determined by the setpoint setting. The up and down movement of the scan stage to maintain a constant Fmax is recorded as surface topography.

Adhesion

The maximum adhesion force is determined from the adhesion peak of the DPFM curve. This signal forms the adhesion image of the sample.

Stiffness

The gradient of the rising slope of the repulsive force signal (see Fig. 1) is determined during each cycle of the pulsed force curve. This gradient is related to the local stiffness of the sample. On soft parts of the surface, this gradient is smaller than on hard parts. This signal thus forms an image of the sample stiffness.

Parameters

Follow the steps in the general procedure. Further adjustment (10. c) for DPFM is explained in the following:

1. The recommended value for the Setpoint is 0.5 V.
2. Check the P-Gain and I-Gain. (5 is a good starting value for both.)
3. Enter a Driving Amplitude pk-pk [V] of about 2 V (depends on cantilever and adhesion of sample).
4. As initial value for the approach the Fmax window should cover the entire range. Set the Fmax Window Width [°] to 360 and the Fmax Window Start [°] to 0.

Approach

5. Click on Start Approach and wait until it is finished.

DPFM Parameters

The maximum force between tip and sample acts at the maximum of the sinusoidal curve, which is also the point at which the cantilever starts to retract from the sample.

After the approach the graph window will either show a sinusoidal curve as shown in Fig. 1 or a pulsed force curve similar to the one in Fig. 2. In the first case, the modulation of the cantilever is too small to completely detach the cantilever from the sample, refer to step 8.

6. Change the Excitation Phase parameter of the PFM Control until the maximum force is in the first half of the oscilloscope graph window (as shown in Fig. 1).
7. Select the Fmax window as shown in Fig. 1 using the corresponding Listen parameter from the PFM Control mark the range by mouse.

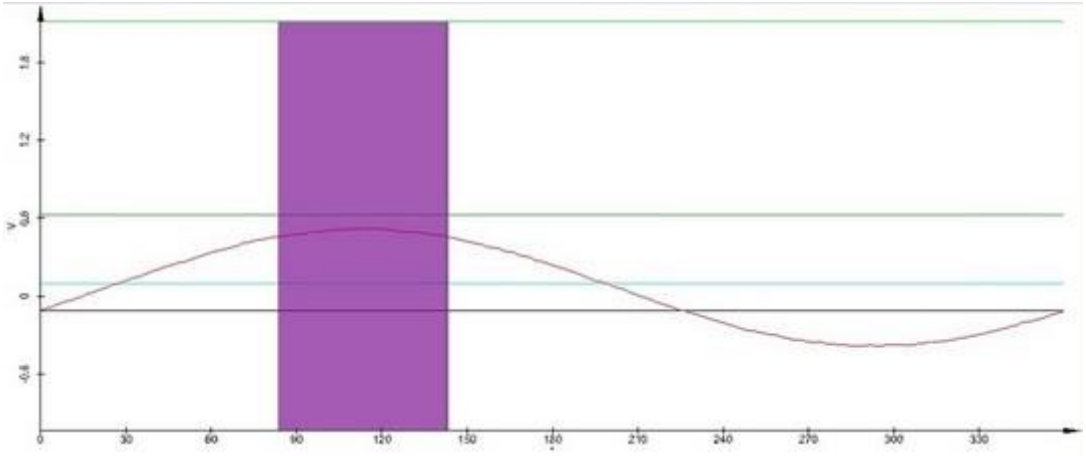


Fig. 1: Sinusoidal curve with Fmax search window

8. Increase the Driving Amplitude pk-pk [V] until a pulsed force curve is displayed in the graph window as shown in Fig. 2.
9. Select the search windows for adhesion and stiffness as shown in Fig. 2 using the

corresponding Listen function.

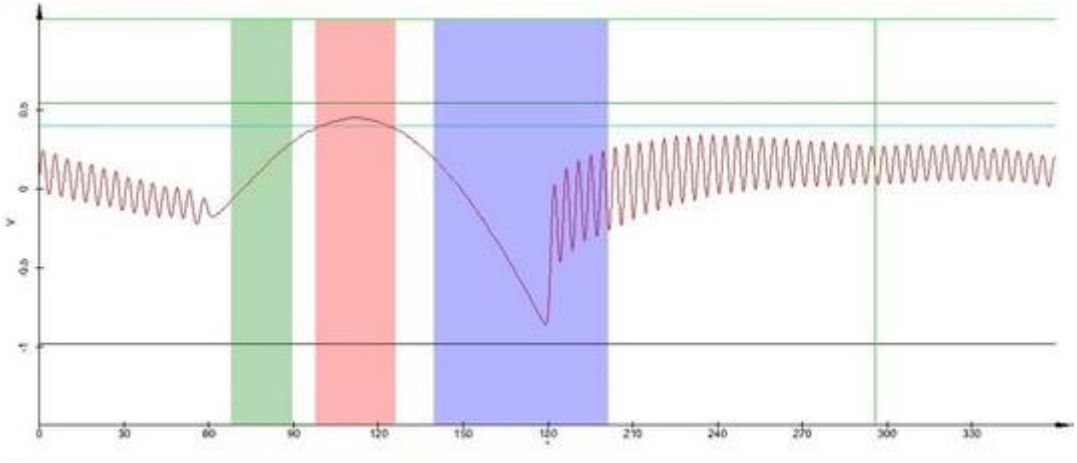


Fig. 2: Pulsed force curve with Fmax (red), Adhesion (blue) and Stiffness (green) search window

Measurement

10. Define the parameters for the Image Scan.
11. Optional: Select Store Data in order to save pulsed force curves in a .wsd file. The user is prompted to enter a file name before the measurement starts.

The size of this additional file can be very large (up to several GB).

12. Click on Start Scan.

Further information:

Feedback settings, PFM Control, Data channels (Adhesion, Stiffness, Topography, Feedback)

Hints

- For high sensitivity in the adhesion image use a low setpoint.
- For high sensitivity in the stiffness image use a high setpoint.

Data Evaluation

Theory

The aquired data of the DPFM measurement, i.e. the adhesion and stiffness images, have voltage as unit. For a mathematical conversion of the measured volts into physical units, such as N for the adhesion image or N/m for the stiffness image, further information related to the experimental conditions is required. The following section describes the required formulas and parameters to convert the voltage values into the appropriate physical values.

The nature of the interacting forces however is not taken into account. The obtained physical values can nevertheless be used for modelling of the tip sample interface.

Please note that the extraction of quantitative values from DPFM measurements depends strongly on environmental conditions such as temperature and humidity. In addition, no changes of the tip during measurements are taken into account in the procedure described in the following.

Adhesion

A calibration of the adhesion force is possible according to the following formula:

$$Adhesion = V_{adhesion} \cdot k \cdot S \qquad (1)$$

with the measured voltage $V_{Adhesion}$, the spring constant k of the cantilever and the sensitivity S of the beam deflection system.

$V_{Adhesion}$ is the voltage determined by the negative value of the adhesion peak.

The approximate spring constant can be found in the cantilever's datasheet. Another approach is the cantilever spring constant calibration using the Sader method. Further information and an online calculation form can be found under http://www.ampc.ms.unimelb.edu.au/afm/calibration.html. The frequency can be determined by a frequency sweep. The quality factor Q is calculated using the cantilever frequency f and and $FWHM$ of the frequency peak:

$$Q = \frac{f}{FWHM}$$

The sensitivity S can be determined by performing force distance curves on a surface which is stiff in comparison to the cantilever, such as a silicon wafer. The sensitivity is shown as the inverted slope of the force curve in the repulsive area (thus having the units nm/V). The values are highly dependent on the cantilever type (particularly its length) and the alignment of the laser spot on the segmented photo diode. Therefore it is necessary to recalculate S every time the cantilever is changed.

Stiffness

To calibrate the stiffness knowledge about some additional quantities is required. These quantities must be determined before measurements are taken. An important measure in this case is the modulation amplitude M of the tip itself. If a direct observation of that quantity is not possible (by an interference method, for example), the modulation amplitude can be estimated from a linear relation between the applied driving amplitude to the modulation piezo and response amplitude of the photo detector.

For this purpose a preliminary measurement on a hard Si wafer, similar to the determination of the cantilever sensitivity S, is required. By applying a small driving amplitude a_{ds} to the modulation piezo while the cantilever is in contact with the Si wafer, a sinusoidal modulation a_{osc} is expected as

response from the photo detector. The ratio U of the amplitude of these two modulations should not change as long as the cantilever is not detaching from the sample and can be used to determine the modulation M during DPFM measurements.

$$U = \frac{a_{osc}}{a_{ds}} \quad (2)$$

$$M = U \cdot S \cdot A \quad (3)$$

with A denoting the driving amplitude applied during DPFM imaging and S the sensitivity.

Using the width of the stiffness search window, the angle σ, the penetration depth Δz can be determined:

$$\Delta z = M \cdot \left(1 - \cos\left(\frac{\sigma}{180°} \cdot \pi\right)\right) - V_{stiffness} \cdot S \quad (4)$$

$V_{Stiffness}$ is the voltage measured in the stiffness image.

If the local stiffness of the sample is defined as:

$$Stiffness = \frac{Force}{penetration\ depth} \quad (5)$$

then it is:

$$Stiffness = \frac{V_{stiffness} kS}{\Delta z} \quad (6)$$

This relation allows the DPFM data to be calibrated in the same manner as the adhesion. Since the conversion of the stiffness and adhesion data into physical units is repetitious, it can be achieved by using the calculator function of WITec Project.

An example on how to convert the voltages supplied by the DPFM measurements to physical units is described on the next pages.

Further Reading

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Preliminary measurements

Parameters like the sensitivity S and the modulation factor U have to be determined by additional measurements. Please note that these additional measurements have to be performed with the same cantilever as the DPFM measurements, as well as with the same alignment of the cantilever and of the beam deflection system.

For the example measurement an arrow contact mode cantilever (Nanosensors) was used with a nominal spring constant k:

k = 0.2 N/m

Force Distance Measurements

As described in the theory section, the sensitivity S is required for further calculations. This sensitivity describes the calibration of the laser detection system. With the initial alignment of the segmented photo detector, the laser spot is adjusted to the center of the four quadrant diode (maximum sum signal, top minus bottom (T–B) signal to 0 and left minus right (L–R) signal to 0). As soon as the cantilever touches the sample, the cantilever bends and the laser spot is displaced from the center position. This results in a T-B signal different from zero, which increases with increasing bending of the cantilever. In a force-distance curve the bending of the cantilever as a function of the piezo z displacement is recorded. For the calibration of the photo detector it is recommended to record force-distance curves on a stiff sample (e.g. Si wafer or glass cover slip). Under these conditions the elastic properties of the sample can be neglected and the displacement of the laser spot on the photo detector is only a result of the cantilever bending.

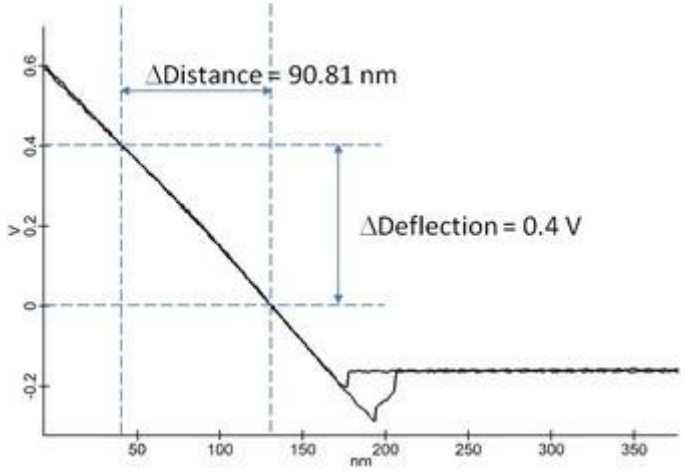


Fig. 1: Force-distance curve measured in contact mode on a Si wafer.

The rising slope of the force distance curve is calculated as:

$$Slope = \frac{\Delta Deflection}{\Delta Distance} \quad (7)$$

For the example shown in Fig. 5 the result is:

$$Slope = \frac{0.4\,V}{90.81\,nm} = 0.0044048\,\frac{V}{nm} \quad (8)$$

With this value it is then possible to convert the voltage change of the photo detector into distances. Multiplying the sensitivity S with the measured voltage (T-B signal) converts it into distance:

$$S = \frac{1}{Slope} \tag{9}$$

For the above mentioned measurement, the sensitivity is:

S = 227 nm/V

Modulation factor U

For calculations of the stiffness, the modulation amplitude of the tip is required. If interference methods are not integrated in the AFM used, the modulation factor U can be determined from a preliminary measurement described in the following:

1. Approach the cantilever under DPFM control to a stiff surface (e.g. Si wafer).
2. Select a small driving amplitude a_{ds} in the PFM control parameter group such that the tip is always in contact with the surface. On the oscilloscope a sinusoidal modulation signal will be visible (Fig. 2 (a)).
3. Measure the peak-to-peak amplitude of this sinusoidal modulation a_{osc} from the oscilloscope display.
4. Increase the modulation amplitude slightly but stay in the range where the cantilever is not detached from the sample and measure the peak to peak value on the oscilloscope again (Fig. 2 (b) and (c)). An example of the curve shape if the applied voltage is too high is shown in Fig. 2 (d).

With equation (2) the modulation factor U for the specific experiment can then be calculated.

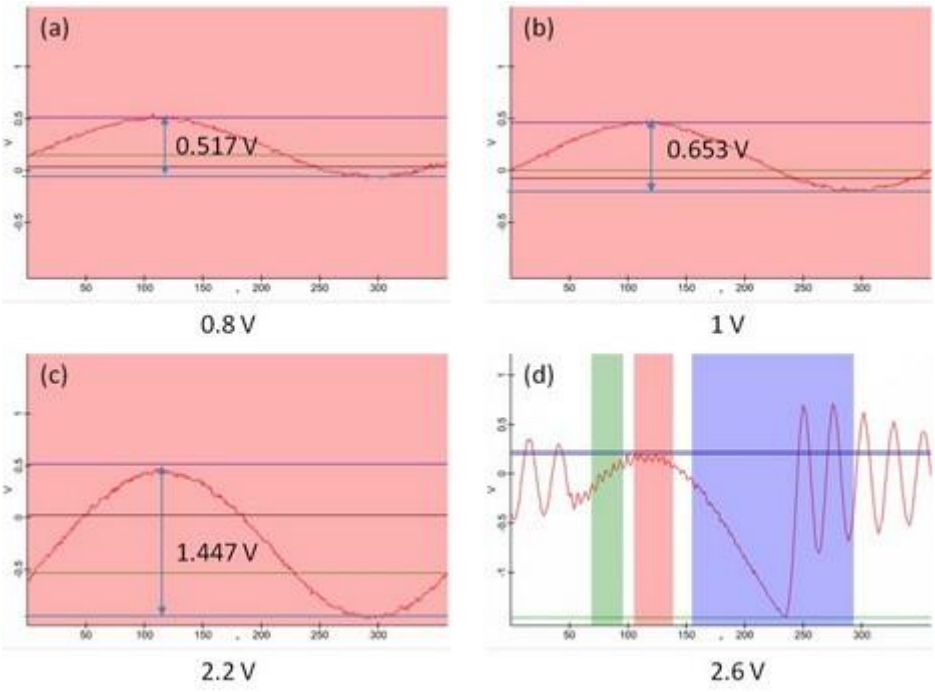


Fig. 2: Illustration of the oscilloscope window while measuring the modulation factor on a Si wafer at various driving amplitudes of (a) 0.8 V, (b) 1 V, (c) 2.2 V and (d) 2.6 V (where the cantilever is already detaching from the sample).

For this example the parameters listed in table 1 apply:

Table 1: Example of measured voltages for the determination of the modulation factor *U*.

Source	a_{ds} **[V]**	a_{osc} **[V]**	U
Fig. 6 (a)	0.8	0.517	0.646
Fig. 6 (b)	1.0	0.653	0.659
Fig. 6 (c)	2.2	1.447	0.657
Average Modulation Factor U:			0.654

The force distance curves and the modulation factor U have to be measured again, as soon as the cantilever is changed or the laser spot is readjusted on the cantilever or on the segmented photo detector.

The results obtained from the preliminary experiments and settings are summarized in table 2.

Table 2: Summary of the data required for DPFM data evaluation from preliminary measurements on a Si wafer.

k [N/m]	S [nm/V]	U
0.2	227	0.65

DPFM Images

In the next two sections data evaluation is described on the basis of an example DPFM measurement. The sample used here is a polymer blend consisting of PS (polystyrene), SBR (styrene-butadiene rubber), and EHA (ethyl-hexyl-acrylate). In this section only the acquired images are considered for evaluation. The second section will focus on the evaluation based on the acquired pulsed force curves.

Fig. 1 shows the topography, adhesion and stiffness images as measured using the parameter listed in table 1. A typical pulsed force curve from this dataset shows the window selection for stiffness, Fmax, and adhesion.

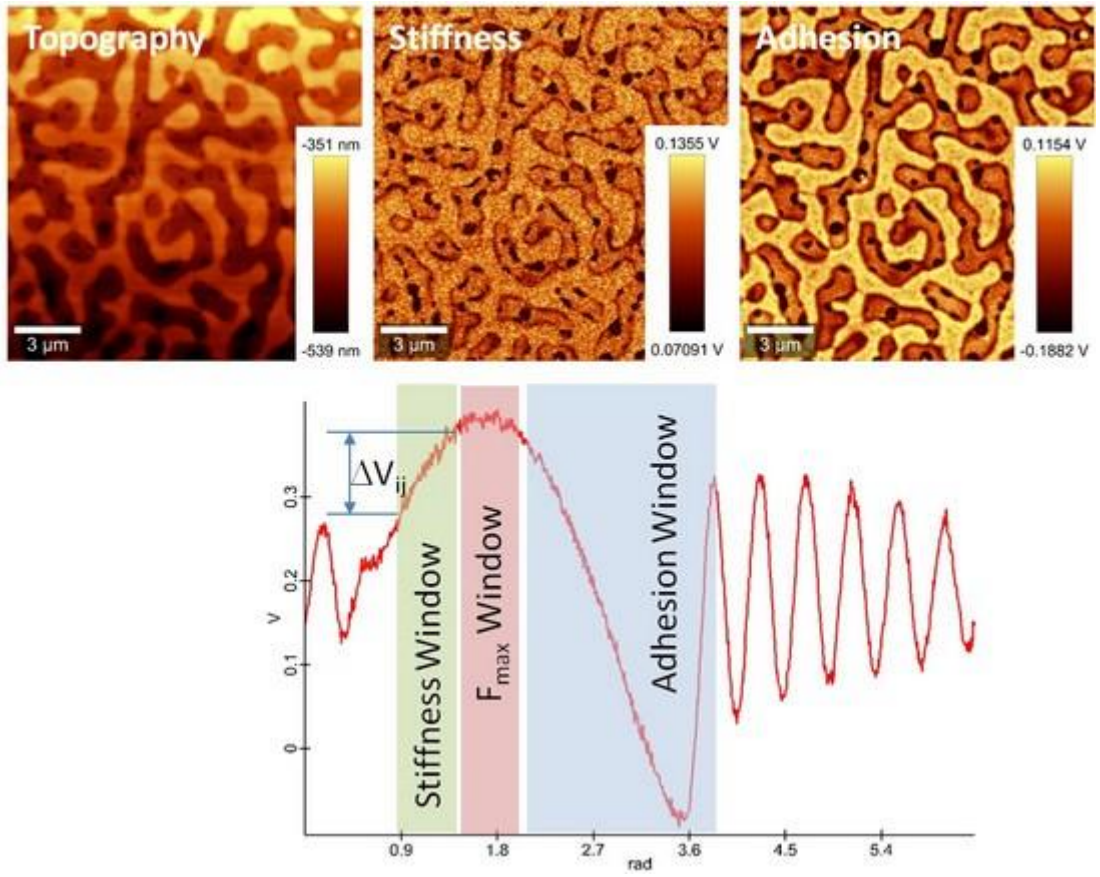


Fig. 1: Results of the DPFM measurement on the polymer blend

Remarks:

- the shown topography, adhesion and stiffness images are raw data with the corresponding z-color scale in nanometer and volts respectively.
- The stiffness image displays the changes in ΔV_{ij} as shown in the pulsed-force curve in Fig. 1.
- The adhesion image displays large adhesion in dark color because the adhesion values are negative values in the DPFM curve.
- The DPFM curve is acquired at a certain offset, indicating a small drift in the T-B signal during the approach or the measurements. This is not unusual with soft cantilevers. As a result of this offset, the measured voltages in the adhesion image vary from -0.1882 V to 0.1152 V even though the theory predicts that they should always be negative.

Table 1: Parameters of the example measurement (important information is highlighted in green and red)

Image Scan:

Points per Line:	256
Lines per Image:	256
Scan Width [µm]:	16.000
Scan Height [µm]:	16.000
Scan Speed [s/Line]:	1.000
Retrace Speed [s/Line]:	1.000

P-I Controller:

Setpoint [V]:	0.40000001
P-Gain [%]:	6
I-Gain [%]:	8
Controlled Channel:	Fmax

PFM-Control:

Driving Amplitude [Vpp]:	2.5999997
Driving Frequency [Hz]:	1000
Sampling Rate [Hz]:	999998.31
Excitation Phase [°]:	0
Fmax Window [°]:	(Start = 87.114723, Width = 33.543232)
Fmax Window [µs]:	(Start = 241.99, Width = 93.176)
Adhesion Window [°]:	(Start = 139.38011, Width = 143.92336)
Adhesion Window [µs]:	(Start = 387.17, Width = 399.79)
Stiffness Window [°]:	(Start = 56.406136, Width = 27.873955)
Stiffness Window [µs]:	(Start = 156.68, Width = 77.428)

Adhesion

For the calculation of the adhesion, the adhesion image $V_{Adhesion}$ has to be converted into a force unit. The voltages are represented in the measured adhesion image as color scale, where dark areas correspond to higher adhesion than bright areas.

> When determining voltages from an image, ensure that no average or background subtraction was applied to the image.

Using the equation (1), with k = 0.2 N/m and S = 227 nm/V in this specific case, it is possible to calculate the adhesion values measured at each image pixel using the relation (10) in the calculator tool (Fig. 2).

$$Adhesion\ [nN] = 0.2\left[\frac{N}{m}\right] \cdot 227\left[\frac{nm}{V}\right] \cdot -V_{adhesion}\ [V] = 45.4\left[\frac{nN}{V}\right] \cdot -V_{adhesion}\ [V] \quad (10)$$

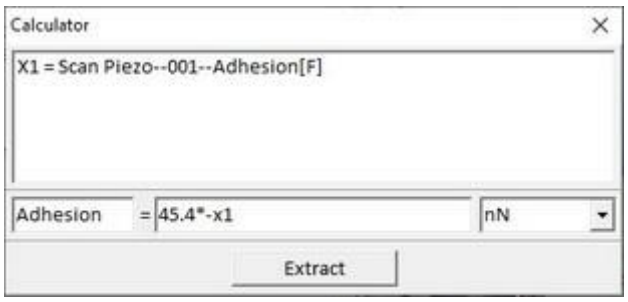


Fig. 2: Calculator tool with equation (10) applied

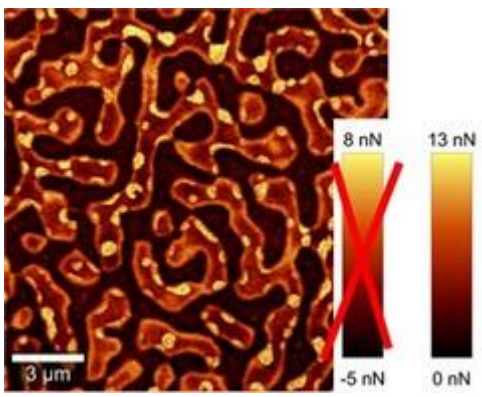


Fig. 3: Adhesion image with converted force units (bright color shows high adhesion)

The result of the calculation varies from negative to positive values, which has no physical meaning. These negative values arise from the offset of the pulsed force curves shown in Fig. 1. In DPFM measurements only relative adhesion forces can be determined and as color scale a leveled color scale can be exported as shown in Fig. 3. The color scale of the adhesion image is now converted into the proper force unit.

Stiffness

The local stiffness is defined by equations (5) and (6), where the nature of forces which contribute to the stiffness measurements are neglected. The resulting local stiffness values obtained from these calculations can be used for i.e. modelling of the tip-sample interface.

The stiffness image recorded in Fig. 1 shows a contrast between the PS and EHA. Again, dark colors correspond to lower stiffness values than bright colors. In order to calculate the stiffness for these two areas, the calculation of the penetration depth Δz is required, which also implies the calculation of the cantilever modulation M.

The modulation M is calculated using equation (3). The parameters for this example are summarized in Table 2 (Preliminary measurements). The voltage A applied to the cantilever is marked green in Table 1.

$$M = U \cdot S \cdot A = 0.65 \cdot 227 \left[\frac{nm}{V}\right] \cdot 2.6[V] = 383.6\ [nm] \tag{12}$$

With the collected data (modulation M from equation (12), sensitivity S from Table 2 (Preliminary measurements), σ is the red value in Table 1), it is now possible to calculate the penetration depth Δz of the cantilever using equation (4):

$$\Delta z = 383.6[nm] \cdot \left(1 - \cos\left(\frac{27.874[°]}{180[°]} \cdot \pi\right)\right) - V_{stiffness}[V] \cdot 227 \left[\frac{nm}{V}\right]$$

$$\Delta z = 44.5[nm] - V_{stiffness}[V] \cdot 227 \left[\frac{nm}{V}\right] \tag{13}$$

The parameter X1 in the calculator (Fig. 4 (c)) is the initial measured stiffness image (Fig. 4 (a)). The resulting image is shown in Fig. 4 (b).

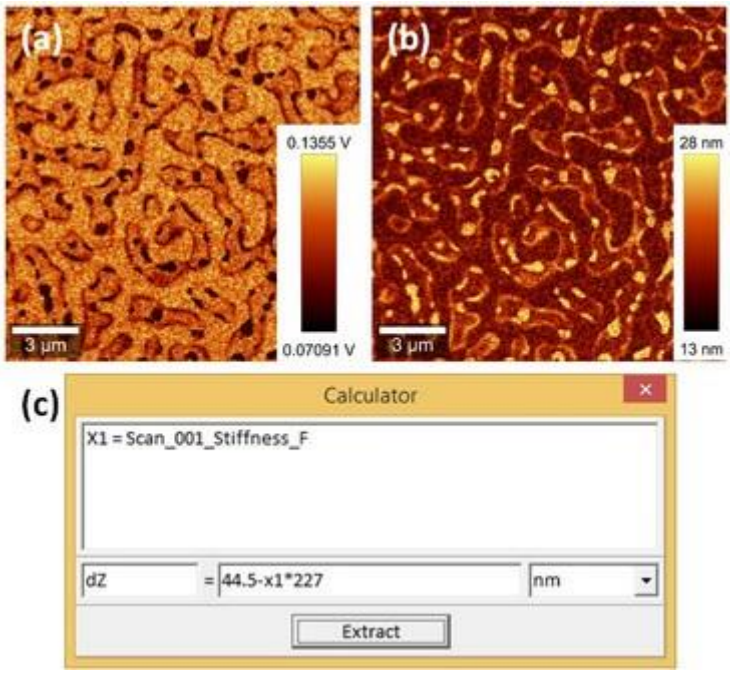


Fig. 4: Measured stiffness image (a), evaluated penetration depth (b) using equation (19) and the calculator tool (c)

The stiffness image is obtained by using equation (6), with the appropriate parameters for this example measurement:

$$Stiffness\left[\frac{N}{m}\right] = \frac{0.2\left[\frac{N}{m}\right] \cdot 227\left[\frac{nm}{V}\right] \cdot V_{stiffness}\,[V]}{\Delta z\,[nm]} \qquad (14)$$

Equation 14 can be used directly in conjunction with the calculator tool as shown in Fig. 5 (d).

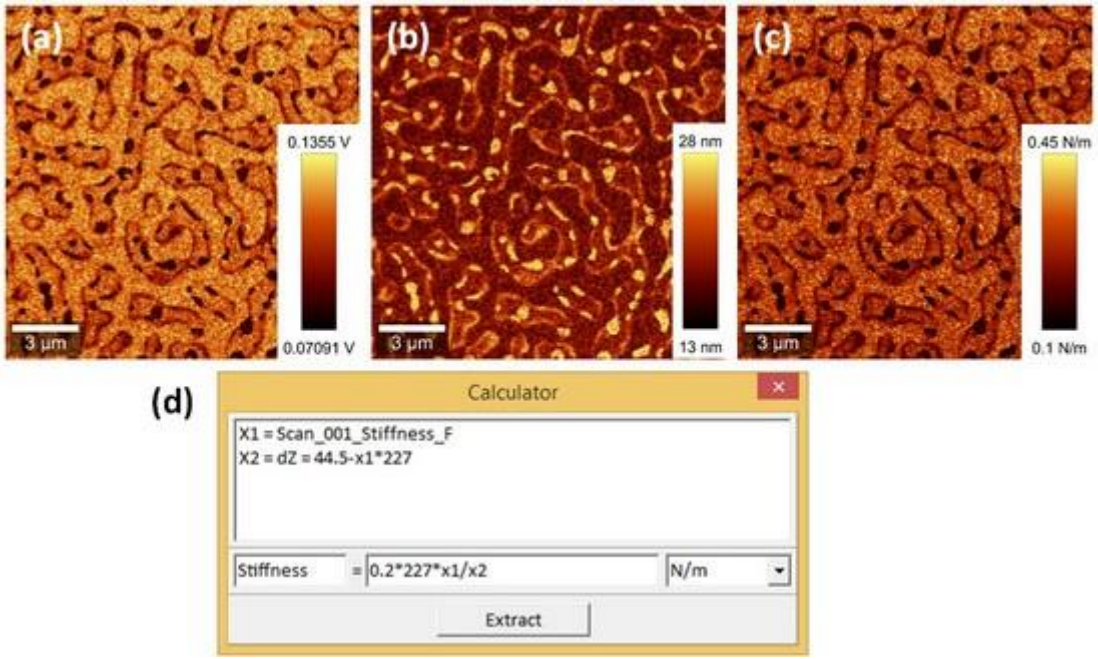


Fig. 5: Measured stiffness image (a), penetration depth (b), stiffness image evaluated using equation (14) and the calculator tool (d)

On samples with high topographical features a high contribution to adhesion and stiffness measurements results from changes of the contact area between tip and sample. Adhesion measurements are also sensitive to capillary forces interacting with the cantilever. These forces are due to water contamination on the sample from air environment. To overcome capillary forces, higher modulation amplitudes are recommended.

DPFM Curves

Import of DPFM data

The pulsed force curves are stored in a .wsd file, if selected before starting the measurement. For the analysis of the data it is necessary to import it into the project using File > Import > DPFM Data. After selecting the appropriate .wsd file, the menu shown in Fig. 1 opens. Select the T-B Channel without any data reduction (enter 1 for Data Reduction) and click Extract All.

The imported pulsed force curves appears as new graph data object and can be treated similar to spectral data arrays.

The new .wip file can be very large and you may run out of memory. Check your Memory options.

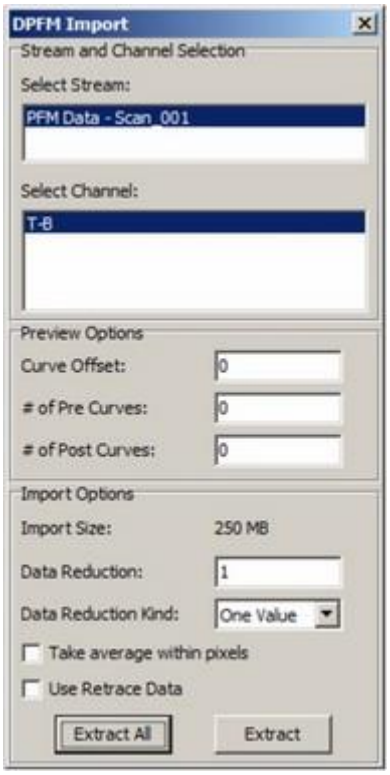


Fig. 1: DPFM import window.

Offset correction

As first step a background subtraction should be performed on the imported pulsed force curves. A slight drift of the T-B signal during approach or the measurement can affect the adhesion values. The background correction eliminates this error. Use a zero order polynom (Fig. 2 (a)) over the range

highlighted as blue area on the curve (Fig. 2 (b)). The result of the background subtraction is shown in Fig. 3 (blue curve).

Change the x axis unit of the curves from deg to rad using the Graph Viewer Context Menu (Double-click on the data object to open the data in the graph viewer).

All the following operations are performed using the background corrected curves in **rad**.

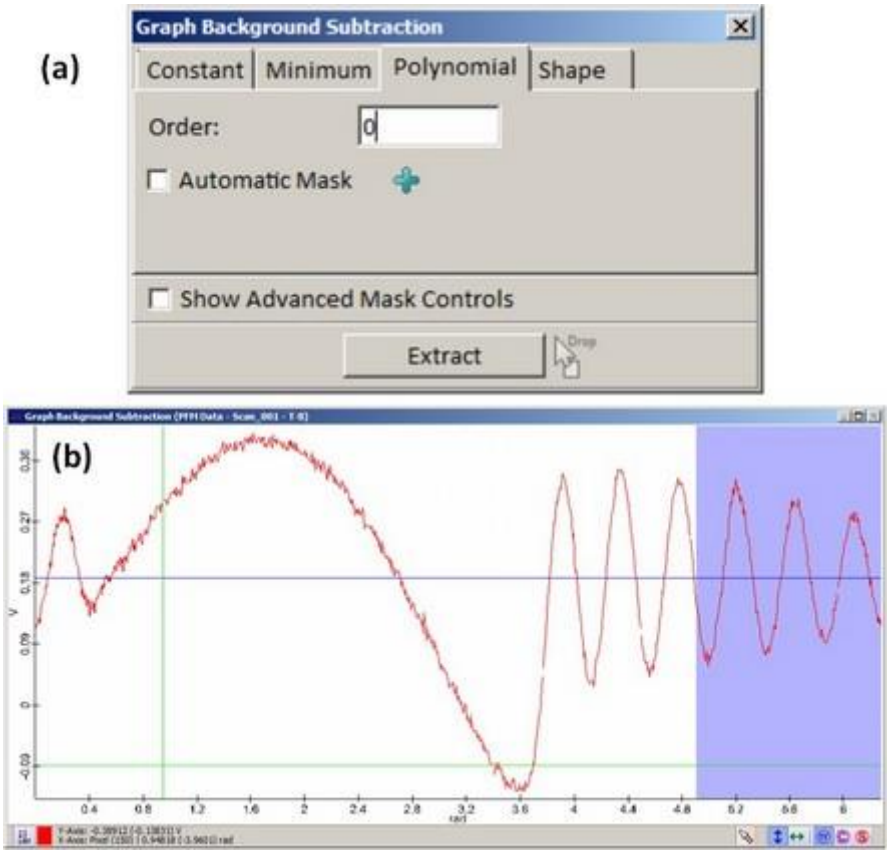


Fig. 2: Graph background subtraction menu (a) and example for range selection (b).

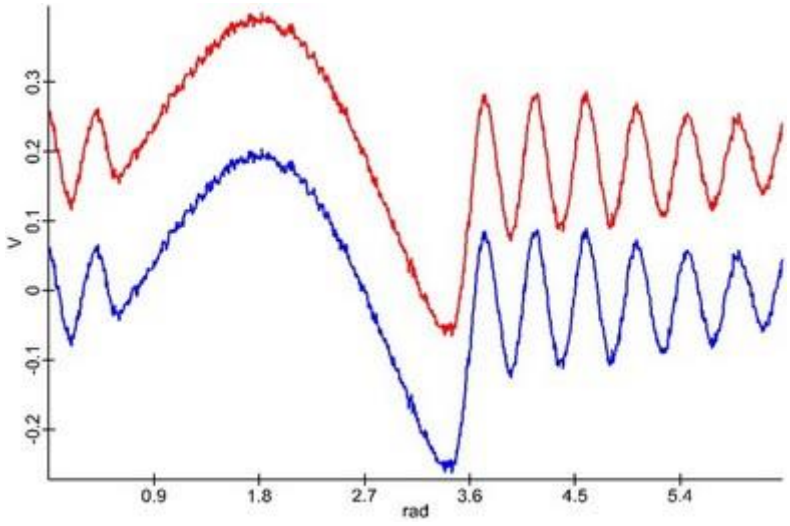


Fig. 3: Result of the background subtraction: original curve (red) and corrected curve (blue)

Evaluation of adhesion and stiffness from the DPFM curves

Adhesion

In order to create the adhesion image use the Filter Viewer with a minimum filter. Select the appropriate range for this filter (Fig. 4 (a)). Based on the resulting image in V (Fig. 4 (b)) the adhesion image in nN (Fig. 4 (c)) can be calculated following the steps in the DFPM Images section.

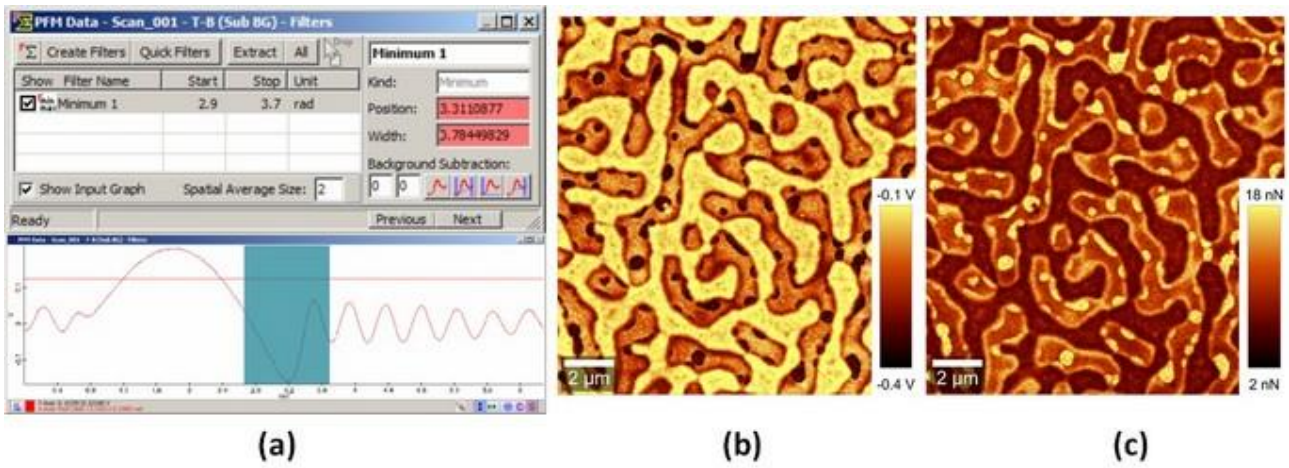


Fig. 4: Evaluation of adhesion from the PFM curves using the filter manager of WITecProject in conjunction with the Minimum filter and the appropriate filter selection range (a), extracted image from the filter manager (b) and the evaluated adhesion image (c).

Stiffness

The used Fit and Extract All of the Advanced Fitting Tool is a Plus feature.

The stiffness is defined by the slope of the rising part of the DPFM curve. By using the Advanced Fitting Tool, a 1st order polynomial can be fitted to the curves in every image pixel.

1. Select Polynomial as the category and 1 as the Polynom Order (see Fig. 5 (a)).
2. Select the fit range as shown in Fig. 5 (c) and note the width of the selected window Δ_{rad}.
3. Click on Fit and Extract All.
4. The slope a_1 is the data object ending with (Fit Polynom: a_1) in V/rad.

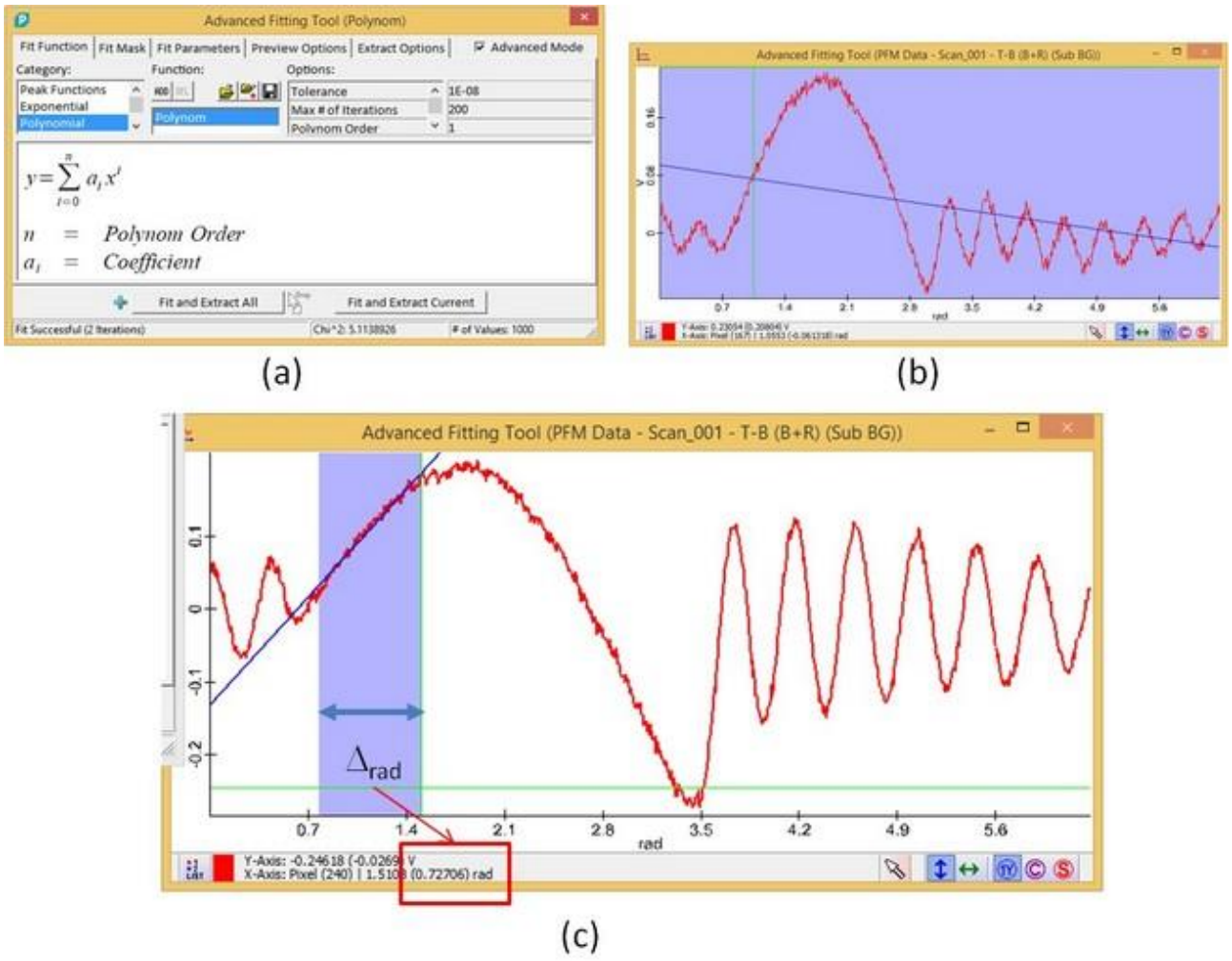


Fig. 5: Advanced fitting tool for 1st order polynomial fit (a), graph viewer which opens together with the fitting tool (b) and graph window showing the selected range of interest together with the blue fitting curve (c).

The slope image can now be used to determine the penetration depth in nm and consequently the corresponding stiffness image using equation (6) and also according to the DPFM Images section. The example for this specific measurement is shown in the two equations below and the resulting images in Fig. 6.

$$\Delta z = M \cdot (1 - \cos(\Delta_{rad})) - a_1 \cdot \Delta_{rad} \cdot S \tag{15}$$

$$\Delta z = 383.6[nm] \cdot (1 - \cos(0.727[rad])) - a_1 \left[\frac{V}{rad}\right] \cdot 0.727[rad] \cdot 227 \left[\frac{nm}{V}\right]$$

$$Stiffness = \frac{a_1 \cdot \Delta_{rad} \cdot k \cdot S}{\Delta z} \tag{16}$$

$$Stiffness \left[\frac{N}{m}\right] = \frac{a_1 \left[\frac{V}{rad}\right] \cdot 0.727\,[rad] \cdot 0.2 \left[\frac{N}{m}\right] \cdot 227 \left[\frac{nm}{V}\right]}{\Delta z\,[nm]}$$

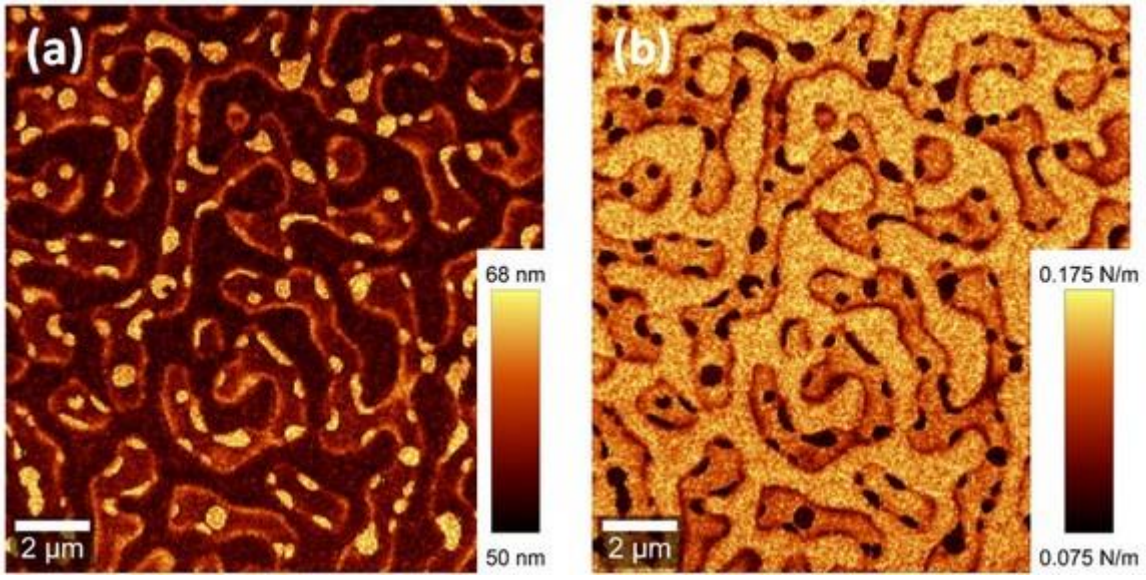


Fig. 6: Penetration depth (a) and stiffness (b) images evaluated using the advanced fitting tools and equations 15 and 16.

Force Distance curves from DPFM Measurements

The DPFM curves can be displayed as force-distance curves:

1. Fit a sinus to the Fmax region of the DPFM curves.
    a. Click on Load user fit functions from file in the Advanced Fitting Tool and select `04 Sine.wps` in the Examples folder.
    b. Select the appropriate range in the Fmax region (Fig. 7 (b))
    c. Activate Extract Fit Curve with Input Data Supporting Points and Extract Fit Curves in the Extract Options tab.
    d. Click Fit and Extract All
2. Multiply the resulting f_{sinus} function (the data object ending with (Fit Sine: Curves)) with the modulation M to get a distance vs. time function ($f_{modulation\text{-}distance}$).
3. Open the DPFM curves and the $f_{modulation\text{-}distance}$ curves in one graph viewer like shown in Fig. 9 (a). (Mark both and press Enter)
4. Switch to the parametric view to show force-distance curves of all measured DPFM curves (Fig. 9 (b)). In this view higher distance values correspond to a position closer to the sample.

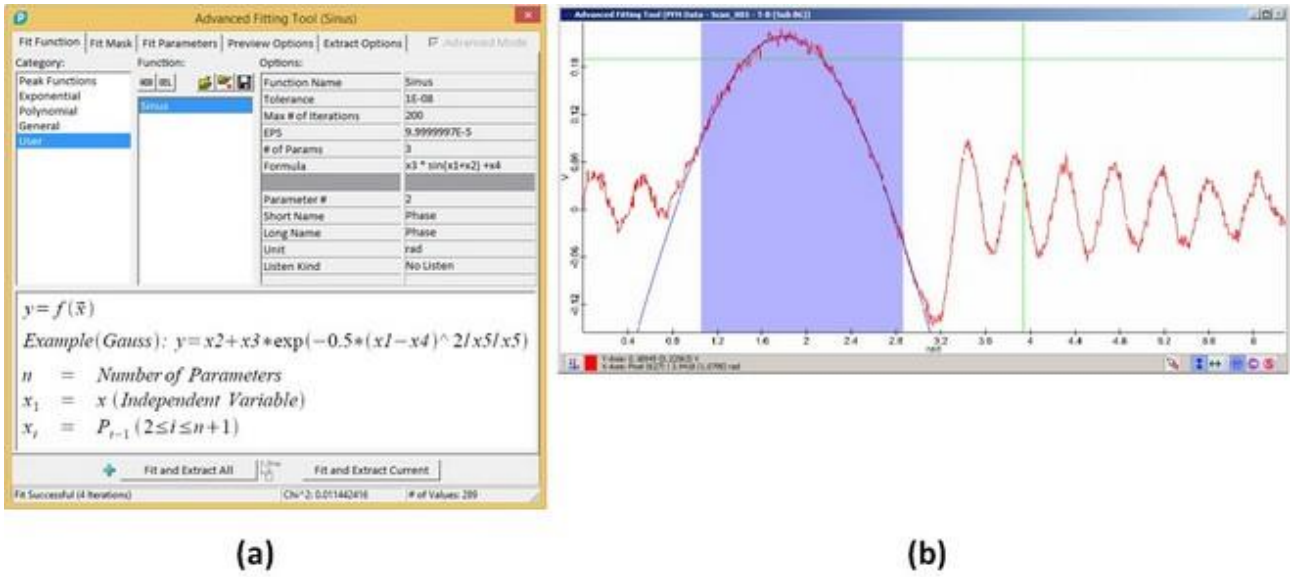


Fig. 7: Example of sinus fitting function in the advanced fitting tools (a) and region selection in a PFM curve for the fit (b).

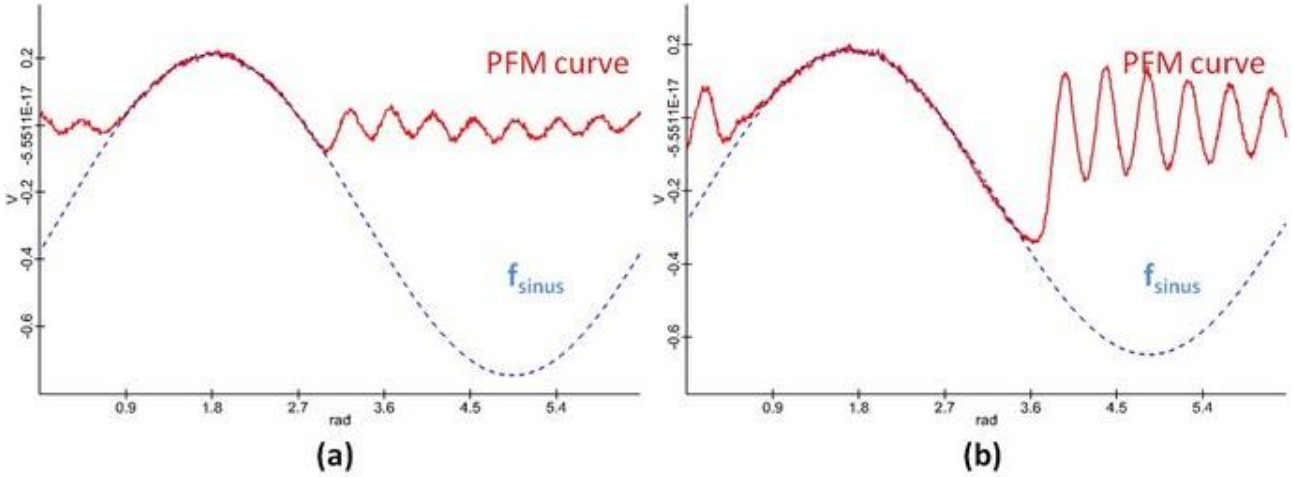


Fig. 8: PFM and f_{sinus} curves from a stiff (a) and soft (b) measuring point represented with the same y-axis.

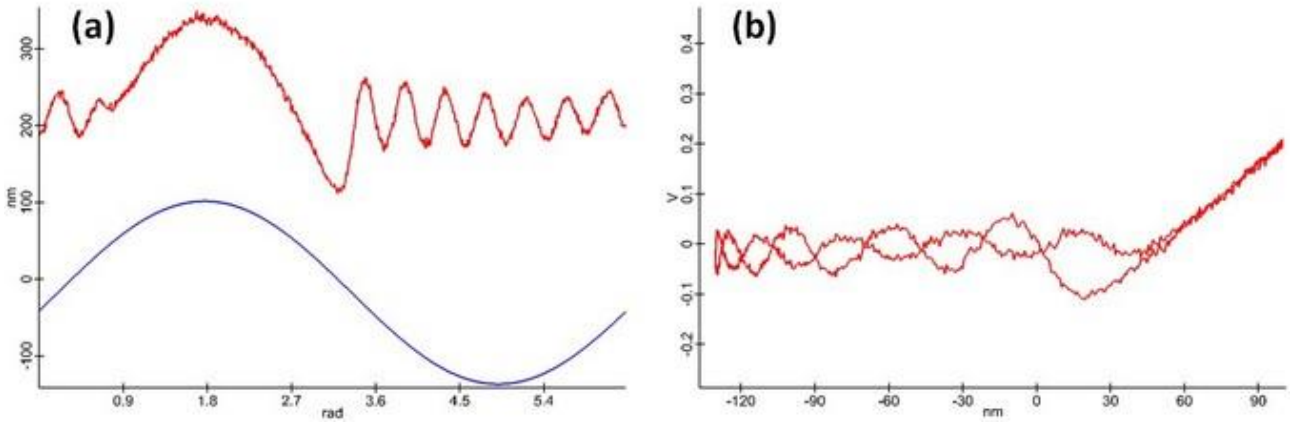


Fig. 9: Example of DPFM and $f_{modulation\text{-}distance}$ curve displayed in one graph viewer (a) and force-distance curve obtained from a DPFM measurement (b).

Conductive AFM

C-AFM Overview

Conductive Contact is an AFM mode to determine the electrical conductivity of a surface in combination with the sample topography. When measuring in contact mode, the tip constantly touches the surface. This way, when the tip and sample are conductive and a potential is applied, a small current can flow between sample and tip. This is especially interesting for doped semiconductor structures, but also to characterize metallic composite surfaces or thin layer coatings.

Topics:

- Theory
- Sample Mounting
- Setting up a measurement
- Example measurement

System requirements:

- EFM package with EFM arm and clamps
- Low Noise Current Amplifier (e.g. femto)
- Circuit plate sample holder (recommended)

Required License feature:

- EFM Mode

Recommended Cantilevers:

The cantilever needs to be electrically connected to the mounting ring (refer to EFM). A diamond coated cantilever for Contact mode is recommended.

Theory

When in constant physical contact with the sample surface, this enables an electrical current to flow between tip and sample, providing additional information about the local resistivity or conductivity of the sample. Though it is not to be confused with a tunneling current in Scanning Tunneling Microscopy, where this current also serves as a feedback signal.

With an applied bias potential U_{Bias}, the current I can be measured flowing between tip and sample according to Ohm's law:

$$I = \frac{U_{Bias}}{R}$$

The resistance here relates to the whole system, not just the immediate sample surface, including the sample holder and its electrical connections, as well as the cantilever. In addition, the measured current highly depends on the contact area between tip and sample. It can vary due to microscopic structural changes of the tip and also with the sample topography.

Another obstacle is the passivation of surfaces. Metals oxidize very quickly under ambient conditions, and in addition, most surfaces have adsorbate layers consisting of humidity and other residuals from ambient air. These act as insulating layers which need to be pierced through in order to form an electrical connection.

This presents high requirements to the tip though, which needs to be conductive as well as stable despite mechanical forces. Metallic coating and metallic tips are more ductile than silicon and usually wear off very quickly. Often, tips with diamond coating are used.

This method is also related to Scanning spreading resistance microscopy (SSRM), which is operated at forces that pierce through oxide layers.

Further Information

- https://en.wikipedia.org/wiki/Conductive_atomic_force_microscopy

Sample Mounting

For sample mounting, it is recommended to use the circuit board sample holder supplied by WITec. This has proven to offer reliable electrical connections.

The sample needs to be connected to a bias voltage, which is supplied by AUX out. Therefore the three grey marked pads on the circuit board can be used. The sample can be glued directly on one of the pads by conductive paint to connect from below or can be connected by cable. Anyway conductive paint is suitable to glue the sample to board because it can be easily removed afterwards with solvents. It is important that the sample or at least the parts of interest are not connected to ground.

For connecting the cantilever arm a short direct cable connection between board an arm is necessary.

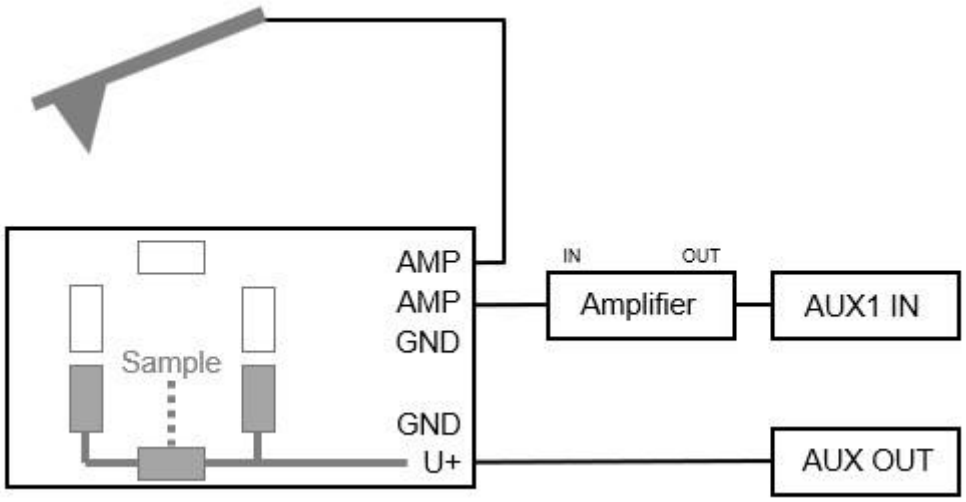
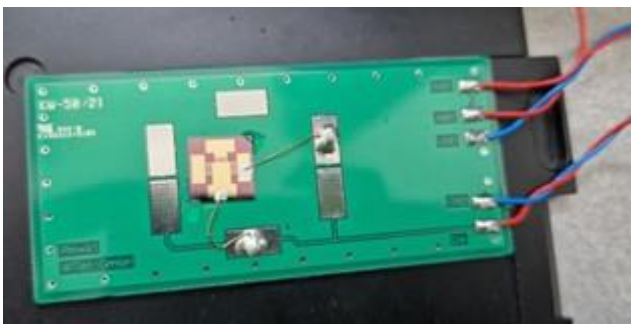


Figure 1: Wiring diagram of the sample mount and the external amplifier.



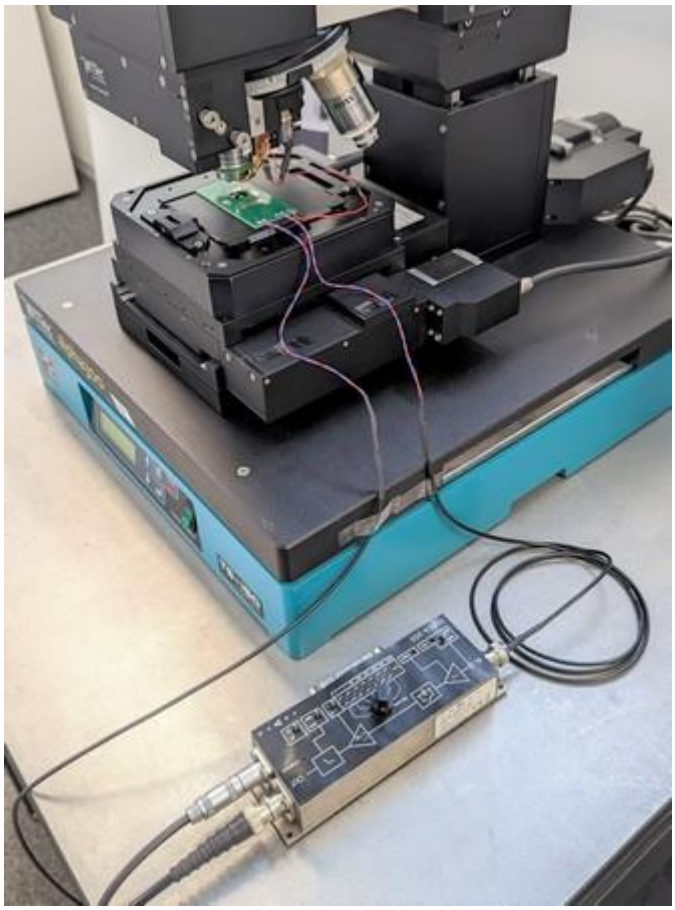


Figure 2: Reference sample fixed on the sample holder (left) and holder mounted in the alpha300 (right).

Hints

- Use adhesive tape to fix the cables to the setup, not to extort too much force on the fragile soldering spots.
- Use a multimeter to test the connectivity at multiple times during the setting up process: If there is any problem, it is usually a connection issue at some point.
- Another method to test for proper connection is to apply a discrete voltage in the EFM controls and measuring this on the sample holder.

Parameters

Follow the steps in the contact mode procedure.

- A setpoint of 0.7 V is good to pierce through an eventual oxide or adsorbate layer.
- Rotate the scanning direction (gamma) 90°, so the fast scanning axis runs parallel to the cantilever.
- Recommended scan speed is 3 s/line.
- Start by measuring without the amplifier turned on. If there is sample contrast visible in the AUX channel, it indicates crosstalk between the wiring.
- The bias voltage can be controlled in the EFM control
- The bias should range from 1 – 3 V. A value of 1.5 V works suitably. Above 3 V there is a risk of melting and damaging the tip apex (resistive heating due to high current flow through a small

cross-section).

Interpretation of values

The qualitative interpretation of numeric values is challenging, since the measured current is dependent on the actual area of electrical contact. This cannot be determined sufficiently enough and can even change during the measurement.

To express the measurement in actual measured current, refer to the photocurrent section, where the calculation is explained.

Reference Sample

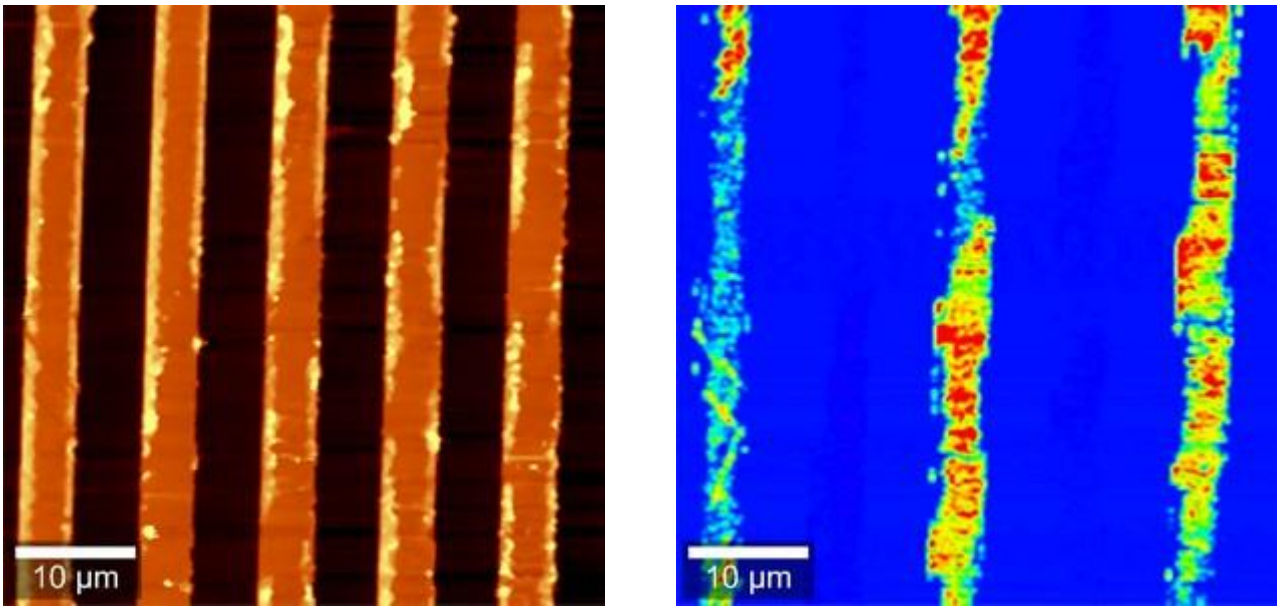


Figure 1: Topography (left) and conductivity (right) image of conductive traces.

The EFM sample is used here. The traces are alternately connected to ground and to voltage.

Table 3: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	50 x 50
Pixel	256 x 256
Time per Line [s]	3
Setpoint [V]	0.8
Bias voltage [V]	1.5

AFM AC Lift Mode

AC Lift Mode Overview

Lift Mode is a two-pass technique that interleaves AC mode scan lines at the sample surface with scan lines at a designated height above the surface. There forces on the cantilever can be measured excluding the influence of the topography.

Topics:

- Magnetic Force Microscopy (MFM)
- Electric Force Microscopy (EFM)
- Setting up a measurement
- Hints section

Measurement modes:

- Image Scan (Multi Pass): acquisition of AFM AC Lift mode images
- Image Scan: acquisition of AFM AC mode images (only for optimizing parameters)

System Requirements:

- alphaControl with a serial number 120-1030-XXX (Marvin 3b) or higher

Required License feature:

- Lift Mode

Parameters

Follow the steps in the general procedure. For further information refer to the AC mode adjustment.

> The Z Offset depends on the chosen free amplitude and the forces between tip and sample. Always work exactly on the resonance frequency of the cantilever for highest sensitivity, because there is no damping of the cantilever during the second pass.

After tip approach:

1. Set the Time / Line (Trace) [s] under Image Scan.
2. Click on Start Scan.
3. Optimize the Feedback settings.
4. Stop the Image Scan. (Data can be deleted.)
5. Adjust the Z Offset for Measurement at the section Image Scan (Multi Pass).
6. Click on Start Multi Pass Scan.

Recorded Channels

Each data object in a Multi Pass scan is created twice marked with [L] or [M]. The data objects marked with [L], which stands for Learn, is measured during the first pass at the surface. The data objects marked with [M], which stands for Measure, are recorded during the second pass above the surface. By default, both Phase and Topography images with the according graphs are opened (Figure 2).

Contrasts due to changing forces between tip and sample can be observed in the measured Phase image (Phase [M]).

Further information:

Image Scan (Multi Pass), Image Scan

Hints

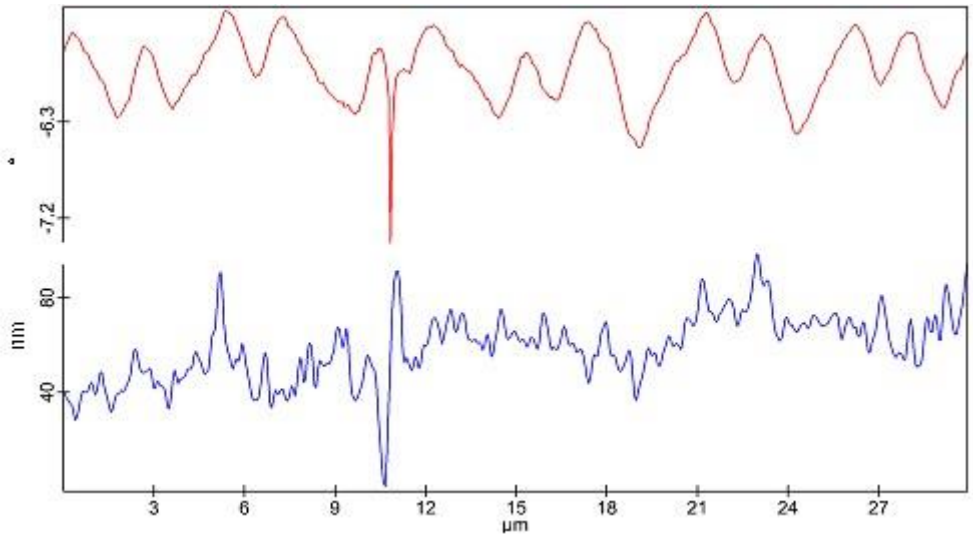


Figure 1: Phase [M] (red) with artifact and corresponding Topography [L] (blue)

If the cantilever collides with the sample during the second pass an artifact will be created in the measurement. It can be seen in Figure 1 that the anomaly at around 10.5 µm in the Phase of the second pass correlates with a big step in the topography. If this happens, a higher z-offset can be chosen. This is also necessary for a high topography in general. A z-offset that is too high, however, will lead to lower contrast because most of the forces are stronger close to sample. Therefore, an alternative is to use the Look Ahead value, which leads to reaction on the topography before it is really reached.

To speed up the measurement the Min. Time for Retrace parameter can be reduced. This could also lead to more stress for the tip on the other hand. So, the appropriate compromise is important.

Further information:

Magnetic Force Microscopy

Overview

In Magnetic Force Microscopy (MFM) the magnetic force gradient along the sample surface is measured using the Lift mode. It can be used to investigate e.g. magnetic recording materials, superconductors, magnetic nanoparticles, etc.

Topics:

- Setting up a measurement
- Example measurement
- Lift Mode hints section

Recommended cantilevers:

For MFM special cantilevers with magnetic coated tips are needed.

Reference sample

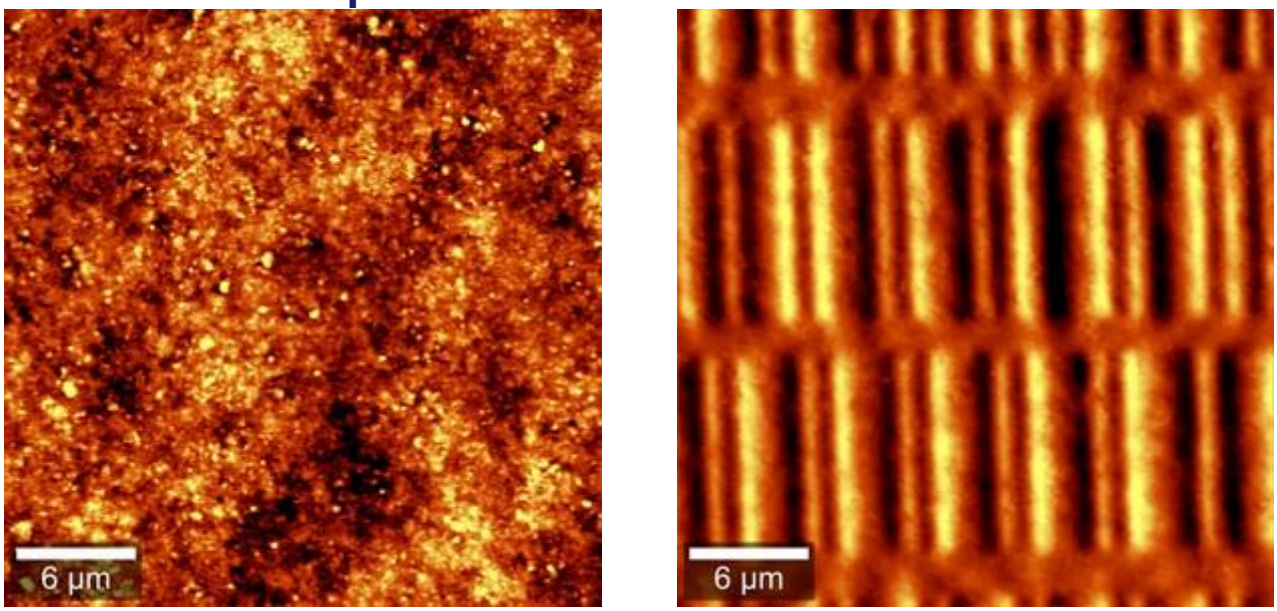


Figure 1: Topography (left) and MFM (right) image of a floppy disc

Besides commercial MFM samples a part of a floppy disc or hard disc platter is a good sample to test and practice MFM. The MFM image in Figure 1 shows the single bits on different tracks.

Table 4: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	30 x 30
Pixel	512 x 512
Time per Line [s]	1
Z Offset [nm]	200

Look Ahead [%]	0

For MFM a high z-offset is necessary in order to see a contrast and really detach the tip from the sample surface.

Electric Force Microscopy

Overview

Electric Force Microscopy (EFM) measures electric field gradient distribution along the sample surface using the Lift mode. EFM is used e.g. for electrical failure analysis, detecting trapped charges, mapping electric polarization, and performing electrical read/write.

Topics:

- Voltage Supply
- Setting up a measurement
- Example measurement
- EFM hints section
- Lift Mode hints section

System requirements:

- EFM package with EFM arm and clamps

Required License feature:

- EFM Mode

Recommended Cantilevers:

The cantilever needs to be electrically connected to the mounting ring. Therefore, a silver conductive paint is used (Figure 1). A metal coated cantilever is recommended.

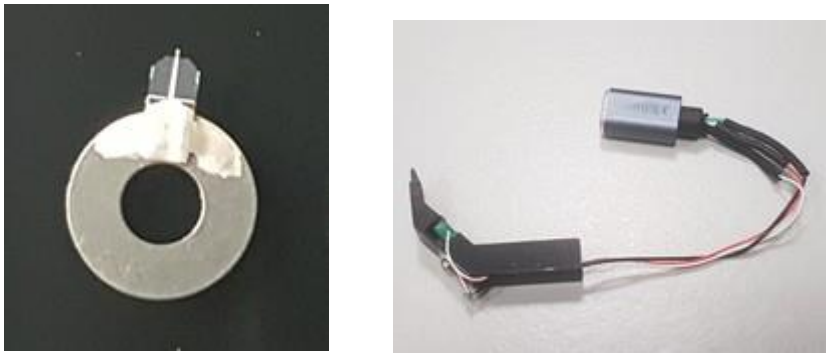


Figure 1: Cantilever connected with silver conductive paint (left) and EFM arm with electric connection (right)

Voltage Supply

Connecting the cantilever:

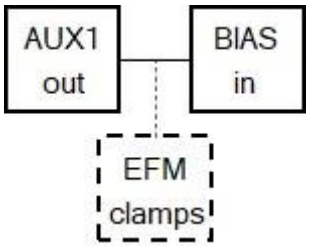


Figure 1: Wiring diagram

- Make sure BIAS-in connector of the alphaControl electronics is connected with AUX1-out via a BNC cable (Figure 1).
- If you want to use the EFM-clamps, connect them over a T-piece adapter with BIAS-in and AUX1-out (Figure 1).
- The sample has to be connected to ground over the black EFM-clamp or by an external potential.
- Make sure the red clamp has no contact to ground.
- Mount a contacted cantilever with the EFM cantilever arm.

EFM Control:

The section EFM Control is used to apply a voltage between tip and sample. Select Aux1 DAC as Output DAC and switch EFM Output to Enabled. Now, the voltage can be adjusted at DC Component. Setup these settings before starting the EFM measurement.

For optimization the voltage can be changed or switched on/off also during the measurement.

Hints

- If the contrast in the Phase image is too low, check the electric connections:
  - connection to the cantilever
  - connection of the sample
  - To check the connection the EFM Control could be used to apply a DC voltage between tip and sample
- Check if the used cantilever is connected properly using silver conductive paint.
- Try another cantilever, if the result should not improve.

Reference sample

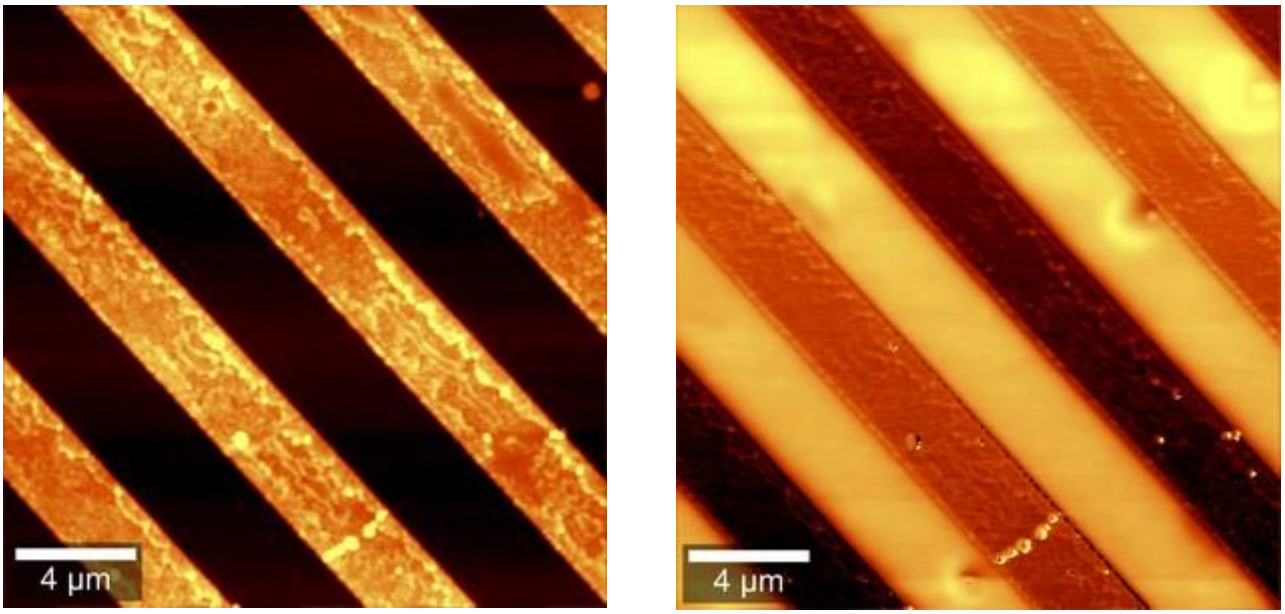


Figure 1: Topography (left) and EFM (right) image of electric traces

For EFM, reference samples are available (e.g. from WITec) with electric traces that can be put on different potentials. The EFM image in Figure 1 shows the traces where potentials of 0 V and 1 V are applied. The isolating material can also be clearly distinguished (dark stripes in the topography, bright stripes in the EFM image).

Table 5: Exemplary parameters for the above measurement

Parameter	Value
Size [µm]	20 x 20
Pixel	512 x 512
Time per Line [s]	1
Z Offset [nm]	50
Look Ahead [%]	10
EFM voltage [V]	4
Sample potential [V]	0 and 1

Kelvin Probe Force Microscopy

KPFM Overview

Kelvin probe force microscopy (KPFM) is an AFM mode to determine the work function of surfaces at the nano scale.

Topics:

- Theory
- WITec implementation
- Sample mounting
- Setting up a measurement
- Example measurement
- Interpretation of values

Measurement modes:

- Image Scan (Multi Pass): acquisition of AFM AC Lift mode images
- Image Scan: acquisition of AFM AC mode images (only for optimizing parameters)

System requirements:

- alphaControl with a serial number 120-1030-XXX (Marvin 3b) or higher
- EFM package with EFM arm and clamps

Required License feature:

- Kelvin probe

Recommended Cantilevers:

The geometry of the cantilever and the tip is a critical factor defining resolution and accuracy of the acquired KPFM images. Long and slender but slightly blunt tips on cantilevers of minimal width and surface area are the best choice.

The cantilever needs to be electrically connected to the mounting ring. Therefore, a silver paint is used (Figure 1). As cantilever a metal coated one (like for EFM) or one made of highly doped silicon (FM cantilevers) can be used.

> Metal coats have a poor stability. The tip electrode often loses parts of its coating during scanning. As a result, the tip electrode will act as an unstable reference since its surface potential distribution is changing during the measurement.

Figure 1: Cantilever connected with silver paint

Theory

Kelvin probe force microscopy (KPFM) is an AFM mode to determine the work function of surfaces at the nano scale. The technique uses an electric field between the sample surface and the probe. The voltage ΔV_{sp} needs to be adjusted to a value such that the gradient Φ is flat and the electric field is dissolved (Figure 1). In this case the following equation is valid, where W_s is the work function of the sample and W_p is the work function of the probe.

$$\Delta W = W_s - W_p = e\Delta V_{sp}$$

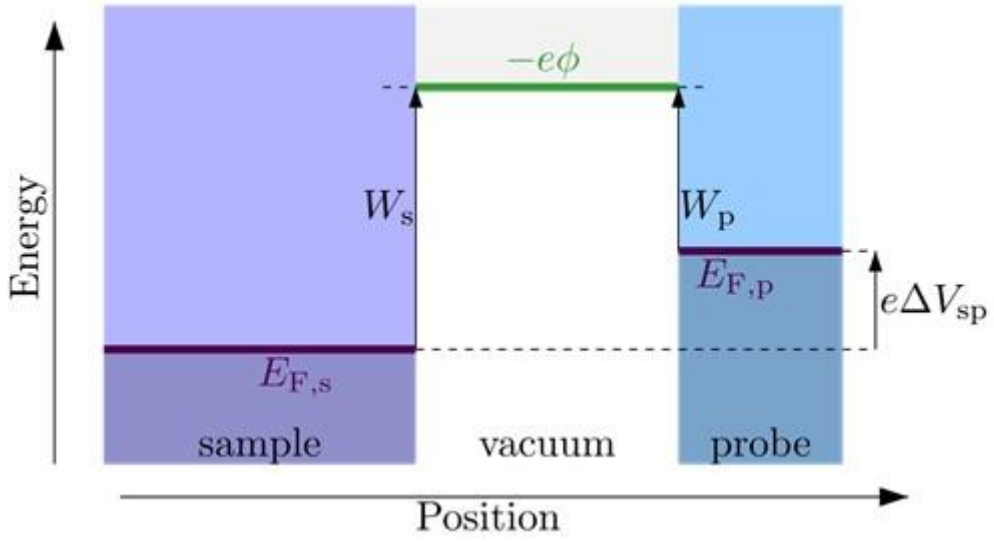


Figure 1: Energy diagram (source: https://en.wikipedia.org/wiki/Work_function)

The difference between the work functions is also denoted like this, where V_{CPD} is called the contact potential difference:

$$\Delta W = -eV_{CPD}$$

To measure the work function using an AFM tip as a probe a voltage is applied between tip and sample. This voltage consists of a DC-bias V_{DC} and an AC-voltage $V_{AC}\sin(\omega t)$ of frequency ω. The total potential difference between tip and sample is

$$\Delta V = V_{DC} + V_{AC}\sin(\omega t)$$

The electrostatic force in a capacitor may be found by differentiating the energy function with respect to the separation of the elements and can be written as

$$F = -\frac{1}{2}\frac{\partial C}{\partial z}(\Delta V)^2$$

where C is the capacitance, z is the separation, and V is the voltage, each between tip and surface. Substituting the previous formula for voltage (V) shows that the electrostatic force can be split up into three contributions, as the total electrostatic force F acting on the tip then has spectral components at the frequencies ω and 2ω.

$$F = F_{DC} + F_{\omega} + F_{2\omega}$$

$$F_{DC} = \frac{\partial C}{\partial z}\left(\frac{1}{2}(V_{DC} - V_{CPD})^2 + \frac{1}{4}V_{AC}^2\right)$$

$$F_{\omega} = \frac{\partial C}{\partial z}(V_{DC} - V_{CPD})V_{AC}\sin(\omega t)$$

$$F_{2\omega} = -\frac{1}{4}\frac{\partial C}{\partial z}V_{AC}^2\cos(2\omega t)$$

The DC component, F_{DC}, contributes to the topographical signal, the term F_{ω} at the characteristic frequency ω is used to measure the contact potential and the contribution $F_{2\omega}$ can be used for capacitance microscopy. The formulae are valid if the voltage is applied to the tip. If the voltage is applied to the sample, V_{CPD} is added not subtracted.

Further information

- https://en.wikipedia.org/wiki/Work_function
- https://en.wikipedia.org/wiki/Kelvin_probe_force_microscope

Implementation

The KPFM mode is accomplished as a two-pass measurement to minimize crosstalk using the lift mode like for MFM or EFM measurements. In the first pass the topography is recorded using Tapping mode and the Primary Lock-in. In the second pass the cantilever is not mechanically stimulated for a vibration anymore. The AC voltage $V_{AC}(\omega t)$ is applied between sample and tip. The cantilever starts to vibrate with ω due to the electric forces and the signal is analyzed by the Secondary Lock-in, which delivers the amplitude. To search for the value of V_{DC} where the minimum amplitude of the cantilever occurs, the V_{DC} voltage is modulated with 100 Hz (by default).

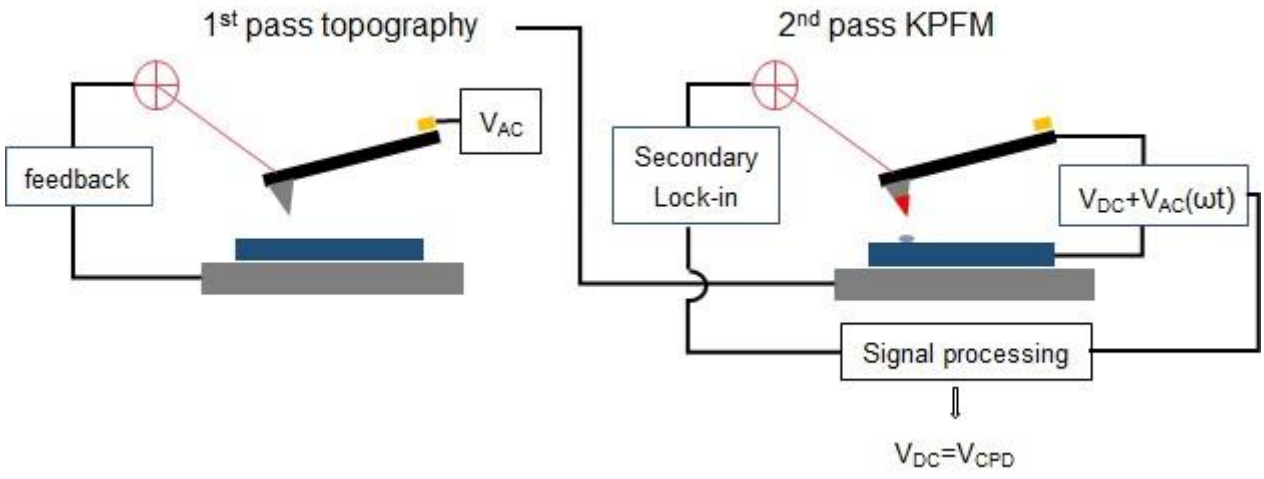


Figure 1: KPFM measurement principle

This kind of implementation is also called KPFM-AM. The advantage of KPFM-AM is a higher sensitivity but compromises the achievable resolution. This is due to the fact that not only the tip contributes to

the measurement but also the whole cantilever.

Sample Mounting

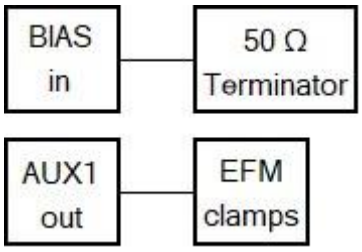


Figure 1: Wiring diagram

- Terminate BIAS-in with a 50 Ω Resistor (grounds the cantilever).
- Connect the EFM-clamps with AUX1-out.
- Make sure the sample is isolated and not connected to ground.
- Connect the red EFM-clamp over the sample clamp and put it on the sample surface.
- Mount a contacted Cantilever with the EFM cantilever arm.

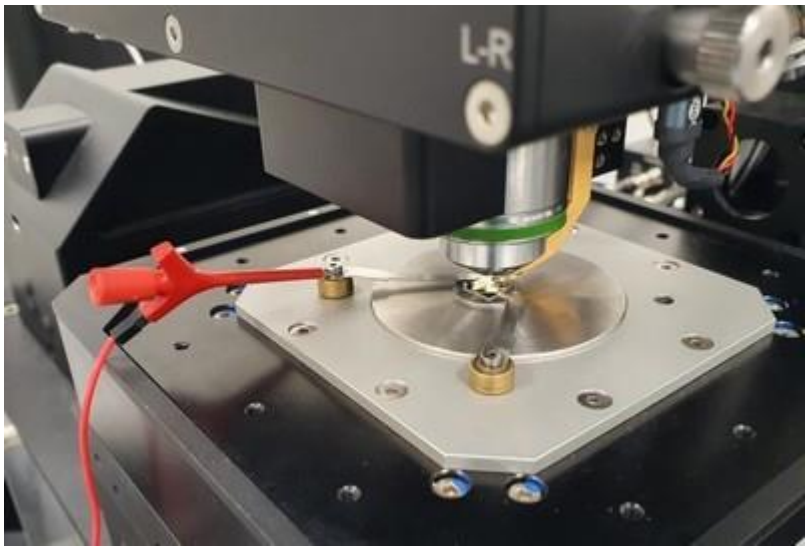


Figure 2: Reference sample mounted on the microscope

Parameters

Follow the steps in the Lift mode procedure.

The Time / Line (Trace) [s] should be set dependent of the Points per Line value. Allow at least 0.01 s for each pixel to prevent digitizing (2.56 s for 256 points).

The Z Offset for Measurement should be negative (e.g. -150 nm) in most cases because the amplitude of the cantilever is much smaller in the second pass.

CPD shows the KPFM image and curve.

Figure 1 shows the PFM Control window during the second pass of a KPFM measurement. The red line

shows the T-B signal, the blue line is the smoothed amplitude of the T-B signal (the phase shift is due to filtering), the red area is the search range for the minimum, the black line (by default it is white) shows the V_{DC}. The value of V_{DC} at the minimum value of the T-B signal is taken as value for the CPD image. The blue line should show two sharp minima. If not check the sample mounting and connections.

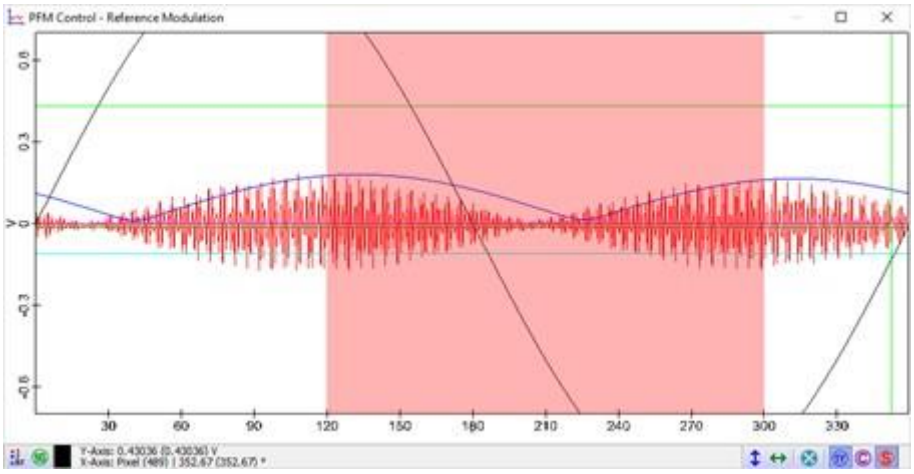


Figure 1: PFM Control window

All settings related to V_{AC} and V_{DC} are set up automatically when the measurement starts. Nevertheless, it is possible to change settings after the measurement started. All usefull parameters are summarized in the Kelvin Probe Control section.

Hints

- Before measuring a sample, the performance of the cantilever should be checked with the reference sample.
- If the sensitivity is too small check the electric connections:
  - connection of the cantilever to ground.
  - connection of the sample to Aux 1 Out.
  - To check the connection the EFM Control could be used which makes it possible to apply a DC voltage between tip and sample like for EFM using Aux 1 Out.
- If the KPFM curve shows digitizing check that the Time per Line fits to the VDC Driving Amplitude. (i.e. 2.56 s for 256 points @ 100 Hz)
- If the resolution is too low check the Z offset (needs to be negative).
- Check if the used cantilever is connected with silver paint.
- Try another cantilever if the result should not improve.
- Compare your results only with such measured under ambient condition, not in ultra-high vacuum.

Reference Sample

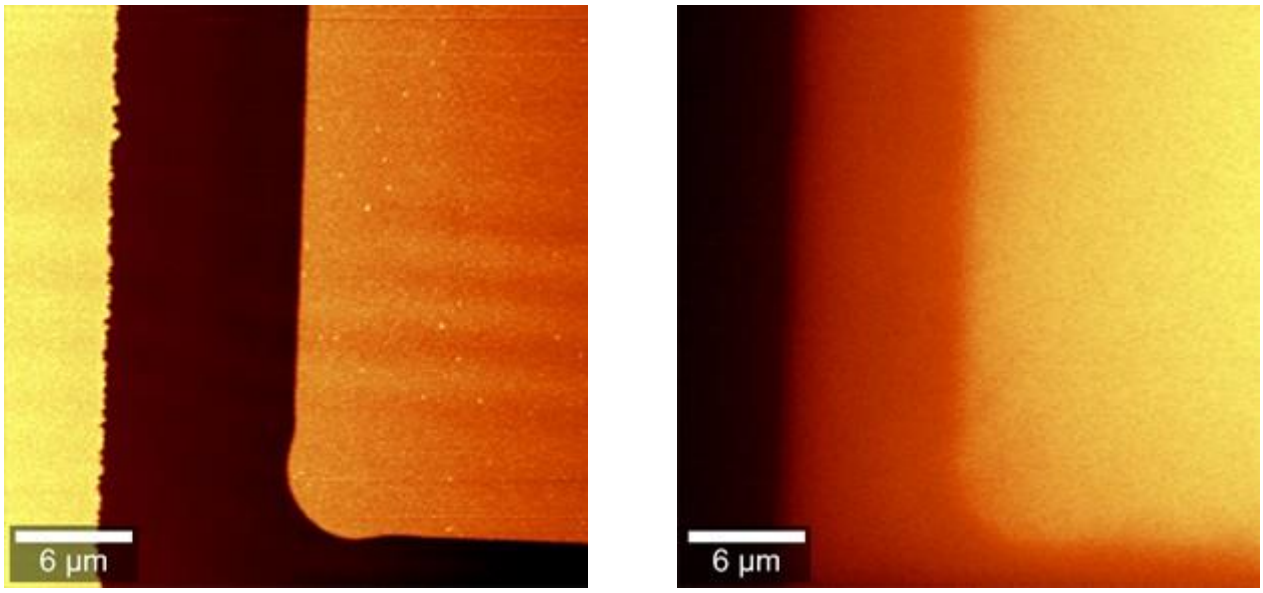


Figure 1: Topography (left) and CPD (right) image of the reference sample

A good reference sample for KPFM measurements is the PFKPFM-SMPL from Bruker.

Table 6: Exemplary parameters for the above measurement

Frequency sweep	
Driving Amp. pk-pk [V]	0.040
Driving Frequency [Hz]	50917.28
Feedback settings	
Setpoint [V]	0.60
P-Gain [%]	8
I-Gain [%]	8
Image Scan (Multi Pass)	
Z Offset for Measurement [nm]	30
Look Ahead (X-Axis) [%]	0.0
Image Scan	
Points per Line	256
Lines per Image	256
Width [µm]	30
Height [µm]	30
Time / Line (Trace) [s]	2.56
Min. Time for Retrace [s]	0.5

Interpretation of Values

The V_{DC} values measured during the KPFM measurement cannot be equaled to V_{CPD}, because they are offset affected (due to the phase shift) and inverted. It is also important to note that these values are always a weighted average, and all surface elements of the tip and the sample affect them. The main idea of the measurement is to see contrasts between materials or areas with different work function. To measure quantitively a system which is capable to work in ultra-high vacuum is needed.

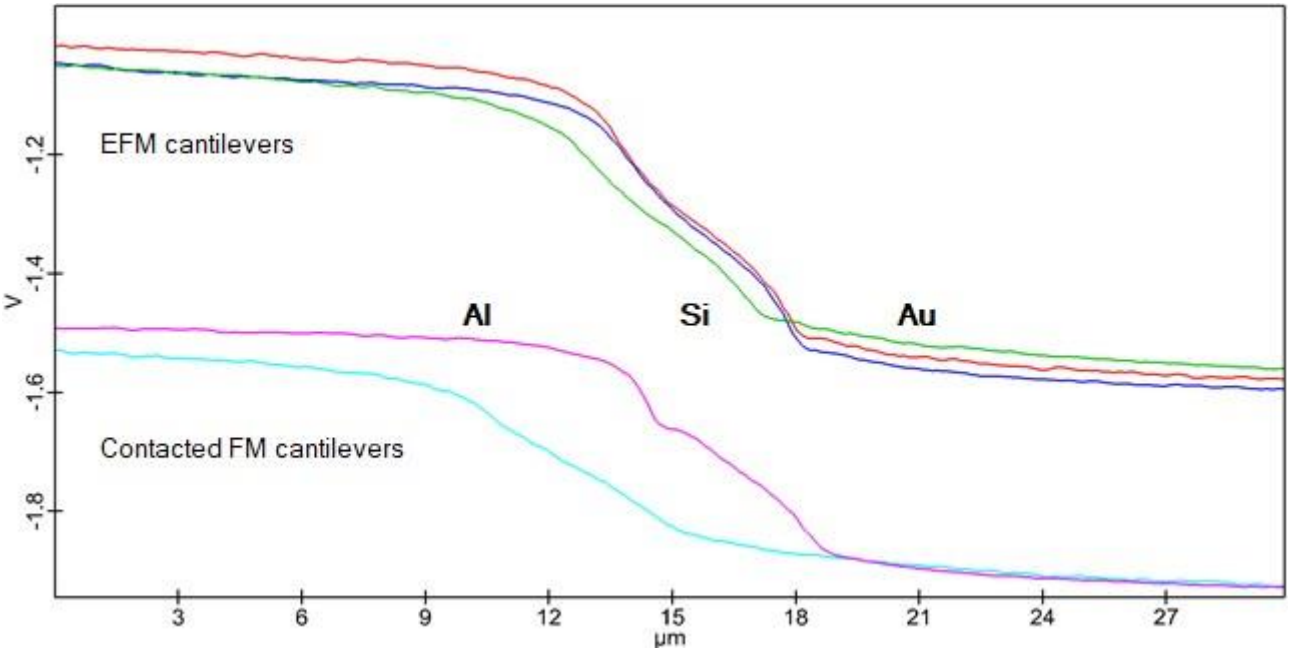


Figure 1: Typical results for 5 different cantilevers on the Bruker reference sample showing the cross section over the edge from Al over Si to Au

To get a more useful value the following equation can be used by determine V_{offset} against a reference sample with the used cantilever.

$$V_{CPD} = -V_{DC} + V_{offset}$$

Nevertheless, if values for the work function should be calculated a calibration on a reference sample is necessary before a sample can be measured. This has to be done for every time the cantilever is exchanged. Values for the work function are needed from literature for the reference material. In order do to do a linear calibration at least two different materials are needed. To calculate values of the work function from the measured V_{DC} the following equation is needed:

$$\frac{W_s}{e} = sV_{DC} + V_{offset}$$

The values of s and V_{offset} are calculated from the reference values and the measured values on this material:

$$s = \frac{\frac{W_{Au}}{e} - \frac{W_{Al}}{e}}{V_{DC,Au} - V_{DC,Al}}$$

$$V_{offset} = \frac{\frac{W_{Al}}{e} V_{DC,Au} - \frac{W_{Au}}{e} V_{DC,Al}}{V_{DC,Au} - V_{DC,Al}}$$

$$\frac{W_{Al}}{e} \approx 4.2V; \frac{W_{Au}}{e} \approx 5.3V$$

Using the Calculator Drop Action it is possible to calculate values of the work function for each point of a KPFM image or cross section (Figure 2).

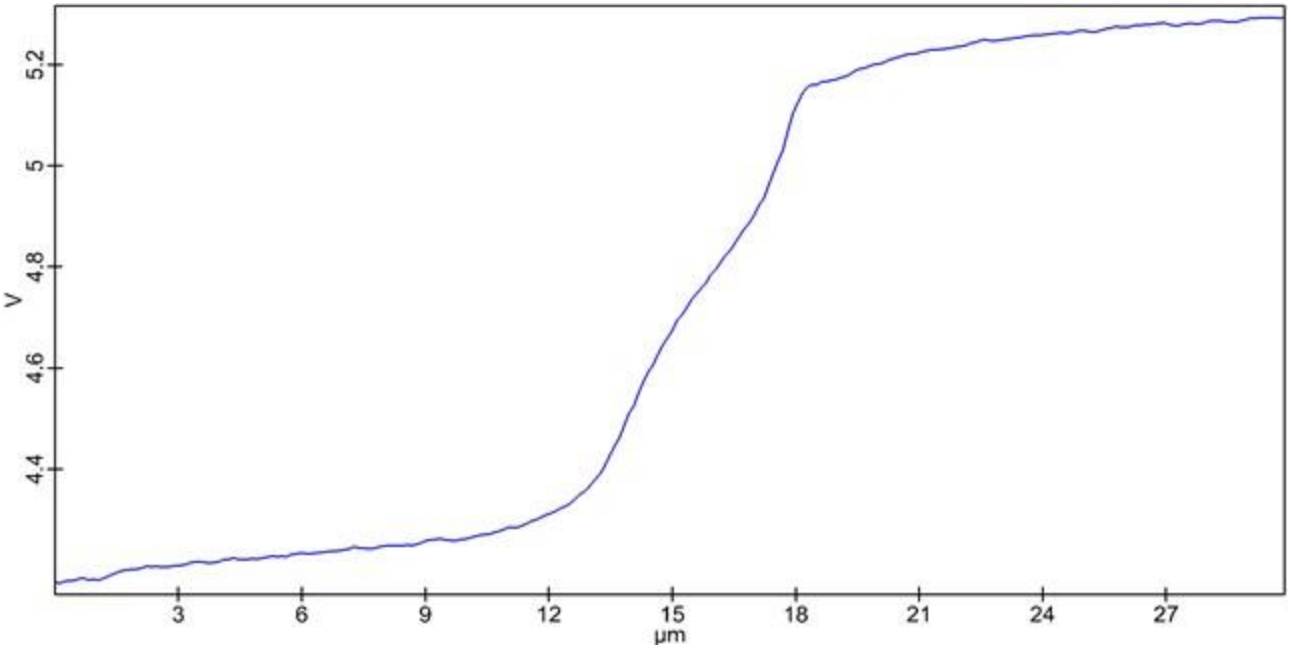


Figure 2: Corrected cross section over the edge from Al over Si to Au with an EFM cantilever

Figure 3: Corrected KPFM image over the edge from Al over Si to Au with an EFM cantilever

The measured values are strongly dependent on the resolution of the method. As already mentioned, the resolution of KPFM-AM is limited. This means that measured values for small spots are always averaged with the surrounding material. Furthermore, the cantilever changes during the measurement, which will also affect the calibration. To get more reliable results, the measurement should be repeated with another cantilever.

Piezoresponse Force Microscopy

PRFM Overview

Piezoresponse force microscopy (PRFM) is an AFM mode to image domains of piezoelectric or rather ferroelectric materials. In order to induce vibrations to the material an AC current is applied to the sample by a conductive AFM tip. The resulting very weak response of the material is than demodulated by a lock-in amplifier. By analyzing either the T-B or the L-R signal, vertical or lateral movements of the material can be detected. With this technique it is possible to image the topography and piezoelectric or ferroelectric domains at the same time.

Topics:

- Connecting devices
- Sample mounting
- Setting up a measurement
- Example measurement

Measurement modes:

- Image Scan: acquisition of PRFM images.

System requirements:

- alphaControl with a serial number 120-1050-XXX (Marvin 4b) or higher
- EFM package with EFM arm and clamps
- Piezoresponse components

Recommended cantilevers:

The cantilever needs to be electrically connected to the mounting ring. Therefore, a silver conductive paint is used. A metal coated cantilever is recommended.

Connecting devices

Transformer

- The transformer increases the voltage by a factor of 4.5 (i.e. from 20 Vpp to 90 Vpp). The EFM clamp is not affected, if connected correctly.

1. Connect the transformer BIAS-out with BIAS-in using the angle piece.
2. Connect the T-piece from AUX1-out and the EFM-clamps with AUX-in of the transformer.

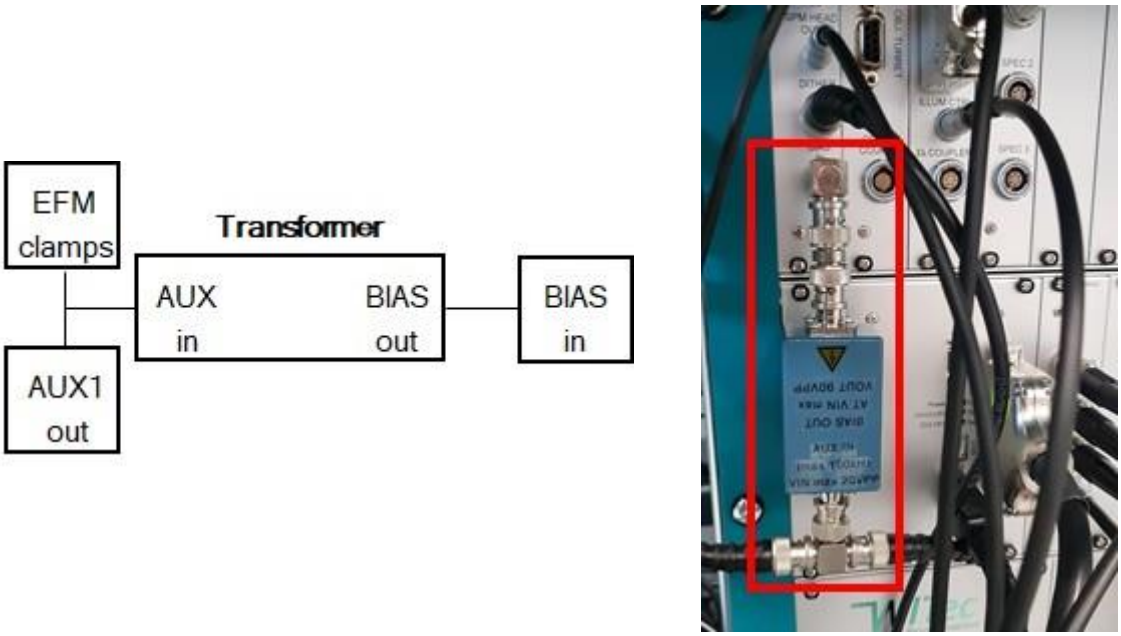


Figure 1: Wiring diagram (left) and picture (right)

Make sure that the transformer is only connected to the BIAS in. Electronic boards or even the whole electronics could be destroyed!

Be careful with the higher voltage at the cantilever!

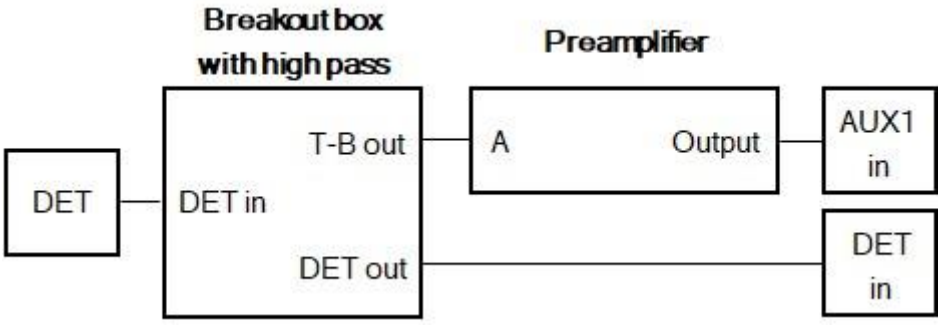
Remove the transformer before using EFM or KPFM mode.

Preamplifier

- The high pass filter removes the DC-offset from the T-B signal.
- The preamplifier amplifies the weak signal. If the amplification is too low, digitalization steps become visible in the signal.

alphaControl with a serial number 120-1060-XXX (Marvin 5) and above:

1. Connect the DET cable with DET-in of the breakout box.
2. Connect DET-out of the breakout box with DET-in of the controller.
3. Connect T-B-out of the breakout box with A of the preamplifier.



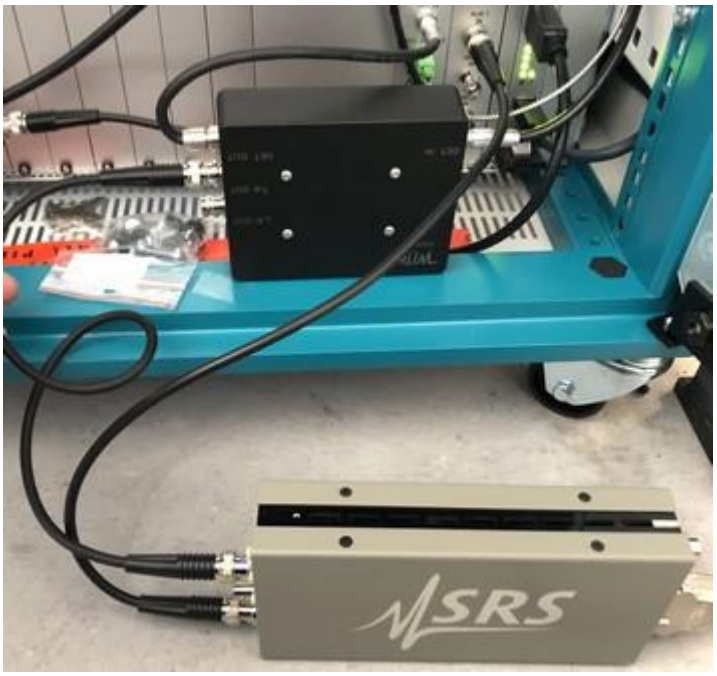
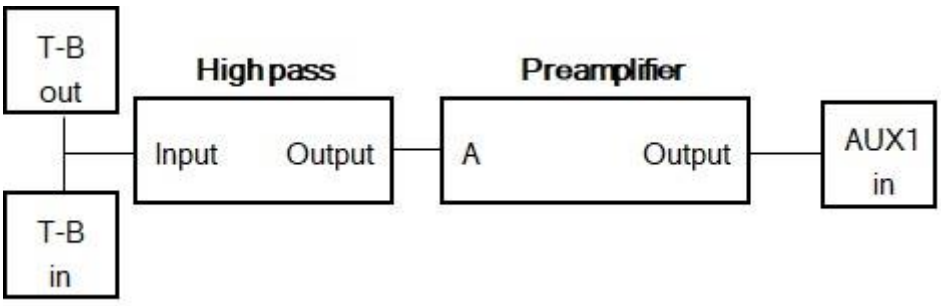


Figure 2: Wiring diagram (top) and installed components (bottom)

alphaControl with a serial number 120-1050-XXX (Marvin 4b):

1. Connect the T-Piece with T-B-in and with the high pass filter (Figure 3).
2. Connect the high pass module with A of the preamplifier.



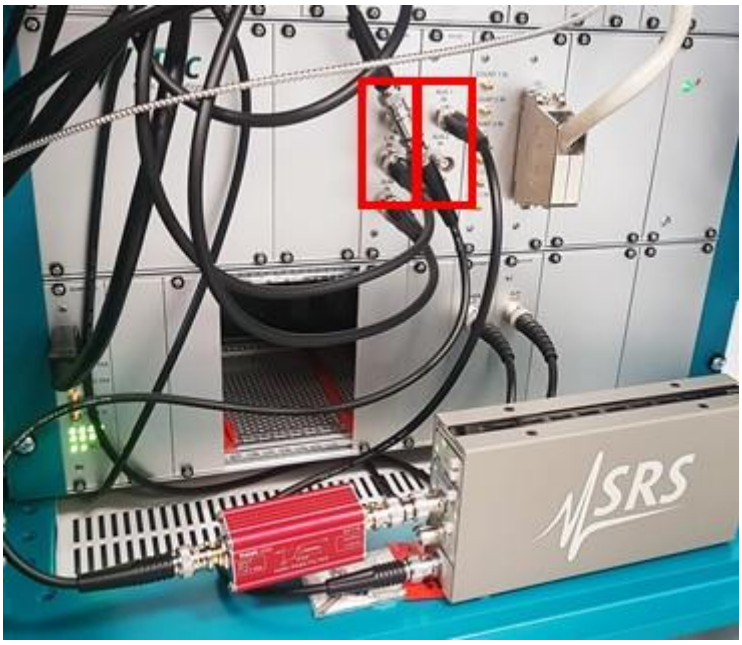


Figure 3: Wiring diagram (top) and installed components (bottom)

Both versions:

1. Connect Output of the preamplifier with AUX1-in. (Figure 2 and 3)
2. Connect the preamplifier with its power supply.
3. Switch the preamplifier gain to 100x and the Input to A and AC (Figure 4).

Figure 4: Preamplifier settings

Sample Mounting

- The sample backside must be connected to ground.
- Ground the sample with the black EFM-clamp over the sample clamp. Make sure the red clamp is not connected with something and has no contact to ground.
- Mount a contacted cantilever with the EFM cantilever arm.

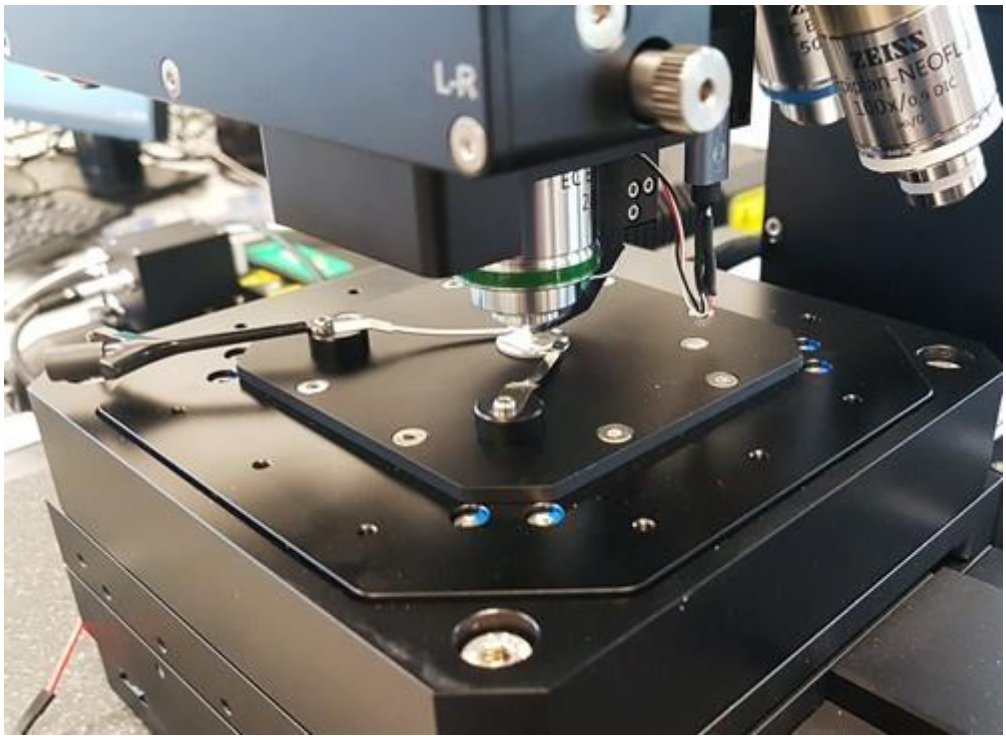


Figure 1: Reference sample mounted on the microscope

Parameters

Follow the steps in the contact mode procedure.

- PRFM is a contact mode configuration, using an AC voltage on the tip for electrical stimulation of the piezoactive material and a lock-in for analyzing the signal.
- For the Piezoresponse configuration the low pass filter of the T-B signal is deactivated.
- Refer to Piezoresponse Control
- Filter frequency is a parameter of the lock-in
    - High values result in more noise and a quick signal response.
    - Low values result in a smooth signal, but a greater time delay.
    - This creates a shift between forward and backward signal and softer edges.
- Low I-Gain is recommended.
- The driving frequency has virtually no influence in the result.

Reference sample

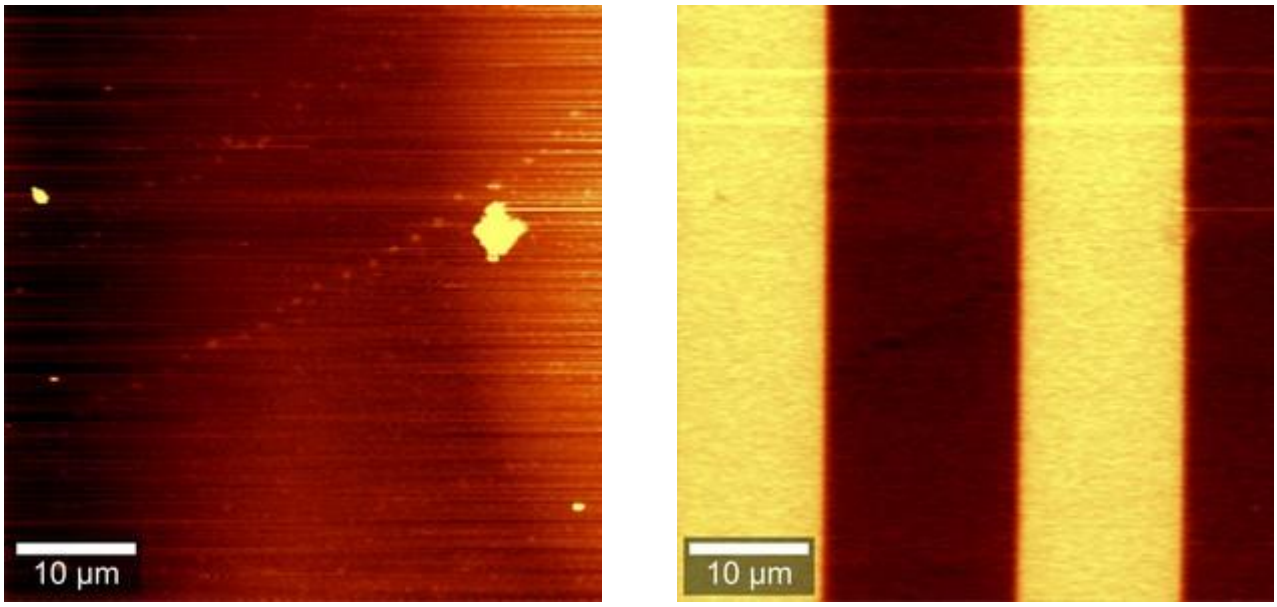


Figure 1: Topography (left) and Phase (right) image of the reference sample

A recommended reference sample to test the appropriate PRFM settings is the PFM-SMPL of Bruker, which consists of periodically poled $LiNbO_3$. The piezoactive structures are present in the center of the sample. Do not measure parallel to the structure. Try to change the scan gamma angle to achieve this.

Table 7: Exemplary parameters for the above measurement

Parameter	**Value**
Size [µm]	50 x 50
Pixel	256 x 256
Time per Line [s]	3
Setpoint [V]	0.5
Driving Amplitude [V]	20
Driving Frequency [Hz]	35000
Filter Frequency [Hz]	10

Raman AFM

Raman AFM Overview

This imaging mode enables simultaneous Raman and AFM measurements in contact or AC mode.

Topics:

- Setting up a measurement

Configurations:

The following configurations are available for all CCD cameras:

- Raman AFM Contact CCDX
- Raman AFM AC CCDX

Measurement modes:

- Image Scan: acquisition of Raman and AFM contact or AC mode images

System Requirements:

- Raman (RA and RAS systems)

Advanced experiments:

Since this configuration just combines the AFM with the spectroscopy capabilities more experiments are possible besides just doing Raman-AFM.

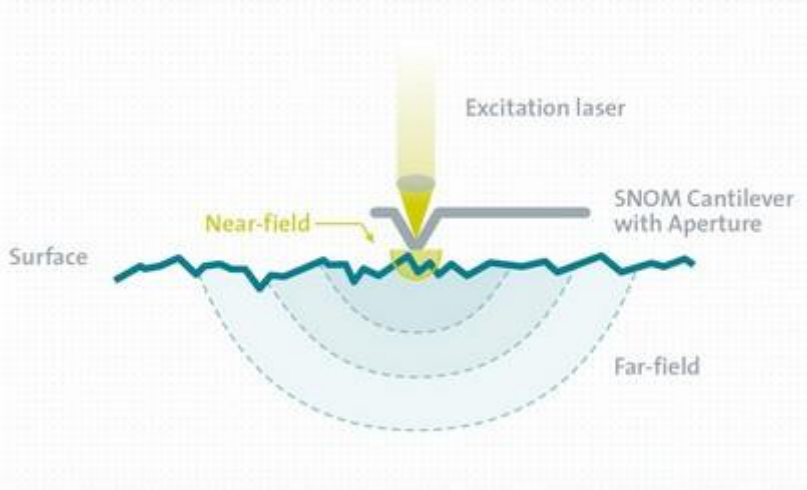
- AFM-PL
- Tip-enhanced Raman spectroscopy (TERS)
- SNOM-PL
- SNOM-Raman (in most cases signal intensity will be too low)

Parameters

Follow the steps in the general procedure. Further information can be found in the Contact mode or AC mode section depending on the used configuration. For information about the spectroscopy part refer to Raman.

SNOM

SNOM Overview



Scanning Near-field Optical Microscopy (SNOM) is a technique to achieve lateral resolution below the defraction limit using an aperture and a point detektor.

Topics:

- Theoretical Background
- Setting up a measurement
- Setting up a measurement in Pick-Up mode
- Setting up a measurement in reflection mode

SNOM modes:

- Contact Mode – standard SNOM mode
- AC Mode – for soft samples, material contrast by phase image
- DPFM – distance depended SNOM using an analog PMT
- SNOM-PL or SNOM-Raman – for combination of SNOM with spectroscopy refer to Raman-AFM

Configurations:

The following configurations are available depending on the equipped photon counting device:

- SNOM Contact PMT/APD
- SNOM AC PMT/APD

Measurement modes:

- Oscilloscope: displays the output channel of a photon counting device as a function of time similar to the display of an oscilloscope.
- Image Scan: acquisition of SNOM images
- Line Scan: acquisition of distance curves along a line
- Distance Curve: acquisition of distance curves at the current position

Recommended Cantilevers:

SNOM cantilevers feature a hollow pyramid as the aperture for SNOM measurements and can be purchased from WITec with apertures of < 150, < 90 and < 60 nm.

System requirements:

- SNOM (S, AS and RAS systems)

Theory

The resolution in classical (as well as confocal) microscopy is limited by the wavelength of the excitation light. This was investigated in detail by ABBE around 1890. According to him, at least the first diffracted order of an object (e.g. a grating) has to be captured by the lens system to resolve the object in image space. This is the reason for the importance of the numerical aperture for the resolution of an optical system.

In a perfect lens system with circular aperture, the image of a point object will be an Airy-pattern. On integrating the irradiance, one finds that 84 % of the light arrives within the central spot and 91 % within the bounds of the second dark ring.

If one brings two point objects close together, so that the maximum of the first Airy pattern is at the first minimum of the second, we are still able to resolve the two spots. This is the Rayleigh limit and the resolution defined by this (arbitrary) criterion is:

$$\Delta x = 0.61 \cdot \frac{\lambda}{NA}$$

where λ is the wavelength of the light and $NA = n \cdot \sin \alpha$, the numerical aperture of the lens system, while n is the index of refraction of the surrounding medium.

The maximum NA for commercially available objectives is about 0.95 when working in air and about 1.4 for oil immersion objectives (sample immersed in oil).

From this formula, one can see that the maximum resolution for conventional microscopy is

- $2/3 \cdot \lambda$ in air and
- $1/2 \cdot \lambda$ using immersion oil.

Using confocal microscopy this resolution can be further improved to

- $1/2 \cdot \lambda$ in air and
- $1/3 \cdot \lambda$ using immersion oil.

These values are only valid for optimum conditions, e.g. thin samples.

The only way to overcome the diffraction limit is to observe very close to the sample in the near-field regime (observer–sample distance $\lambda/5$).

The idea was first proposed by SYNGE in 1928. He suggested using a metal plate with holes much smaller than the wavelength of light, to illuminate this with light from the back side and then scan this plate in close proximity across a sample. If the plate-sample distance is much smaller than the diameter of the holes, the resolution is limited by the diameter of the light source (holes) and not by the wavelength of light.

It was not possible at that time to prove this idea and it took until 1972 before ASH and NICOLLS could verify the theory with electromagnetic waves in the microwave range (3 cm wavelength, 0.5 mm resolution $\Rightarrow \lambda/60$). The first results in the optical regime where obtained by POHL and BETZIG (1986), who used pulled optical fibers and shear-force feedback for distance control.

The WITec SNOM solution goes a step further and uses microstructured cantilever sensors and beam

deflection feedback (well known from AFM) for distance control. To take advantage of the high resolution obtainable with SNOM, one should consider the following points:

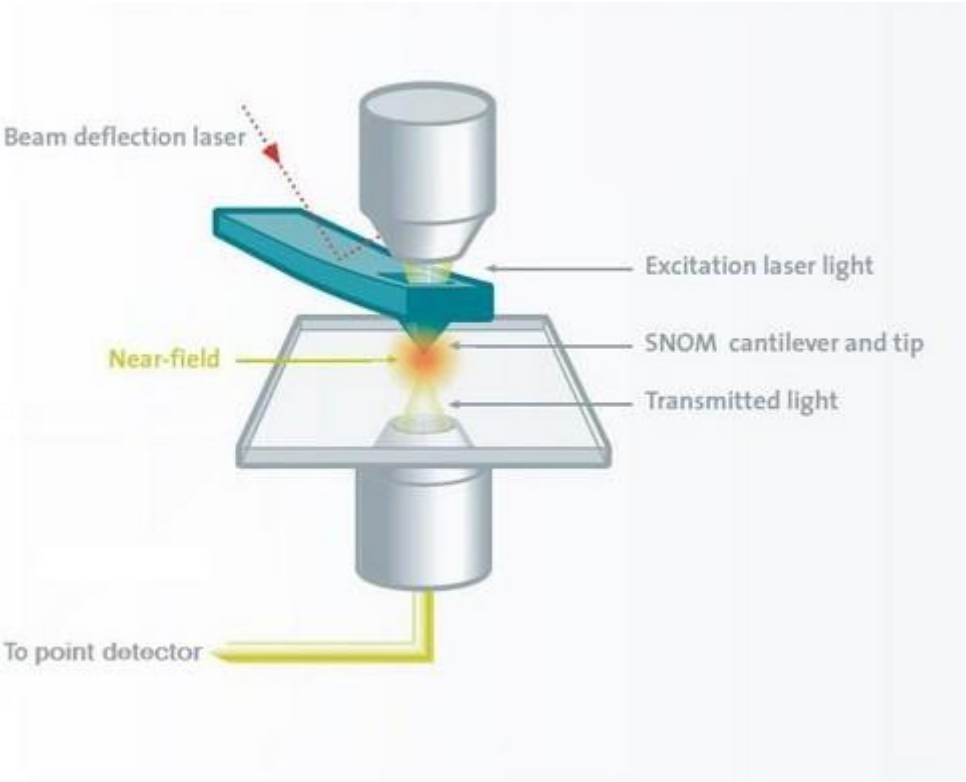
The maximum resolution is given by the aperture of the sensor, but this resolution is only possible in the near-field. Therefore, the distance between tip and sample should be less than the radius of the aperture (< 50 nm for a 100 nm aperture), otherwise the resolution decreases. This resolution can only be obtained at the surface of a sample (one can not look inside the sample as in confocal microscopy).

As a result of the small distance between tip and sample there is always an interaction between topographic features and light emitted by the aperture, which gives rise to artifacts. The user should always be aware that these artifacts might be present. On the other hand, the importance of these artifacts should not be exaggerated.

A careful comparison of the optical image with the simultaneously obtained topographic image can help to identify artifacts. If one finds an optical feature at exactly the same position as a topographic step, one should be skeptical. Particularly if the change in optical contrast is only a few percent of the overall intensity.

If there is a topographic step, the distance between tip and sample changes as does the coupling of the electrical field (near-field) between them, which may cause a change in detected intensity.

Usually, the optical aperture is slightly shifted (50–150 nm) from the point of contact. This is due to the fact that the mechanical contact is somewhere at the rim of the optical aperture. The hollow aperture is surrounded by a 100–150 nm thick layer of aluminum providing the mechanical contact. Therefore, a genuine optical contrast should show a shift between the optical and topographic images of 50–150 nm.



Working principle of the WITec SNOM solution in transmission

The preferable mode for near-field optical microscopy is transmission. In this mode, the light transmitted through the transparent (or fluorescing/luminescing) sample is collected by the detector and topographic artifacts are usually a minor problem.

In reflection mode, the light transmitted through the aperture and reflected from the sample surface

is collected with auxiliary optics. In this mode, topographyinduced artifacts are most likely and can even dominate the image. The reflection mode is most often used when the sample does not transmit the excitation light. In this case, the light transmitted through the aperture must cross the extremely small gap between tip and sample. If the tip is scanned across a topographic step, it is very likely that the detected light intensity would change and give rise to topography-induced artifacts. Here again, a careful comparison between topography and optical images is very helpful in distinguishing actual sample features from artifacts.

The following near-field optical modes are possible with the WITec system:

- near-field transmission
- near-field fluorescence/luminescence transmission (using additional filters)
- near-field reflection (with the reflection mode upgrade)
- near-field reflection fluorescence/luminescence (with the reflection mode upgrade and additional filters)

Setting up a measurement

This section describes how to start a SNOM measurement in transmission.

Sample mounting and focusing

1. Select the appropriate SNOM configuration.
2. Make sure the magnetically fixed cantilever arm is removed.
3. Focus on the sample using the AFM objective and search for the region of interest.
4. Optional: Set the Microscope-Z user position to zero for your reference.
5. Focus on the sample using the inverted objective including the steps marked with "For transmission".

Coarse alignment

6. Follow the steps in the general procedure for AFM of the sections Cantilever mounting and Adjustment. Ignore the second hint in article 9.a. in the general procedure and regard to the following points:
    - Position the cantilever like shown in Fig. 1 so that the laser is focused into the aperture. Open the laser shutter for that and activate the Laser Shutter Lock (for TruePower).
    - For contact mode: 1 V is a good starting value for the setpoint.

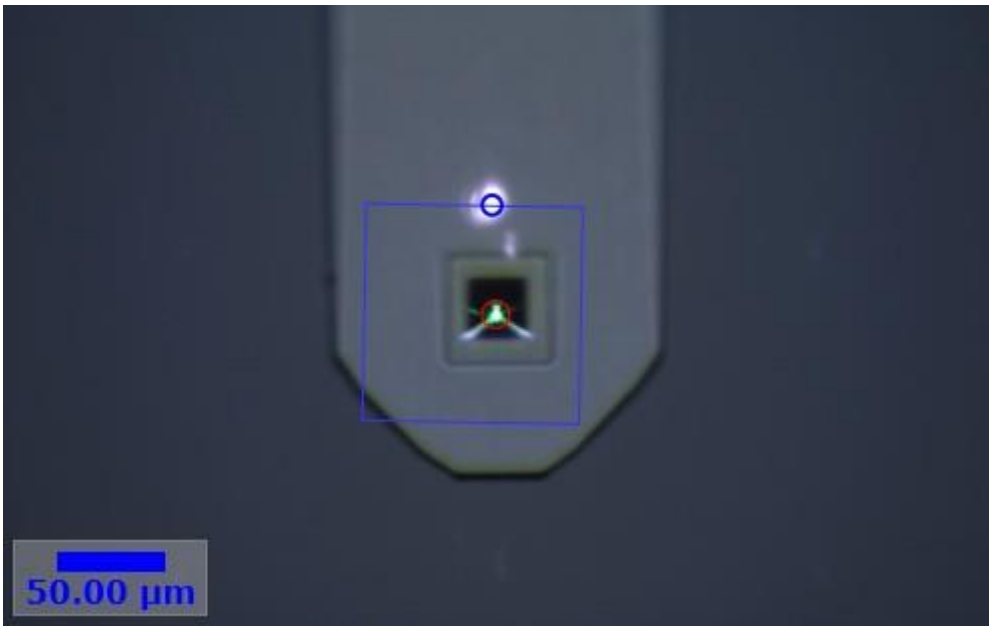


Fig. 1: SNOM cantilever with laser focused into the aperture (top view)

Fine alignment

7. Check that the beam spot is centered on the four quadrant diode.
8. Click on Start Approach.
9. Optional: As soon as the tip is in contact with the surface (T-B signal reaches the setpoint and the scan table starts to retract) the approach can be aborted by clicking Stop.
10. After double-checking that the Retract Distance [µm] is set to 50 µm, press Retract Tip.
11. Select the bottom camera increase the top illumination and/or use the bottom illumination, if present.
12. Move up the inverted objective until the tip of the pyramid is in focus (about 50 µm). (Refer to the hints section.)

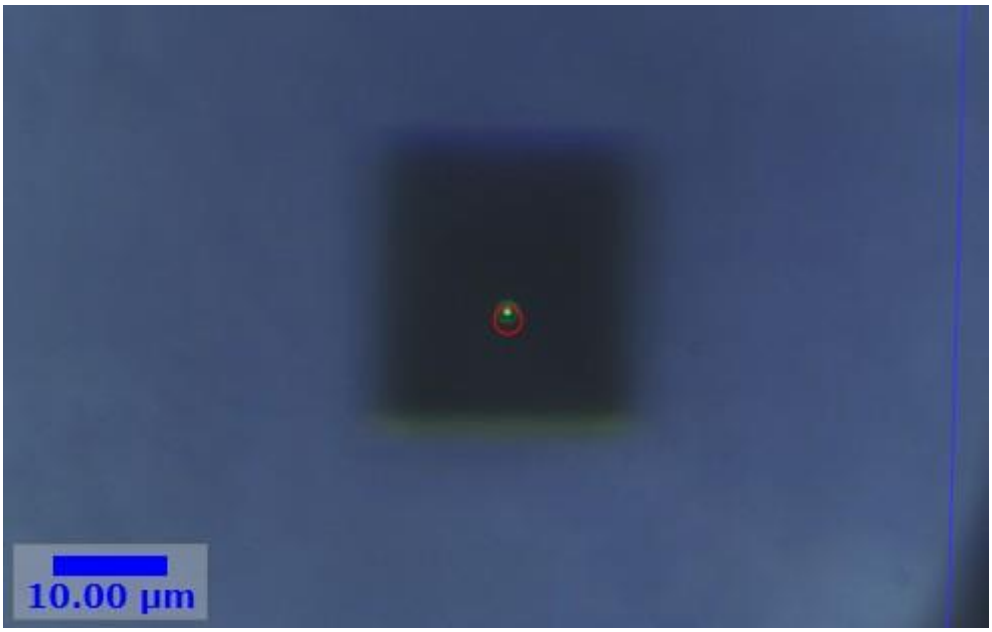


Fig. 2: SNOM cantilever with transmitted light (bottom view)

13. Switch to Raman Mode. For non-automated systems: Configure the upright beampath for Raman.
14. Increase the brightness of the video image and if necessary the laser power until you see some laser light. (Refer to the hints section.)
15. If there is no light spot visible at the aperture like in Fig. 2, slightly change the cantilever position in the x-y direction until you see it.
16. Focus the inverted objective on this spot.
17. Fine-adjust the cantilever position in order to maximize the throughput through the cantilever.

Approach and measurement

18. Check that the beam spot is centered on the four quadrant diode.
19. Activate the checkbox Track Z Difference in the inverted objective section.
20. Click on Start Approach.
21. Click on Move Z Difference in the inverted objective section to focus on the tip again. (If necessary improve the focus manually.)
22. Move the inverted objective in the x-y direction until the laser spot is in the green circle.
23. Reduce the laser power until it is barely visible.
24. If necessary: Set up the inverted beampath for measurement.
25. Click on Start Oscilloscope. (Refer to the hints section.)
26. Maximize the signal by moving the inverted objective in x, y and z direction. (Refer to the hints section.)
27. Adjust the laser power for an appropriate signal intensity.
28. Stop the Oscilloscope.
29. Start the measurement.

Further information:

Feedback settings, Detection, AFM Contact mode, Data channels (Topography, Count Rate)

Hints

Always double-check that you selected the appropriate device before you move anything. In case of the cantilever position or the inverted objective the corresponding window needs to be open.

- **For 12.:** If you are not able to see the tip of the pyramid, focus on the edge of the cantilever and then move the inverted microscope 8–10 µm down.
- **For 14.:** The new user might have difficulties in positioning the cantilever in order to obtain light through the cantilever. The following steps can be performed if no light can be obtained through the cantilever:
    - The laser intensity can be increased. This is only possible up to a certain point since the scattered light will start to dominate the image if the laser intensity is too high. Note also, that by increasing the laser power the power dissipated into the cantilever increases. Since it acts similar to a bi-metal strip this will change the bending of the cantilever. Again move the cantilever position in X and Y in order to find the transmitted light.
    - Follow the hint for 12. This ensures the proper focusing onto the tip of the pyramid. Again move the cantilever position in X and Y in order to find the transmitted light.

- Move the cantilever to the side in order to allow the laser beam to hit the inverted objective directly. You most likely will have to decrease the laser intensity in order to locate the exact position of the laser spot. Then verify that this position is where the red circle on the video screen is. Adjust the position of the inverted microscope in X and Y if necessary. Once this is optimized, move the cantilever back and move the cantilever in X and Y in order to find the transmitted light.

- **For 25.:** Make sure the detector is not overloaded. Refer to Detection.
- **For 26.:** If the maximum signal is found at a light spot position outside the green circle, maybe a pinhole adjustment is necessary.

Pick-Up mode

System requirements:

- Additional filter for blocking the beam deflection laser before detection

Setting up Pick-Up mode

1. Configure the beampath for excitation from below and detection from above. Switch to laser from below in the inverted objective control.
2. Follow the instructions for the standard measurement for sample mounting and focusing and regard to the following points:
   - For focusing the laser on the sample use the Confocal configuration and adjust the piezo stage z-position to 0. (This ensures that the laser is in focus after tip approach.)
   - Change back to the appropriate SNOM configuration afterwards.

Coarse alignment

3. Follow the steps in the general procedure for AFM of the sections Cantilever mounting and Adjustment. Ignore the second hint in article 9.a. in the general procedure and regard to the following points:
   - Position the cantilever like shown in Fig. 1. The aperture needs to be within the green circle and it needs to be focused inside.
   - For contact mode: 1 V is a good starting value for the setpoint.

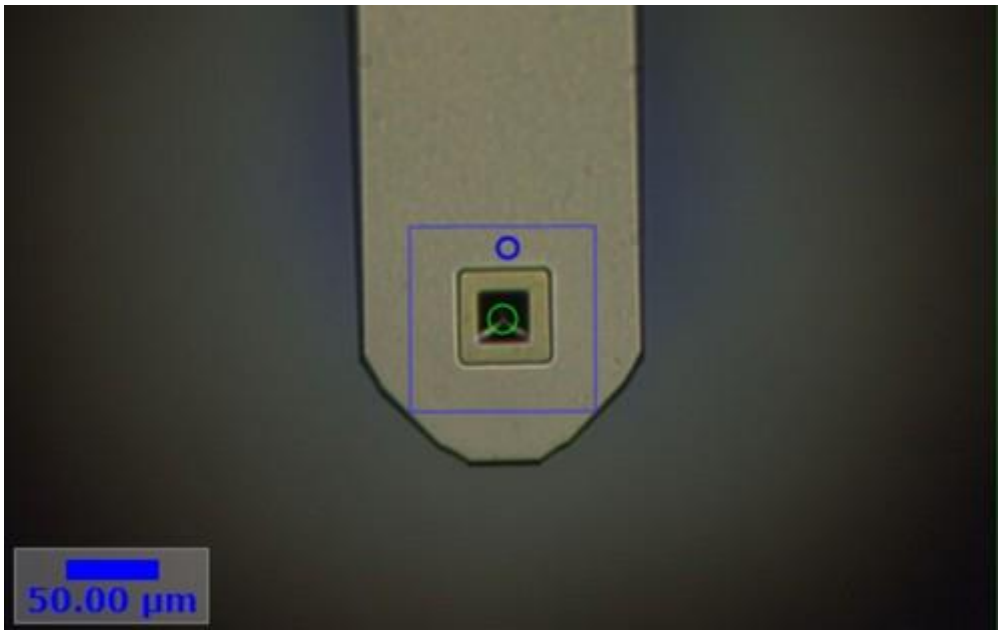


Fig. 1: SNOM cantilever focused into the aperture (top view)

Fine alignment

4. Check that the beam spot is centered on the four quadrant diode.
5. Click on Start Approach.
6. After successful approach adjust the setpoint to -10 V. (This retracts the piezo stage and the cantilever is about 10 µm above the surface.)
7. Switch off the illumination. For non-automated systems: Put the withelight slider to the empty or darkfield position.
8. Open the Laser Shutter.
9. Increase the brightness of the video image and, if necessary, the laser power until you see some laser light.
10. Slightly change the cantilever position to maximize the intensity of the light spot like in Fig. 2. Start with the z-axis.

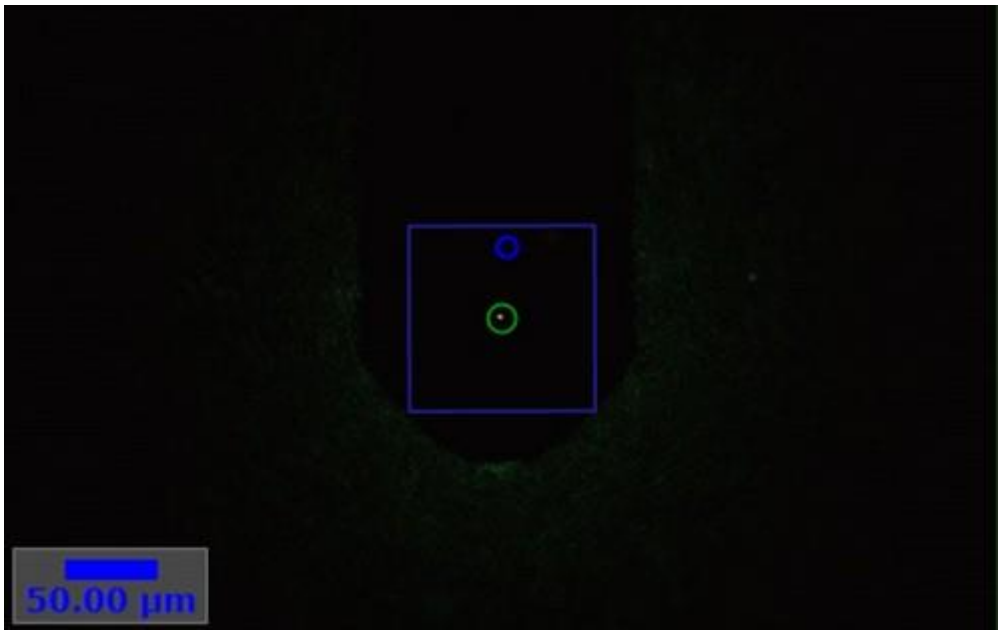


Fig. 2: SNOM cantilever with transmitted light (top view)

Measurement

11. Switch to Raman Mode. For non-automated systems: Remove also the top camera from the beampath.
12. Check that the beam spot is centered on the four quadrant diode.
13. Put the setpoint back to the initial value. (The piezo stage moves up until the cantilever is back in contact.)
14. Reduce the laser power until it is barely visible.
15. Click on Start Oscilloscope.
16. Adjust the laser power for an appropriate signal intensity.
17. Stop the Oscilloscope.
18. Start the measurement.

Reflection mode

System requirements:

- Reflection mode module

Setting up reflection mode

1. Check that the reflection microscope is connected with the photon counting device.
2. Follow the instructions for the transmission mode for sample mounting and focusing (skip 5.) and coarse alignment.
3. Select the Rear camera.
4. Move the reflection microscope using their positioning stage until you see the cantilever from the backside and try to focus on the tip using the forward/backward screw. (Fig. 1)

Fig. 1: SNOM cantilever in rear view with reflection on the sample surface.

Fine alignment

5. Switch to Raman Mode. For non-automated systems: Configure the upright beampath for Raman.
6. Place the stray light protection around the sample or close the front door of the enclosure to reduce ambient light on the sample.

7. Increase the brightness of the video image and if necessary the laser power until you see a small laser spot at the tip. (Refer to the hints section.)
8. Fine-adjust the cantilever position in order to maximize the throughput through the cantilever.

Approach and measurement

9. Check that the beam spot is centered on the four quadrant diode.
10. Click on Start Approach
11. Adjust the reflection microscope (using the left/right and up/down screw) until the light emitted from the cantilever is in the green circle of the video image. (If this is the case the light is hitting the fiber correctly.)

Do NOT alter the adjustment screws of the fiber coupling unit.

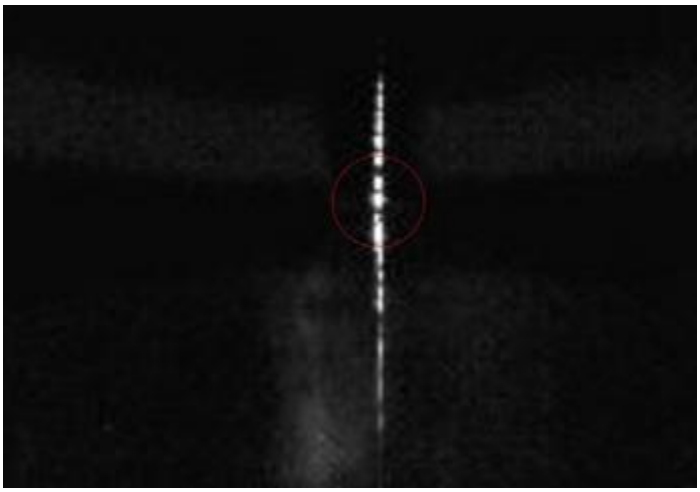


Fig 2: Light spot from the tip and reflections on cantilever and sample.

12. Click on Start Oscilloscope.
13. Optimize the signal by slightly moving the reflection microscope in all three direction. (Refer to the hints section.)

When the tip is in contact, try to avoid any vibrations of the microscope body.

14. Adjust the laser power for an appropriate signal intensity.
15. Stop the Oscilloscope.
16. Start the measurement.

Hints

- Don't be confused by reflections on the sample surface.
- Changing the laser power may also change the bending of the cantilever (acts similar to a bi-metal strip). This can cause the tip to loose the sample contact. If so, readjust the beam spot on the four quadrant diode while the tip is retracted.
- **For 7.:** If you are unable to observe the light from the cantilever aperture:
  - Try to repeat the coarse alignment.
  - Align the cantilever in transmission mode including the fine alignment (until 17.) using a 0.17 mm glass slide as a sample. Then move the microscope up using the microscope z control, replace the cover glass with your sample and move the microscope down again.
- **For 13.:** Reflections close to the tip can even have higher intensity then the near-field signal.
- **For 13.:** If the sample completely blocks the aperture, you will maybe observe no signal. Refer

to the next hint.

- **For 15.:** If you don't observe the structure of your sample carefully adjust the reflection microscope while doing an image scan until you observe the near-field signal.
- If you observe the near-field signal outside of the red circle, you can reposition the red circle (Probe position) in the Menu.

SNOM AC

SNOM AC Overview

SNOM AC mode measurements are especially useful for eliminating topography induced artifacts in reflection mode measurements. Additionally, it may be beneficial for samples that are either soft or weakly bound to the substrate. For these samples, the operation in the intermediate contact regime can be preferable because the AC mode reduces the effect of tip induced sample damage and the dragging of particles over the surface.

One should notice however, that the oscillation of the cantilever can have an influence on the contrast of the near field measurements. If a high oscillation amplitude of the cantilever is chosen, the tip will, on average, be further from the surface. Due to the fact that the contrast in SNOM measurements is strongly correlated with the tip–sample distance, it will decease with increasing amplitude.

System requirements:

- AFM (AS and RAS systems)

Recommended Cantilevers:

SNOM AC mode cantilevers are shorter, stiffer and feature a higher resonance frequency (≈ 45 kHz) compared to SNOM contact cantilevers and can be purchased from WITec.

Parameters

Follow the steps in the general procedure. For information about the AC mode adjustment process refer to AFM AC mode.

Setpoint

In comparison to AFM AC mode measurements, one should note that SNOM AC cantilevers are longer and significantly wider than AFM AC mode cantilevers. This results in a significantly stronger air damping of the oscillation amplitude than is the case for AFM AC mode cantilevers (see Fig. 1).

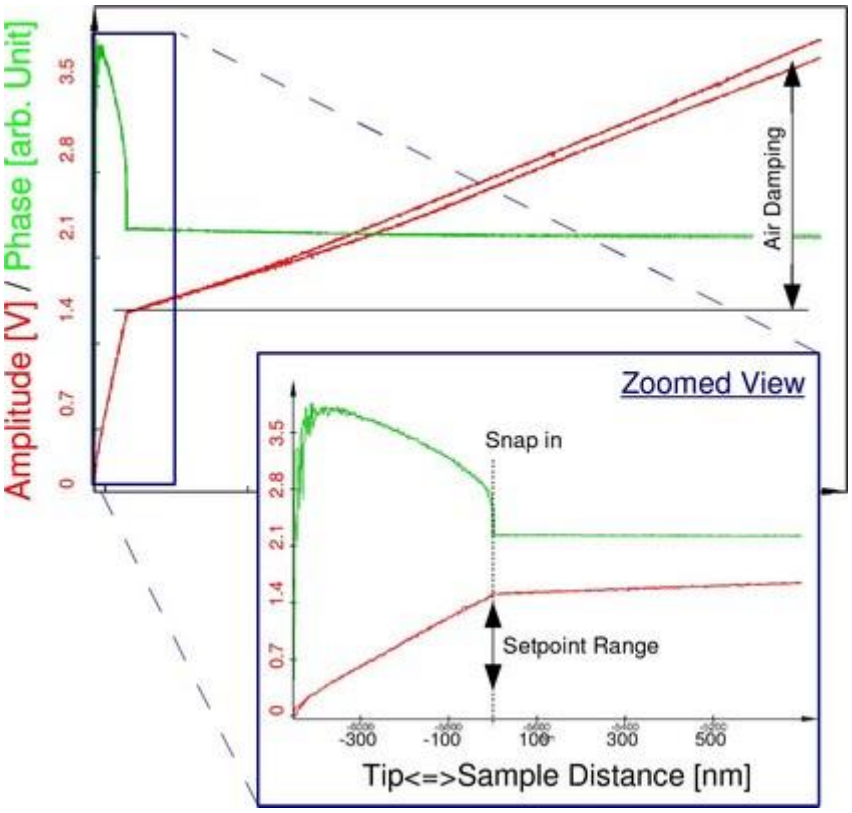


Fig. 1: Typical curves for phase (green) and amplitude (red) as a function of tip?sample distance. The usable setpoint range is indicated in the zoomed view.

The setpoint should be a significantly lower value (e.g. 1/4) than the free amplitude. This is necessary due to the heavy air damping which can be seen from the amplitude- and phase-distance curves in Fig. 1. The total tip–sample distance covered in the curve displayed in Fig. 1 exceeded 10 µm and the oscillation was still damped due to the air cushion between the tip and the sample. It is therefore important to move the tip far enough away from the sample surface (e.g. 100 µm) when determining the free oscillation amplitude.

The setpoint for SNOM AC measurements can be chosen in the range as indicated in the zoomed view of Fig. 1. (A higher setpoint results in a weaker sample–tip interaction.)

Special

Photocurrent

Photocurrent Overview

Photocurrent is the result of light exposure to a photosensitive device, like a photodiode.

Topics:

- Setting up a measurement
- Example measurement

Photcurrent modes:

Depending on the system configuration the following photocurrent modes are possible with a WITec system:

- Photocurrent
- Photocurrent with laser chopper and external lock-in
- Photocurrent combined with Raman or even SNOM

Configurations:

The following configurations are available depending on the used data channel:

- Photocurrent AUX1/EXT1

Measurement modes:

- Oscilloscope: displays the current output from the amplifier
- Image Scan: acquisition of photocurrent images using the piezo scanner
- Large Area Scan: acquisition of photocurrent images using the cross-table

System requirements:

- Raman or SNOM (M+, R, RA, RS, RAS or S system)
- Extension or AUX A/D card for the alphaControl
- Low Noise Current Amplifier (e.g. femto)
- Optional: AUX Out card for the alphaControl for bias voltage supply.
- Optional: laser chopper and external lock-in

Procedure

1. Make sure the current amplifier or external lock-in is connected to either EXT 1 or AUX 1, depending on your photocurrent configuration.
2. Select your photocurrent configuration.
3. Focus on your sample.
4. Adjust the laser to low laser power suitable for your sample.
5. For non-automated systems: Configure the beampath for Raman and open the laser shutter.
6. Start the oscilloscope and check the signal. Adjust the current amplifier amplification to get a signal between between 0.01 V and 1 V.
7. Start your measurement.

Further information:

Data channels (Aux 1, Ext 1)

Hints

- If the amplification is too low, digitalization steps become visible in the signal.
- Controlling the bias over the Aux out channel is accomplished over the EFM Control.

Data evaluation

The measured data is in V. In order to calculate the photocurrent in A, divide the data by the amplification factor of the current amplifier. E.g. the setting in fig. 1 shows an amplification of 10^4. Drop the measured data on the calculator tool. If the amplification is 10^4 type X1/10 behind the equal sign and mA as unit.

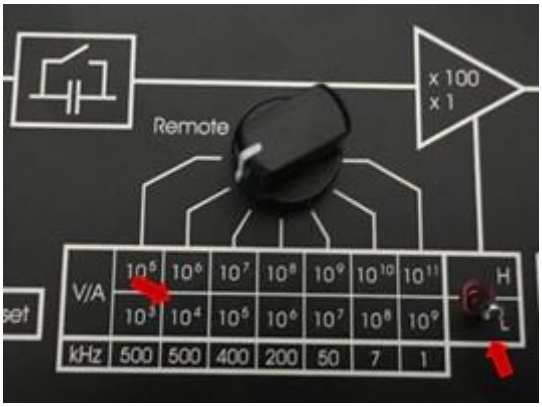


Fig. 1: Example of the amplification setting of the femto current amplifier.

Reference sample

Sample: WITec Photocurrent reference sample

Ensure that the following settings are correctly applied:

- Femto amplifier gain to 10^4 (small switch to "L" position)
- Other Switches: GND/Bias to GND, 10Hz/FBW to FBW, AC/DC to DC
- 532 nm laser with approx. 7 mW
- 20x objective

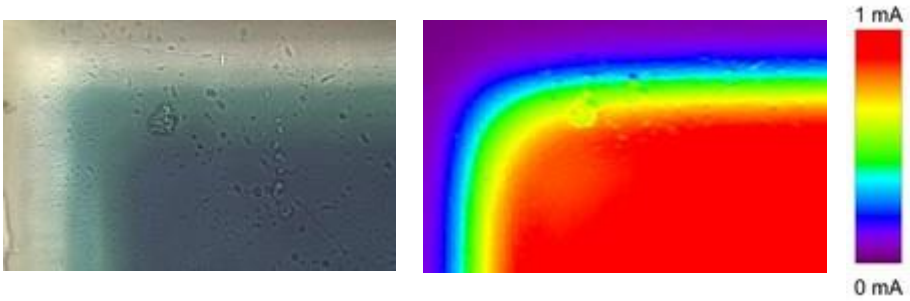
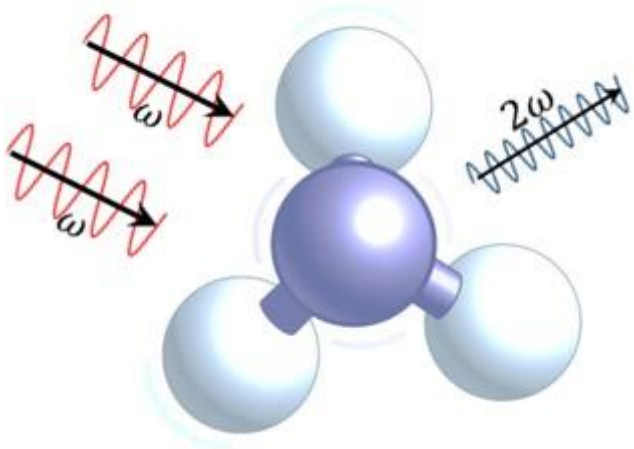


Fig. 1: Left: Video image, Right: current distribution

- The active zone (dark), a transition zone and the frame (bright) can clearly be identified in the video image.
- According to these zones, the measured photo current shows the expected behavior. 0 mA at the frame, up to approx. 1 mA in the center of the active zone. Fig. 1 shows an example of the upper left edge.
- Remark: The photo diode consists of a transparent coating with approx. 170 µm and the silicon layer beneath, focus plane is not that critical.

SHG

Second-harmonic generation (SHG) is a non-linear optical effect. The energy of two photons of the same energy are used to create one photon with twice the energy of the incidence photons.



Useable Configurations:

- Raman to use the spectrometer for detection
- Confocal to use a point detector for detection

Measurement modes:

- Oscilloscope: shows the current spectrum/value
- Image Scan: acquisition of SHG images using the piezo scanner
- Large Area Scan: acquisition of SHG images using the cross-table
- Series Slow: for Polarization experiments

System requirements:

- pulsed 1064 nm laser recommended for SHG
- spectrometer capable of detecting in the VIS range
- point detector in combination with an appropriate low-pass filter

Procedure:

1. Select the appropriate configuration for your detector.

2. Focus on your sample and use an objective with good chromatic correction.
3. Select the SHG laser.
4. For non-automated systems: Configure the beampath for the SHG laser.
5. For use with spectrometer: Select an appropriate spectral center (i.e. 532 nm for 1064 nm excitation).
6. Optimize your focus using the oscilloscope on your SHG sample.
7. Define and start your measurement.

Further information:

Raman, Confocal

Lithography

The lithography module can be used for sample manipulations on the nanometer and micrometer scale using an AFM tip or a laser.

Useable Configurations:

- AFM Contact and AFM AC for using an AFM tip or even a SNOM cantilever
- Confocal for using the laser

System requirements:

- AFM (A, RA or RAS system) for using an AFM tip
- Raman or SNOM (M+, R, RA, RS, RAS or S system) for using the laser

Required License feature:

- Lithography

Creating a script:

- List of commands
- Refer to the several example scripts in the "UserLithography" folder, which is the default folder for choosing a file.

Procedure:

1. Select one of the lithography configurations (see above).
2. Focus on the sample for laser lithography or follow the general procedure for AFM.
3. Choose the file in the Lithography section in the Control tree.
4. Optional: Click on Preview to check.
5. For laser lithography: Configure the beampath for Raman.
6. Click on Start Lithography.

Further information:

Lithography

Hints:

- Include DrawActive.txt and DrawInactive.txt in your script instead of hardcoding i.e. SetLaserShutter. This enables you to easily change the script from laser lithography to AFM or to change setpoint or retract values without changing it in every line.
- Refer to the DrawActive.txt file in the "include" subfolder of the "UserLithography" folder for further information about how to start and stop the writing.

Profilometer

Profilometer is a configuration to use the Confocal Chromatic Sensor of TrueSurface Mk1 or Mk2 just for recording the topography. It is also possible to use this configuration with TrueSurface Mk3.

Measurement modes:

- Oscilloscope: displays the current value
- Image Scan: acquisition of profilometer scans using the piezo scanner
- Large Area Scan: acquisition of profilometer scans using the cross-table

System requirements:

- TrueSurface Mk1 or Mk2

Procedure:

1. Focus between highest and lowest point of your sample.
2. Change to the TrueSurface objective.
3. For non-automated systems: Configure the beampath for TrueSurface.
4. Define and start the Large Area Scan or Image Scan.

WITec Control

Welcome to WITec Control

Welcome to the WITec Control Measurement Software Help.

Main Window	Load/Save projects, select configuration
Video Window	Live video image, illumination, objectives, microscope control, stitching
Control Window	Manage hardware and measurement parameters
Messages Window	Shows progress and warnings or asks for user inputs
Service Monitor	Configuration of CCD Cameras, CCD/Video Device Status
COM Automation	Programming interface for WITec Control

Press the **F1 key** anywhere in the software to open the context help or browse the Help Menu to open the help contents.

Main Window

Tool Buttons

For general Tool Buttons help see WITec Project Main Window

- Auto Save Project (See Program Options - Auto Save Project)

- Information Button (shows help for current configuration)
- Stop Measurement Button

- Measurement buttons (depending on Configuration)
  Left Click: Start Measurement.
  Right click: Open parameter sub tree in Control Window

Main Menu

For general Main Menu help see WITec Project Main Window

View

Point Viewer Window
Shows the Point Viewer to define automated measurements at multiple sample positions.

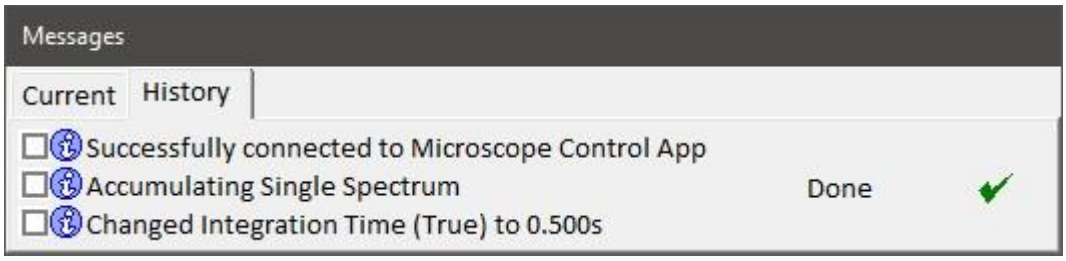
Options

Scantable Load Profiles
Lets you select one of the available Scantable Load Profiles. Load Profiles can be used to load e.g. scan table PI controller settings for heavy samples.

Messages Window

The message window is a message interface between the controller hardware and the user. In the "History" tab, there is a chronological classification of past operations.

The "Current" tab is automatically selected during a measurement and displays the current operation and the remaining time required for the operation.



The nature of the message can be identified by the symbol displayed in front of the message. The following types of messages are displayed:

Information

This class of messages contains only information about ongoing processes performed by the controller. The message description next to this symbol, informs the user about the actual process.

Warnings

If a controller process fails, a warning message is displayed.

User input for an operation

Some routines of the controller are automated. Nevertheless, they cannot be completed without user inputs. Messages which contain the user input symbol describe the action which should be accomplished by the user.

Hazard

Hazard messages indicate severe problems of the hardware. They contain information about communication errors between computer and controller or failures of the power supply.

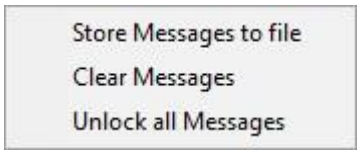
Move the mouse over a message to get a tooltip with more detailed information.

Checkboxes

With the checkboxes it's possible to move messages between the "Current" and the "History" tab.

Context menu

It can be opened by clicking the right mouse button anywhere in the Messages window.



Store Messages to file

Stores the messages to the file `C:\ProgramData\WITec\WITec Suite X.X\Log Files\WITec Control\UserFeedbackLog.txt`.

Clear Messages

Clears all unlocked messages of the upper part.

Unlock all Messages

Unlocks all messages of the lower and moves them to the upper part.

Video Window

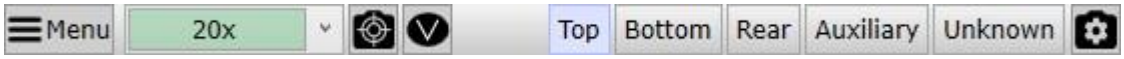
Video Control Overview

The WITec Video Control window is the main user interface for

- showing the video image
- controlling the illumination
- managing objectives
- positioning of devices
- laser control
- spectrometer selection
- controlling further devices ...

Move the mouse over a control in the running software and press F1 for context help!

Top Bar



See Menu

Objective Selection


Objective Video Calibration


Vignetting Correction

Video Camera Selection


Video Camera Settings

Video Image

The video live view shows the video image of the currently selected camera.

You can zoom in and out using the mouse wheel or by holding down the control key on the keyboard and dragging a rectangle.

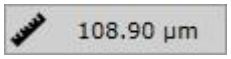
Right Bar

 See Illumination

Bottom Bar

 **Toggle Field Stop**

Toggles the field stop open/focus position. Right-Click to open Field Stop/Aperture Stop Settings.



Mouse Distance

Shows the current mouse distance. You can click the left mouse button to set a reference position.

Turn off listen

In the bottom bar you might see some mouse pointer followed by a label (e.g. Probe Position).

This means that you can click into the video image in order to set or move something e.g. the probe position.

If you click on this Button, the mouse mode will be cleared so no software component will listen to a position anymore.

 **Zoom Out**

The magnifier icon shows that you have zoomed into the video image. Click it to zoom out.

 **Video Image Acquisition**

Saves the current video image in the full camera resolution as a new bitmap into the current project.

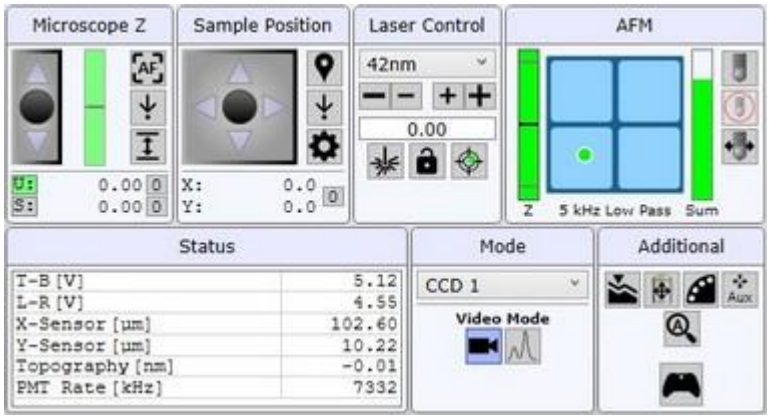
 **Video Measurement**

Opens the Video Measurement Window for doing image stitching and focus stacking.

 **Video Movie Recording**

Opens the Video Movie Recording Window to create movies.

Control Boxes



- Microscope Z
- Sample Position
- Laser Control
- AFM Status / TrueSurface Mk3 Control
- Status Values
- Microscope Modes
- Additional Microscope Controls

State Manager

See State Manager.

Menu

Illumination and Video Camera Settings

Opens detailed settings for the illumination/smart brightness and video camera settings.

Field Stop / Aperture Stop Settings

Opens detailed settings for the automated field stop / aperture stop - if available.

Set Probe Position
Lets you click somewhere in the video image to define the new probe position.
You can turn off this mode by pressing the button again or by pressing the label in the bottom right corner of the video image.

Set Probe Position to Video Center
Sets the current Probe Position to the Video Center.

Start Objective Video Calibration
Starts the Objective Video Calibration. Each objective needs its own calibration.

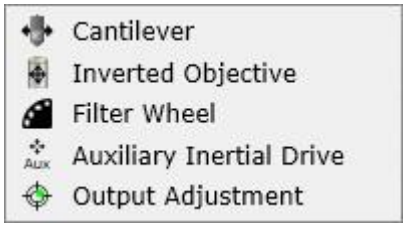
Objective Offset Compensation Wizard
Opens the Objective Compensation Wizard

Objective Turret Configuration
Opens the Objective Turret Configuration

Spectroscopy Settings
Opens the Spectroscopy Settings.

Portable Coordinate System
Opens the Portable Coordinate System Dialog.

Device Control



Cantilever
Shows the cantilever positioning user interface.

Inverted Objective
Shows the user interface for positioning the inverted objective.

Filter Wheel
Shows the user interface to change the filter wheel position (SNOM feature).

Auxiliary Inertial Drive
Shows the auxiliary inertial drive user interface. The name of this menu entry is dynamically set due to the configuration.

Output Adjustment
Shows the Laser Output Adjustment Window.

Advanced
Intended only for the WITec Support Team:

Spectrometer Calibration Lamp Permanently On
This will turn on the spectrometer calibration lamp, no matter which WITecControl configuration is selected.

Use Laser Filter for Raman
If checked, the laser filter is coupled during Raman measurements. Only works with automated filter couplers.

Change Laser Wavelength
Lets you change the exact laser wavelength of the currently selected laser.

Show Laser Power Correction Factor Button
If enabled, a little button next to the laser power edit is visible.
There it is possible to define a laser power correction factor which is automatically stored for each laser.

Show COM Parameter
Shows all remote controllable parameters.

Set/Remove Cantilever Laser Spot Marker
Sets or removes the cantilever laser spot marker. Available in AFM Mode.

Add custom logfile entry
Allows to add a custom logfile entry/comment in case of errors or bad software behavior. This way analyzing errors might be more easy.

EasyLink Controller Help
Shows the EasyLink Controller Online Help (Button Assignments).

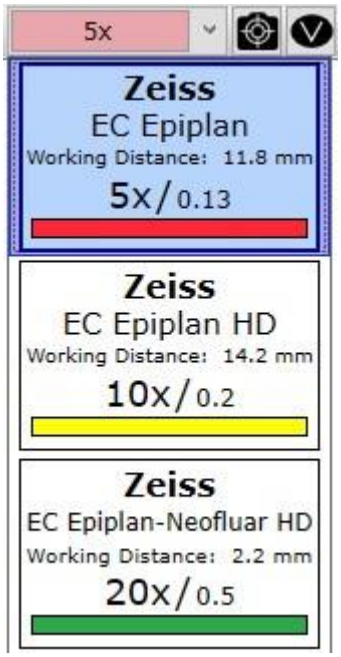
Microscope Control Help
Shows this WITec CHM Help.

About
Shows program information such as version number, license, memory consumption.

Objective Management

Objective Selection

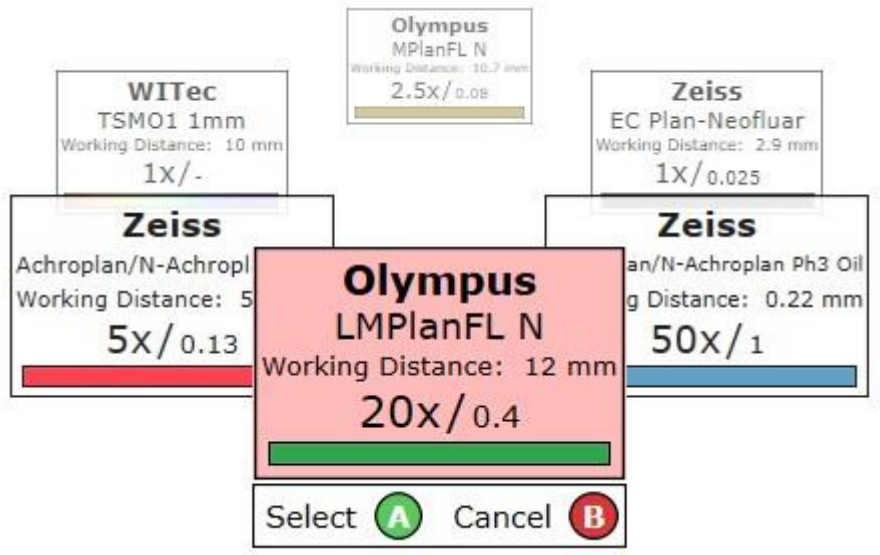
The selected objective is displayed in the top bar of the window and can be changed using the combo box:



Selecting another objective will open the Change Objective Dialog.

Right-click on the combo box will open a context menu containing the Objective Turret Configuration.

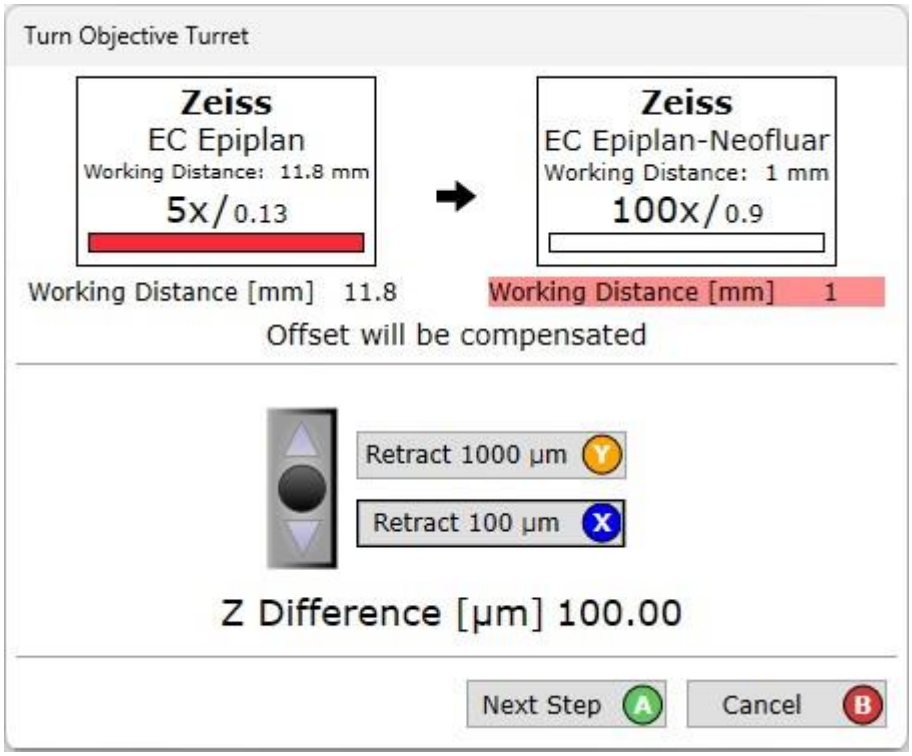
It is also possible to change the objective using the EasyLink Controller by pressing A:



Change Objective Dialog

If you select another objective, the change objective dialog will open.

This dialog is an assistance for safely changing the objective:



Step 1:

You can automatically retract the microscope from the sample by 100 µm or 1000 µm (or use it multiple times) to have enough space for a safe turret rotation. Of course, you can also move the Z stage manually using the UI Joystick Control or the EasyLink Controller.

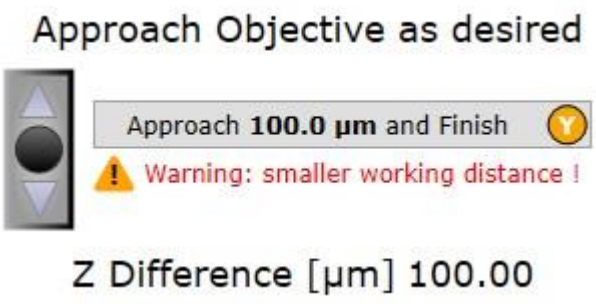
Use the X/Y/A/B Buttons on the EasyLink Controller to press the buttons on the user interface.

After moving up the microscope, you can press Next Step.

If you have a motorized objective turret, the turret will move automatically now; turn the manual objective turret if it is not motorized.

Step 2:

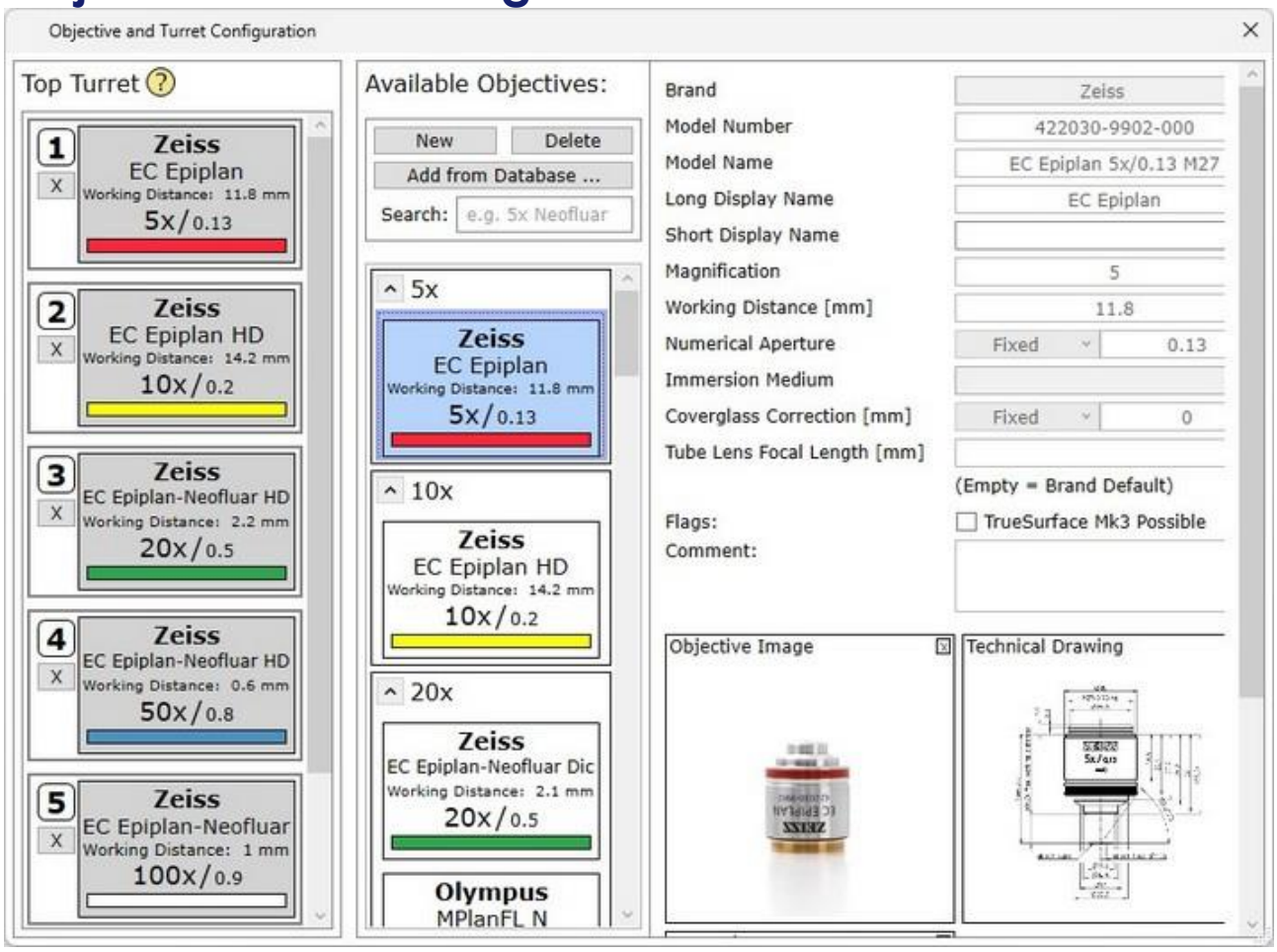
In the second step you can either manually approach the microscope and do the focus as desired or you can press Approach to automatically move the microscope to the same Z-position, where it was before you retracted the microscope:



If you change to an objective with a smaller working distance than the current objective, you will get a warning.

Always make sure that there is enough space around the objective!

Objective Turret Configuration



Available Objectives

Before assigning objectives to your turret slots, you have to add objectives to your available objective list:

Objective Database
You can add objectives to your available objective list using Add from Database ....
Here you can search for your objective in a large objective database.

Create new objective
If your objective is not listed, you can create a new objective using the "New" Button.
Hint: Please enter at least a correct magnification and working distance due to other software components relying on this information.

Turret Slot Assignment

You can simply drag and drop objectives from your available objective list into your turret slots.
To remove an objective from a turret slot, just press the X next to the slot number.

Press on the Help button for a recommended slot assignment.

> If you have configured a new objective, don't forget to calibrate it using the Objective Video Configuration.
>
> If you have changed the objective configuration, don't forget to compensate the objective offset; just open the Objective Compensation Wizard.

Objective Video Calibration

 **Video Calibration**

For image stitching and in order to have a correct spatial correlation between video images and acquired scanned images by a probe, a video calibration is necessary and can be done in different ways.

If a sample positioner or scan table is available, the video calibration will be done automatically using the table (the sample positioner will be preferred).

If no table is available, a manual calibration can be done.

Context Menu

Right-click on the Video Calibration Button and select Use Default Calibration to use a default calibration.

Please use this only in special cases, when there is no scan table or sample positioner available.

Automatic Calibration

If a scan table or a sample positioner stage is available, the video calibration is done automatically if you click on the "Start Calibration" button or menu item.

The automatic calibration may fail on the following conditions:

Wrong Image Content:

- No structures visible
- Periodic/Cycling structures
- Not focused
- Image too bright or too dark
- Laser spot is visible
- Light shade is visible
- Cantilever is visible

Wrong Configuration:

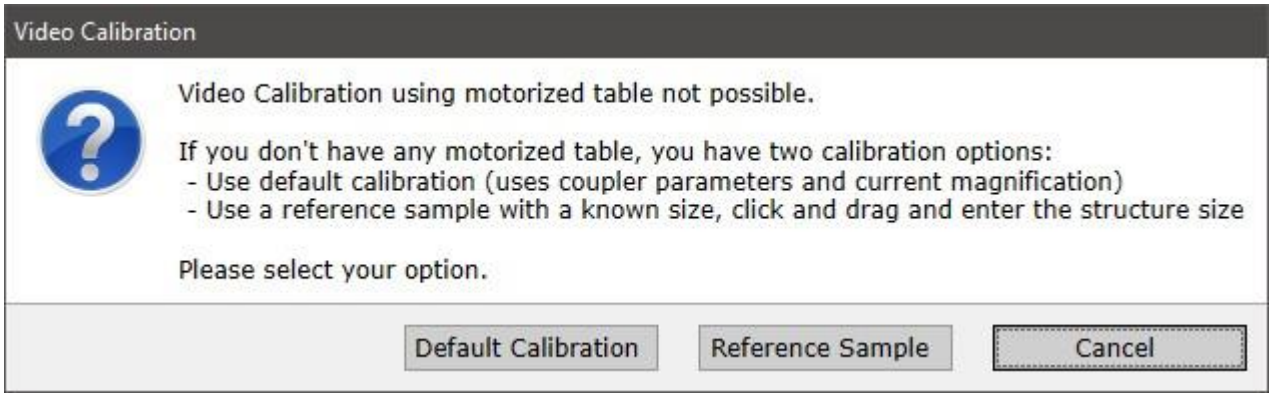
- Wrong objective is selected in the software
- Wrong configuration of the tube lens focal length or video chip size

Hardware Error:

- The scan table / sample positioner is not moving correctly

Manual Calibration

If the video calibration using a motorized table is not possible, you can choose between the default calibration and reference sample:



Default Calibration

Calculates a default pixel size using the current objective magnification, tube lens focal length and video chip size.

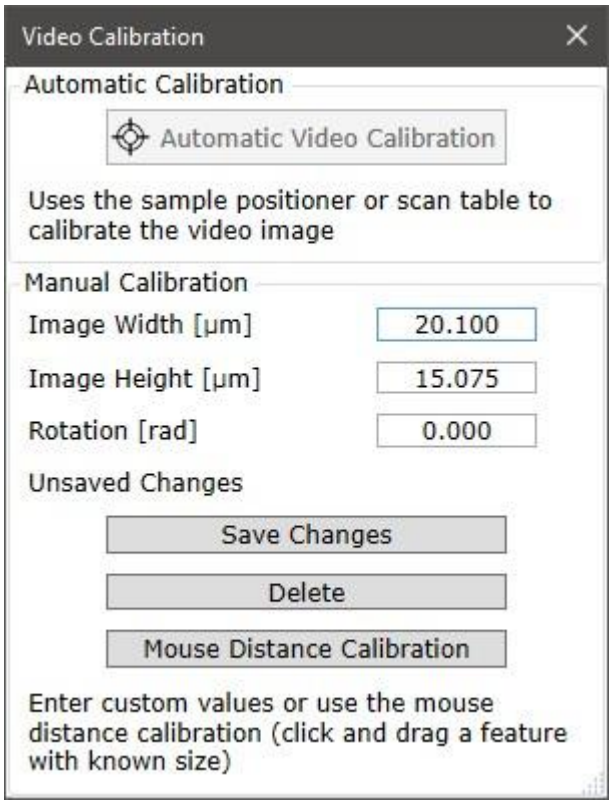
Reference Sample

Here you can drag the size of a feature with a known size in the video image and enter the size.

Advanced Video Calibration Dialog

The advanced dialog can be found in the Main menu -> Advanced Submenu.

Here you can enter custom calibration values:

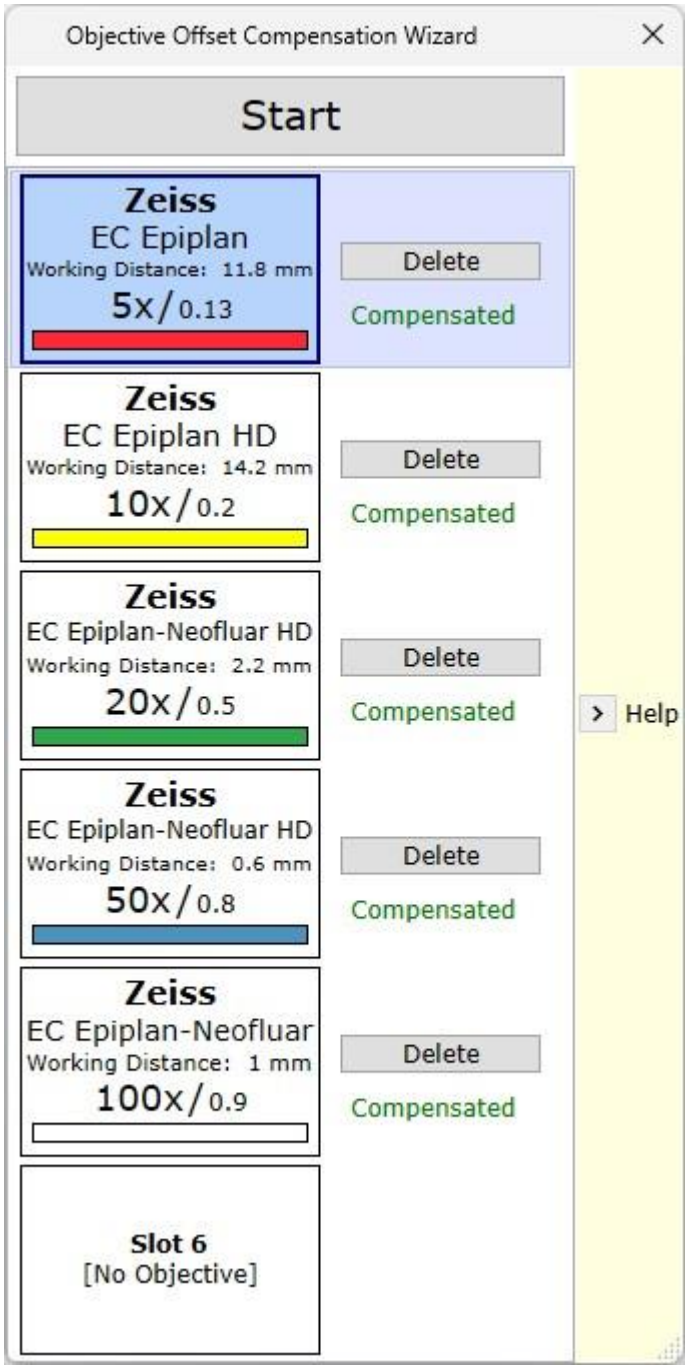


Objective Compensation Wizard

The Objective Compensation Wizard can be used to compensate the small displacement between different objectives.

Depending on whether the X/Y Sample Positioner and the Z Stage is motorized, the compensation is done in all 3 spatial axes.

To start the Objective Compensation Wizard, open the dialog via the main menu:



Click on Help to see a step by step guidance.

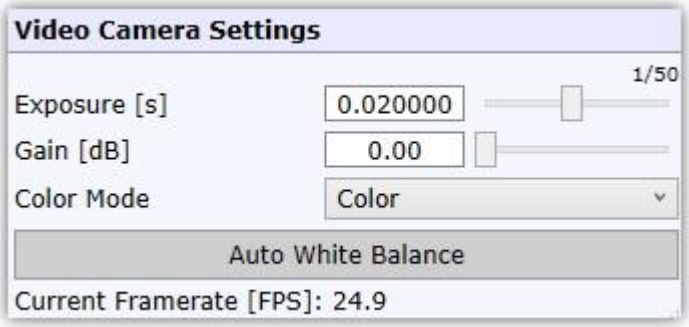
The wizard shows all configured objectives. You can click on an objective to select it and move your X/Y/Z axes to a desired position (e.g. a certain feature on the sample).

Press Start Calibration to tell the software that from now on all offsets should be stored for each objective. If you want to move the sample without saving the displacement, press Stop calibration first.

The compensation is automatically stored upon selecting another objective or closing the dialog.

Device Control

Video Camera Settings



Exposure
Changes the exposure time of the camera. This will also change the frame rate up to a limit of 25fps (or less for a high resolution camera).

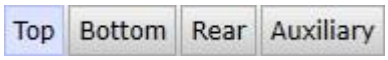
Gain
Changes the gain of the camera. Only use this, if a longer exposure time is not possible and your image is still too dark.

Color Mode
Here you can switch between color and monochrome video images.
This setting is also used for video image measurements (single bitmap, stitching, focus stacking).

Auto White Balance
Press this button once to do an automatic white balance.
A white sample is recommended, e.g. a Teflon band.

Video Camera Selection

You can change the current camera in the top bar of the video control window:

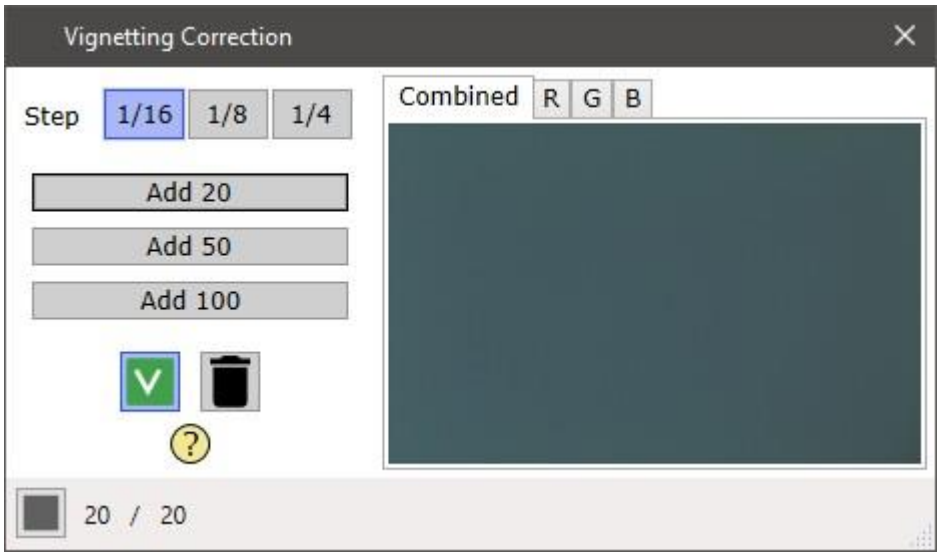


The following video cameras are possible, depending on the system configuration:

- Top: normal brightfield video camera that looks from the top through the objective turret
- Bottom: a camera that looks from below, e.g. available in a SNOM system
- Rear: a camera that looks from the backside
- Alignment: automatically selected and only used for the laser adjustment

Vignetting Correction

The vignetting correction leads to a better video image quality, especially for stitching images.



Step (1/16, 1/8, 1/4)
Defines the step size for the automatic movement (e.g. 1/16 video image size).

Add 20/50/100
Automatically moves the sample positioner and adds video images to the current Vignetting Correction.
You should add images as long as there are structures visible in the preview image on the right.

 **Toggle Use Vignetting Correction**
Lets you activate or deactivate the current Vignetting Correction.

 **Delete Vignetting Correction**
Deletes the current Vignetting Correction.

Combined / R / G / B (Image)
Shows the current Vignetting Correction image.
By switching to R/G/B you can have a look at one of the color channels red/green/blue.

 **Stop Measurement**
Stops the Vignetting Correction image measurement, if currently running.

Illumination

Right-Click anywhere on the right bar to open the Smart Brightness and Video Camera Settings.

Darkfield Mode

This button enables or disables the darkfield mode.
Only available in an automated microscope system with darkfield configuration.

Top Lamp

You can turn on or off the top illumination.
It is automatically turned on upon program start and turned off upon program shutdown.
In automated systems, the white light coupler will automatically couple in or out.

Smart Brightness / Bottom Brightness

With the upper wheel control you can change the Smart Brightness parameter.
With the lower wheel control you can change the bottom lamp brightness.

Auto Brightness

Click this button to trigger the automatic smart brightness using the currently selected video image and Smart Brightness settings.
If the top lamp is turned on, the auto brightness will only use the top lamp.
Otherwise it uses the bottom lamp, if available and turned on.

Hint: Auto Brightness is automatically performed when

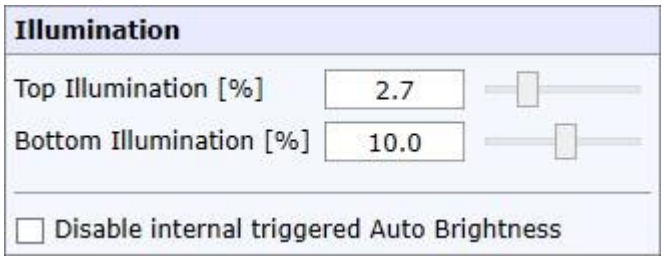
- program starts up
- changing an objective
- toggling darkfield Mode
- changing Aperture Stop Position

Bottom Lamp

You can turn on or off the bottom illumination.

Hints:
The software automatically turns off the illumination when doing Raman measurements.
If a measurement has finished or a video measurement is started, the last used illumination settings are used.

Illumination Settings



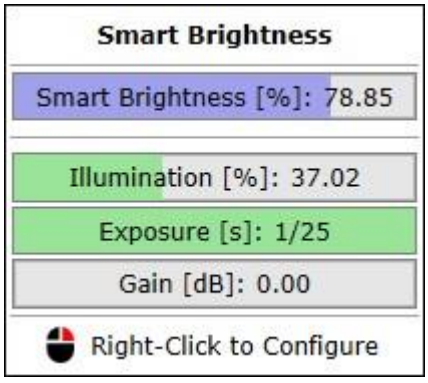
Here you can adjust the brightness of the top and bottom lamp exactly by typing a percentage or using the slider.

Disable internal triggered Auto Brightness
If checked, the auto brightness is NOT done when

- Changing Objective
- Toggling Dark Field Mode
- Changing Aperture Stop Position

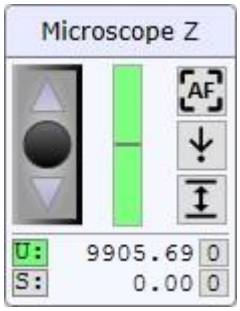
Smart Brightness

The smart brightness parameter changes the illumination as well as the video camera exposure and gain in order to increase or decrease the brightness of the video image - depending on which check boxes in the smart brightness settings are selected.
Exposure has the priority and is increased first, then the illumination, then the gain.
A little pop-up shows all changed parameters when you move the top wheel:



Microscope Z Stage

You can control the Microscope Z Stage using the UI Joystick Controls or the EasyLink Controller:



Microscope Z (Joystick Control)

Controls the Microscope Z Stage. The speed depends on the objective magnification.

Green Bar

Shows the current position of the software controlled, limited Z axis.

If the current position is higher or lower than 95% / 5% of the complete range, the bar gets red.

Auto Focus (Button)

Press the auto focus button in the user interface or on the EasyLink Controller to do an **auto focus**

using the video live image.

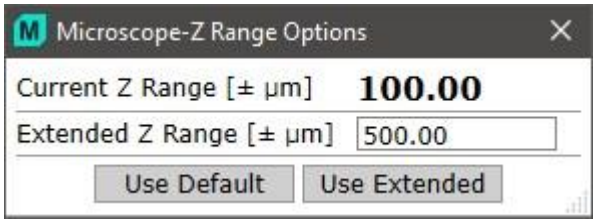
A rectangle is shown in the video image while the auto focus is operating. The rectangle gets green if the auto focus was successful or red if the auto focus failed.

 **Move to Z-Position (Button)**

Opens a little pop-up where you can enter a software limited z position and move to that position.

 **Microscope-Z Range Options (Button)**

Opens a little pop-up which allows to extend the software limited z moving range:



Use Default

Uses the default limits of plus minus 100 µm.

Use Extended

Uses the desired extended z range of e.g. plus minus 500 µm

It is the responsibility of the user to make sure that there is enough space between the sample and the objective.

 **User-z (Radio Button):**

Shows a user controlled, unlimited Z axis position in µm.

It shows all relative movements that the Microscope Z Stage does.

The user can set this value to zero at any time, e.g. to define the focus plane.

This value is saved upon closing the application.

Click to enable the user controlled, unlimited Z axis movement.

 **Software-z (Radio Button):**

Shows a software controlled, limited Z axis position in µm.

This position is used for automated Z Axis movements, e.g. in a depth scan or in TrueSurface mode.

Click to enable the limited Z axis movement.

Z-coordinates used for the geometry definition of measurements are always Software-z-coordinates. This enables refocusing without changing the z-coordinate of the measurement (because the Software-z does not change when changing the focus while User-z (U:) is selected). Focusing with Software-z (S:) selected, will not change the focus for the measurement defined before.

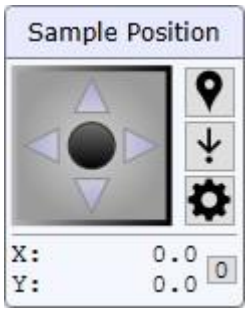
Set Zero (Buttons)

The upper one sets the user controlled, unlimited Z coordinate to zero.

The lower one sets the software controlled, limited Z coordinate to zero.

Sample Positioning

You can control the Microscope Cross Table / XY Sample Positioner using the UI Joystick Controls or the EasyLink Controller:



Cross Table (Joystick Control)

Controls the Sample Positioner. The speed depends on the objective magnification.

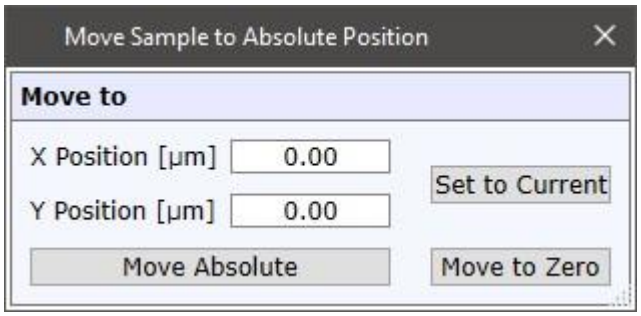
Move Sample to Mouse Position

If active, you can click somewhere in the video image to move the probe position to that position.

You can turn off this mode by pressing the button again or by pressing the Sample Position label in the bottom right corner of the video image.

Move Sample to Absolute Position

Opens a little pop-up where you can enter an absolute XY-Coordinate and move to that position:



X/Y

Shows the current cross table position in µm.

Set to Current

Sets the X/Y edit values to the current position

Move to Zero

Moves the sample positioner to the position 0 | 0.

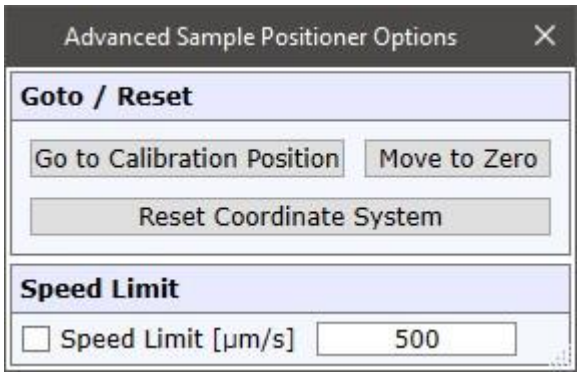
Move Absolute

Moves the sample positioner to the position defined in the X/Y edits.

Set Zero (Button)

Sets the XY position to zero.

Advanced Sample Positioner Options



Go to Calibration Position

Moves the sample positioner to its calibration position.

Reset Coordinate System

Resets the coordinate system.

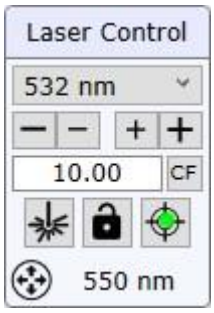
Speed Limit

If checked, the speed of the sample positioner is limited for any movement (manual movement, automatic movement during measurement).

The speed limit is turned off when restarting the software.

Laser Control

You can control the laser with the following user interface. If you have a TruePower Laser, also Laser Intensity and Shutter are available.



Laser Selection (Combo Box)

Here you can switch between multiple lasers. Effects of change:

- For automated microscope systems the beam path will change accordingly.

- Laser wavelength is set to calculate the Raman shift.
- Laser position (red circle in the video image) is restored (can be adjusted in the menu).

If you have multiple spectrometers, the last used laser is automatically selected for the current spectrometer.

Laser Intensity (Buttons and Edit)

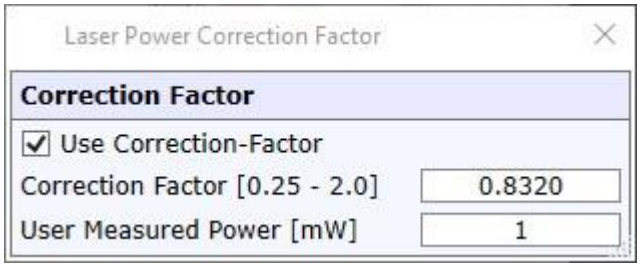
You can change the laser power by either pressing the - or + buttons or by entering an intensity value in milliwatts and press <enter>. The actual reached value might slightly differ from the number you entered.

If the value can not be reached, the software will show an error. Make sure the laser is turned on and warmed up.

Laser Power Correction Factor

Lets you define a correction factor to correct the difference between measured laser intensity in the fiber and the intensity on the sample.

To enable a laser power correction factor, turn on the visibility of the correction factor button in the Main Menu (Advanced).



Enter a user measured power to automatically calculate the correction factor.

Laser Shutter (Toggle Button)

Press on the laser button to open or close the laser shutter.

Laser Shutter Lock (Toggle Button)

If this button is checked, the software will not change the laser shutter state automatically (e.g. by switching to Raman or video state).

This option is only available for AFM configurations.

Laser Output Adjustment (Toggle Button)

Opens the Laser Output Adjustment Window.

Depending on your hardware, here you can adjust the laser output in the fiber or start the automatic calibration.

Only available if an automated laser output adjustment unit is configured.

Laser Fiber Adjustment

In order to do a laser fiber adjustment, please use the True Power Laser Tool in the service monitor.

Raman/Fluorescence Resolution

Shows an estimated optical resolution for the current selected laser and objective.

Click on this element to toggle between lateral and axial resolution display.

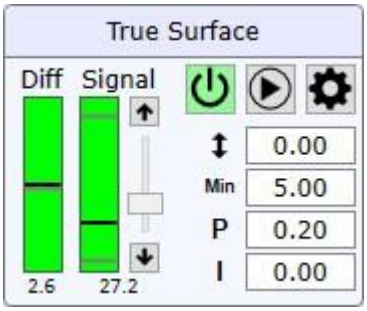
TrueSurface Mk3

The TrueSurface Mk3 device controls the position of the Z-Stage in a way that the sample surface will always have the same distance to the objective, thus leading to a stable Raman signal when scanning on rough samples.

Further information (Operation Guide):

- TrueSurface

> If your system has an AFM and TrueSurface Mk3, then the TrueSurface view is only shown if there is no AFM configuration loaded in WITec Control.



Turn TrueSurface On/Off

Turns the device on or off. This does NOT start the Z-Stage control.

Notice that, as soon as the TrueSurface Mk3 device is turned on:

- The Z-Stage is locked and can not be used for other measurement modes that want to move the Z-Stage
- The Z-Stage can be moved manually by the user, as long as the TSMk3 controller is not enabled / started.
- The Z-Stage manual movement mode switches to the software limited Z mode, because the TSMk3 also can only move within this range.

Start

Enables or disables the controlling of the Z-Stage.

Please stop the controlling immediately, if you hear some strange noise like oscillation of the table.

Diff

Shows the z controller difference between the desired value and the actual value.

The gray bar shows the desired value, the black bar shows the actual value.

Signal

Shows and controls the reflected laser signal strength of the TrueSurface Mk3 sensor.
The strength of the reflected light changes depending on the kind of sample.
Using the slider and buttons you can adjust the laser intensity and thus the measured signal.
The black bar shows the current signal strength. The gray bars show the signal limits.
If the signal goes beyond the limits, the controller does not change the z stage.

Focus Shift

This parameter changes the absolute z offset in order to optimize the Raman signal. (The value is not in µm.)

Min Signal [%]

Sets a minimum signal in percent. If the signal is weaker than this minimum, the controller does not change the z stage.

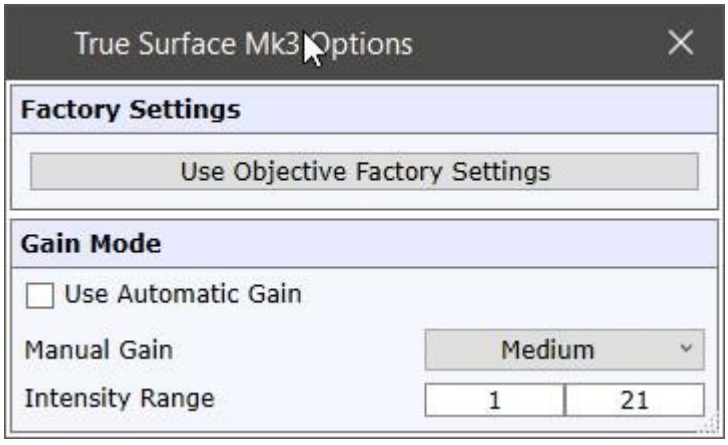
Feedback P-Gain

Sets the P-Gain of the controller unit.
Decrease if the Z-Stage is oscillating. Increase if the Z-Stage is following the surface too slow.

Feedback I-Gain

Sets the I-Gain of the controller unit.

Options



Use Objective Factory Settings

Lets you recall the default settings for the currently selected objective, if defined.
For all parameters, a default value is stored for all objectives intended for TSMk3.
WITec delivers good standard values that might be changed by the user due to changing behaviors on different samples.

Use Automatic Gain

If checked, the gain is automatically selected when changing the intensity.
If not checked, the settings below are used.

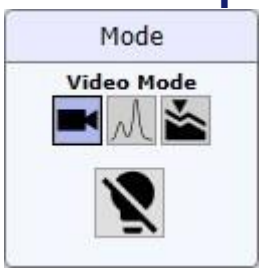
Manual gain

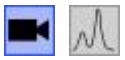
Defines the gain of the TrueSurface Mk3 Unit.

Intensity range

Defines the intensity range that is used when changing the intensity.

Microscope Modes



 **Video / Raman Mode**

The Raman or video modes are selected automatically upon starting or stopping measurements in WITec Control. However, the user is able to switch between those modes at any time.

According to this state, automated microscope systems will adjust their beampath and shutters of true power lasers are closed or opened.

 **TrueSurface**

Only available for systems with TrueSurface Mk2.

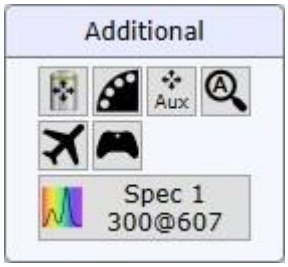
The TrueSurface mode is selected automatically upon starting a TrueSurface or Profilometer measurement. For automated microscope systems it sets up the beampath for TrueSurface.

 **Enable all Light sources**

Only for SEM / RISE Users: enables all light sources.

On startup, all light sources are disabled until the user presses this button.

Additional Devices



 **Inverted Objective Positioning**

Opens the Inverted Objective Positioning Window.

Filter Wheel Position

Opens the Filter Wheel Window.

Auxiliary Inertial Drive

Opens the Auxiliary Inertial Drive Window.

Analyzer / Polarizer Settings

Opens the Analyzer / Polarizer Window.

Portable Coordinate System

Opens the Portable Coordinate System Window.

EasyLink Controller Help

Opens the EasyLink Controller Help Window.

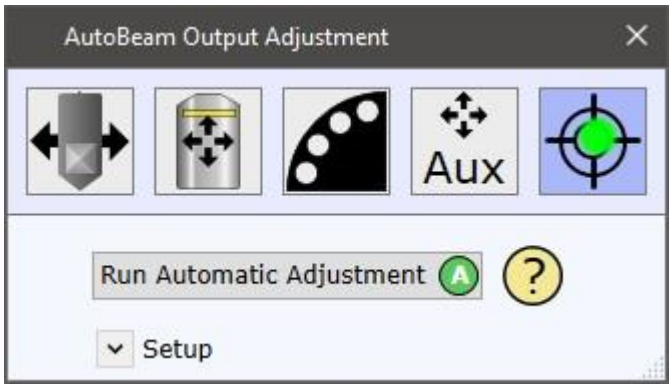
Spectroscopy Settings

Shows the currently selected Spectroscopy System (1, 2, 3) and the selected grating with center position.

Opens the Spectroscopy Settings Window.

Output Adjustment

Adjustment for AutoBeam Output Coupler

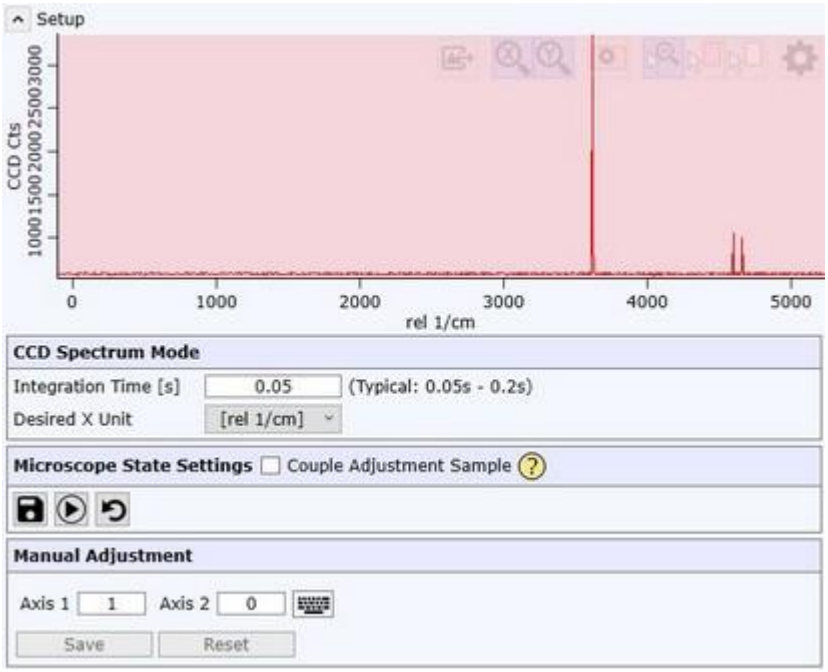


Run Automatic Adjustment

Starts the automatic adjustment sequence and tries to find the maximum output signal.

If this action is successful, the adjustment is automatically saved for the current combination of laser and output.

Setup



CCD Spectrum Mode

Enables to define the integration time and X Unit of the live spectrum

Microscope State Settings

Couple Adjustment Sample

Lets you couple in or out the adjustment sample

Save

Saves the following states for the current laser-output-combination:

- Grating and Position
- Laser Power
- Integration Time
- Spectral Range

Execute

Sets the microscope state which is saved as user settings.

Restore

Restores the factory settings.

Manual Adjustment

Axis 1 / Axis 2

Let you manually move the two axes to adjust the laser output.

You can click on the button to use the arrow keys up/down/left/right to move both axes.

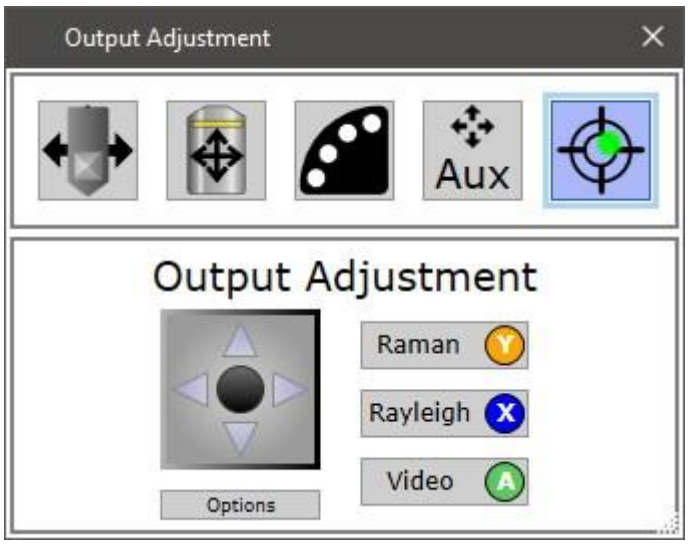
Save

Saves the current output adjustment (axes positions) for the current combination of laser and output.

Reset

Resets the current output adjustment for the current combination of laser and output.

Adjustment for Output Coupler with Alignment camera



To start the laser output adjustment, first start the oscilloscope in WITec Control.

For help moving the inertial drive, see Inertial Drive Control.

Use the UI Joystick Controls or the EasyLink Controller to adjust the adjustment mirror.

The motorized laser output adjustment is only available with an automated output tower.

Raman

Sets up the beam path for Raman signal.

Use this in a first step to align your laser.

Only use single steps in all directions to optimize your signal.

If there is no signal, try using the Rayleigh or video adjustment.

Rayleigh

Sets up the beam path for Rayleigh signal (removes the filter).

Only use single steps in all directions to optimize your signal.

If there is still no signal, use the video adjustment.

The Rayleigh adjustment mode is only available if the laser coupler has an removable filter.

Video

Sets up the beam path for video adjustment and switches the video live view to the adjustment video camera.

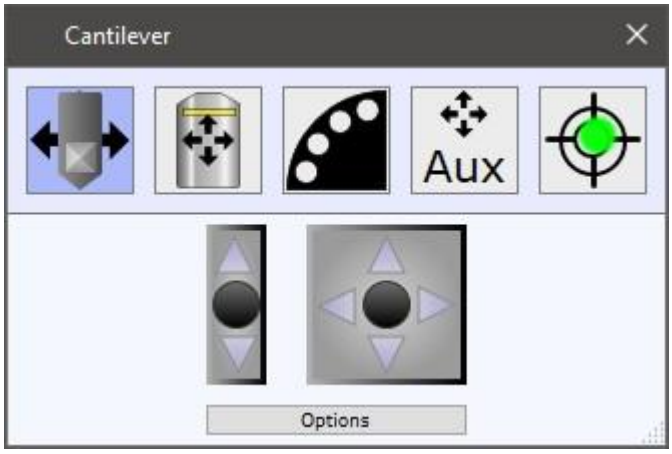
Try to move the laser spot in the red circle.

If the laser spot is far away, use the continuous movement feature.

If you have finished this task, close the window and stop the WITec Control oscilloscope.

Inertial Drive Control

This dialog can be accessed from Additional devices or from AFM Status for Cantilever movement.

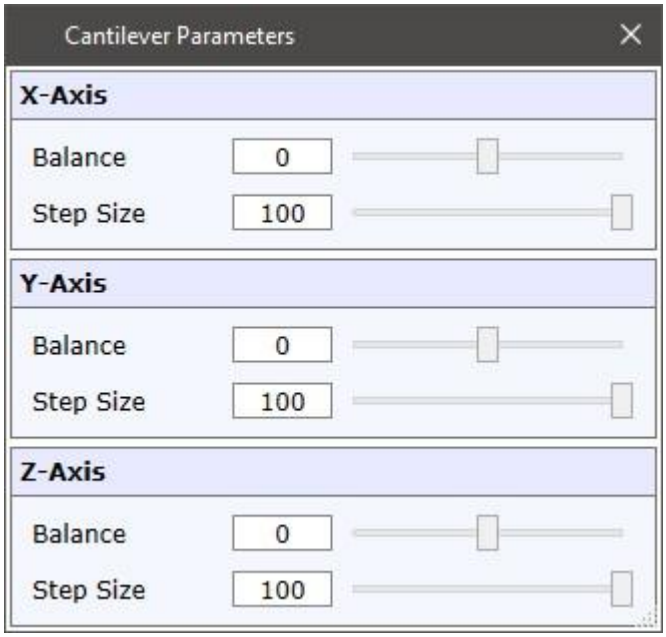


Inertial drives are used to move the cantilever, the laser output adjustment mirror or an auxiliary device.

You can use the UI Joystick Controls or the EasyLink Controller to move the inertial drive.

Pressing the arrows will do a single step.

If the axes don't move symmetrically or one direction does not move at all, open the options and adjust the balance or step size:



Auxiliary Inertial Drive

For the auxiliary inertial drive, there are some more settings for each axis:

Invert Axis

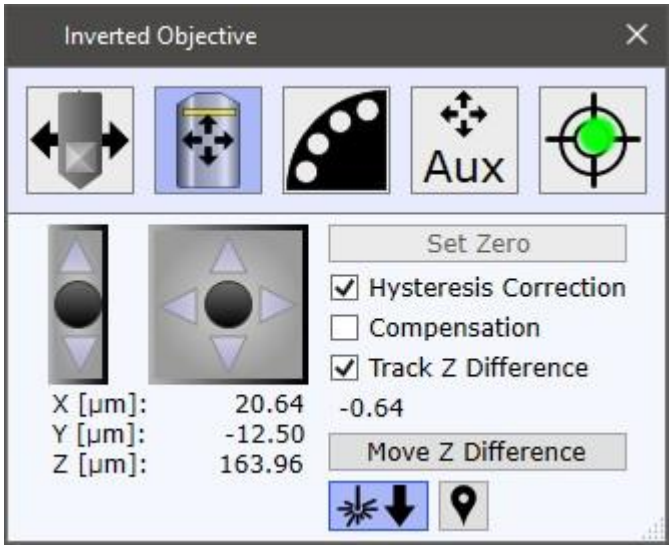
Inverts the movement of the axis.

Hardware Axis

Let's you define which hardware axis should be controlled when using the joystick/easylink.

Inverted Objective Positioning

This dialog can be accessed from Additional devices.



You can control the X/Y/Z axes using the UI Joystick Controls or the EasyLink Controller.

Set Zero

To avoid unwanted contact of the inverted objective with the scan table, the maximum allowed area of movement is limited.

Press set zero to set all values to zero and to enlarge the area of allowed movement.

Hysteresis Correction

If checked, a hysteresis correction will be done when doing single steps using the gamepad or the "arrow buttons".

Compensation

If checked, the inverted microscope compensates X-/Y-Position whenever the probe position changes:

- when changing the top turret objective
- when changing the probe (e.g. laser change)

Track Z Difference

If checked, the difference between the inverted objective Z-Position and the microscope Z-Stepper Position will be tracked.

Move Z Difference

Moves the inverted objective in Z, so that the tracked difference between the inverted objective Z-Position and the microscope Z-Stepper Position is compensated.

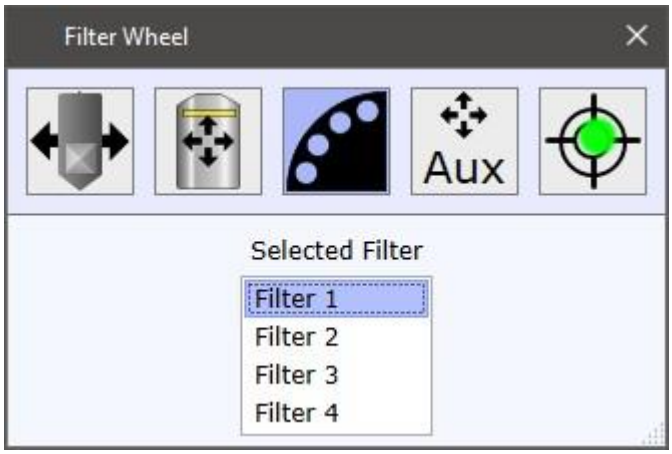
Indicates that the laser light is coming from top and the marker in bottom camera view shows the detector position.

Indicates that the laser light is coming from bottom and the marker in bottom camera view shows the laser position.

If checked, you can click into the bottom camera image in order to move the inverted microscope to the desired position.

Filter Wheel

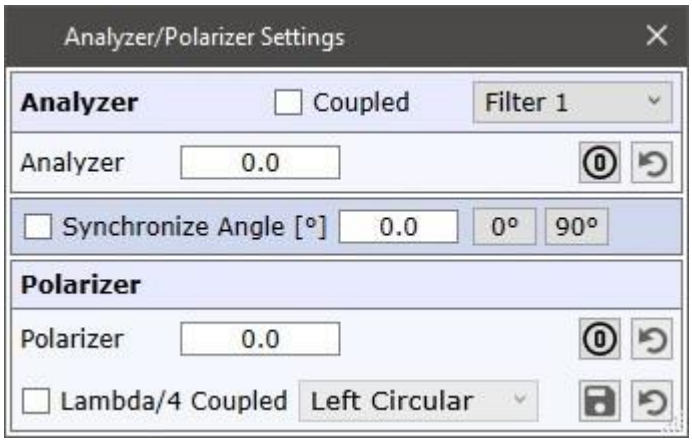
This dialog can be accessed from Additional devices.



Just select your desired filter position from the list box.

You can also push the right stick of the EasyLink Controller up and down to select a filter.

Analyzer Polarizer



Analyzer

Analyzer
Angle of the analyzer.

Coupled
If activated the analyzer is in the beam path.

Filter 1/2/3
If you have more than one filter for the analyzer, select the one used when changing them.
(The zero position may differ between the filters.)

Zero
Set Current Analyzer Position as Zero

Synchronize Angle
If checked, automatically synchronizes a desired angle between the analyzer and the polarizer.

Polarizer

Polarizer
Angle of the polarizer.

Zero
Set Current Polarizer Position as Zero

Lambda/4 Coupled
Defines whether a Lambda/4 plate is coupled.
If checked, you can select one of the following predefined positions:

- Left Circular
- Right Circular

You can still move to a custom polarizer position.

The Combo-Box will then show "undefined" lambda/4 state.

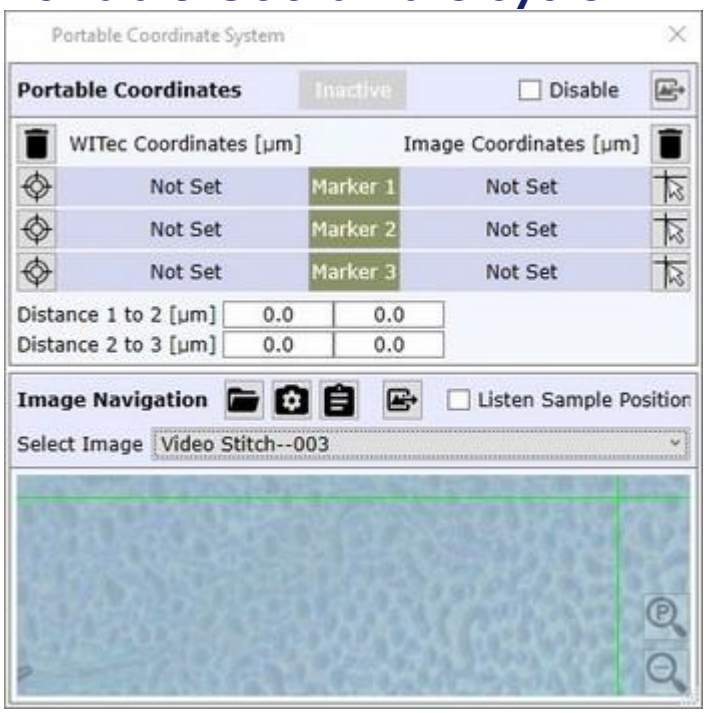
Save Lambda/4 Position

Lets you save the current polarizer position as the Left Circular or Right Circular lambda/4 position (user will be asked).

Restore Factory Settings

Here you can restore the factory settings for the Analyzer or Polarizer zero position or the lambda/4 positions.

Portable Coordinate System



Portable Coordinates enables to learn 3 markers in order to

- correlate measurements of different microscopes
- navigate using an imported image (e.g. macroscopic image from an external camera)

Disable

Disables the usage of portable coordinates in new data objects.

Export Marker Images

Exports the video images acquired at each marker position.

WITec Coordinates

Delete Markers

Deletes all microscope markers

 **Set Marker Position**

Sets the current sample position as the desired marker position

Image Coordinates

 **Delete Markers**

Deletes all image markers.

 **Set Marker Position**

If turned on, you can click into the image in order to define the image marker position.

Marker Button

Click this button in order to move to the sample positioner to the learned marker position.

Distance 1 to 2/Distance 2 to 3

Here you can define the expected distance between marker positions in order to move to the next marker automatically.

Just click on Marker 2 or Marker 3 to move to the next marker.

Image Navigation

 **Load Image from File**

Loads an image from file.

Can be

- Zeiss CZI Image *.czi
- WITec HDF5 File *.h5oiwt
- Bitmap/Gif/Jpeg/Png/Tiff Files

 **Get Image from External Camera**

Here you can get an image from an external camera, e.g. a USB Webcam or a Table Cam.

 **Get Image from Windows Clipboard**

Gets the image from the windows clipboard.

 **Export Image to current Project**

Exports the current image as a new WITec Project bitmap object to the current project, with the spatial position calculated from all markers.

Listen Sample Position

If checked, you can click in the image to move the sample positioner to the desired position.
Only works if all 3 markers are defined for WITec and Image Coordinates.

Select Image
If a loaded image file contains multiple images, you can select which image should be displayed.

Field Stop / Aperture Stop

Field Stop

Position
Sets the position of the field stop.

Toggle Open / Focus Position
Toggles between the open and focus position.
You can define both positions with the "Define" buttons.

Define Focus Position
Defines the current position as the Focus/Close position.

Define Open Position
Defines the current position as the open position.

Aperture Stop

Position
Sets the position of the aperture stop.
When changing the position, the video brightness will be automatically adjusted.

UI Joystick Control

The User Interface Joystick Control allows to control the movement of several axes such as the Microscope Z Stage, the Sample Positioner, the Inverted Microscope, the Cantilever etc.
It can be controlled using the mouse or the keyboard.

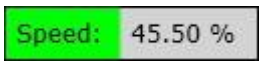
Mouse Control

The black circle in the middle can be dragged with the mouse, which controls the direction and the speed, depending on the mouse movement distance:


The arrows can be clicked for single steps.
Hint: Hold down the control-key on the keyboard to do a continuous movement instead of single steps.

Turning the **mouse wheel** on a vertical joystick will do single steps in the desired direction.

Holding down the right mouse button will change the step size:



Keyboard Control

If the control is just clicked with the left mouse button, it gets the keyboard focus and can be controlled using the arrow keys.

> Hold down the control-key on the keyboard to do a continuous movement instead of single steps.

The speed can be changed using the keys 0, ... 9:

EasyLink Controller

A lot of microscope devices and some software parameters can be controlled using the WITec EasyLink Controller.

Assignment of Buttons

The assignment can change upon switching to another task in the software.
If no special task is currently in process, the default mode is active and the assignment looks like

this:

If a special task is currently in process, e.g. if the change objective wizard is open, then some buttons are handled by this wizard in order to move up or down the microscope automatically. Watch out for buttons showing the EasyLink Controller assignment, e.g.:

Continuous Movement

To move any axis **continuously**, you have to hold down one of the shoulder buttons:
The lower shoulder button will set the moving speed according to the objective magnification and video zoom.
The upper shoulder button will set the moving speed to the maximum speed ("**Turbo**") of the current moving device.
The more the joystick is moved to the boundary, the higher the moving speed.

Single Steps

If no shoulder button is pressed, only a single step is done in the selected direction.
The single step size can be adjusted by pressing on the Joystick or by selecting a custom step size using the right mouse button on a UI joystick control.

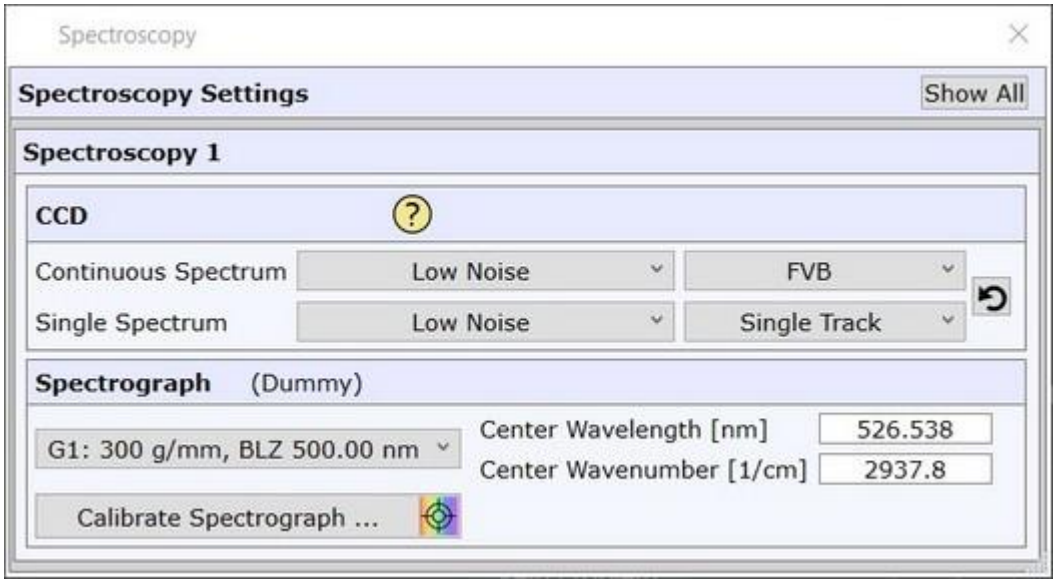
Secondary Options

Hold down the left menu button to enable "Secondary *Options*", which allows to use the same controller buttons for different purposes such as laser control, auxiliary device control, video measurements etc:

Spectroscopy Settings

Further information:

- Raman
- Change laser wavelength
- Camera configuration (Service monitor)
- EMCCD



Show All

Click to show all Spectroscopy Systems, otherwise only the currently selected Spectroscopy is displayed.

CCD - Read Out and Binning Modes

Here you can change the CCD sensor readout mode (left comboboxes) and the binning mode (right comboboxes).

Continuous Spectrum

The settings apply to continuous spectra acquisition used for oscilloscope, fast time series, image scan and continuous modes of the large area scan.

> For longer integration times > 400 ms Single Track can be used as Binning setting to reduce the number of cosmic ray events and the amount of dark current, but will result in a slightly lower signal. For integration times > 1 s Single Track is the recommended setting.

Single Spectrum

The settings apply to e.g. single spectrum, line scan and stepwise raster mode of the large area scan. For these modes speed is less important and default settings are used in almost all cases.

Mode

The mode defines readout speed and dynamic range of the camera.

Low Noise

This setting is optimized for low noise, but can limit the readout speed for very short integrations times.

High Dynamic Range

This setting offers more dynamic range for measuring higher intensities without saturating the camera.

High Dynamic Range (Fast)

This setting offers more dynamic range and also a higher readout speed.

Electron Multiplying

This setting is only available for EMCCDs. For this setting a low signal without background is required. The low signal is then amplified over the readout noise. For longer integration times thermal noise becomes significant over the readout noise and the benefit of the amplification is lost.

Binning

Binning defines the readout of the CCD chip.

Crop (Ultra Fast)

Only a small part of the CCD area is read, but the rest of the CCD is not cleaned. This is the fastest mode and reduces clock-induced charges.

Single Track

Only several lines of the CCD are used for the spectrum, the rest is discarded. This mode collects the least cosmic ray events and has less dark current. The slower readout can result in a lower signal intensity for short integration times.

FVB

For Full Vertical Binning (FVB) the whole CCD chip is binned to one line. This mode is faster than Single Track, but collects more cosmic ray events and more dark current.

None

For line cameras only. No binning possible.

Use Defaults

Sets the default settings for all measurement modes.

Spectrograph

Grating

The grating parameter allows the selection of the gating used for spectrometry. The gratings listed in the the pull down menu depend on the individual configuration but generally have the form [Grating number: Groove density, Blaze wavelength].

Center Wavelength [nm]

The wavelength hitting the center of the CCD chip can be adjusted using this parameter. This wavelength will then also be the central wavelength in the spectra displayed in the software. To change the central wavelength, the software calculates the necessary rotation of the grating selected.

Spectral Wavenumber [1/cm]

The spectral center is identical to the center wavelength. The only difference is that the unit is in Wavenumber relative to the laser wavelength.

Calibrate Spectrograph

Opens the Spectrograph Calibration.

Note that automated microscope systems will automatically couple necessary devices for the calibration, e.g. the automated calibration lamp, output, etc.

Spectrograph Calibration

Further information (Operation Guide):

- Spectrograph Calibration

Calibration (Tab)

Top Bar

Verify only (Checkbox)

If checked, you can verify the quality of the grating calibration, without changing the current calibration.

Cancel

Cancels a currently running calibration or verification.

Current Reports

Shows the latest calibration or verification report for each grating.

Export Reports

For problem analysis, you can export the latest reports into a ZIP file and send it to WITec.

Note that you can also include the latest reports when creating a Support ZIP File.

Delete Old Reports

Deletes reports older than 90 days. This might be reasonable in order to clean up unnecessary data, e.g. when you do calibrations/verifications very often.

Gratings

For each grating, you can see the grating info and its calibration state.

History

Opens the history of all calibration and verification reports of the current grating and let you open and compare any of those reports.

Combobox ("VIS")

Here you can select one of the calibration groups specified for the current spectrograph-grating combination.

Calibrate (or Verify)

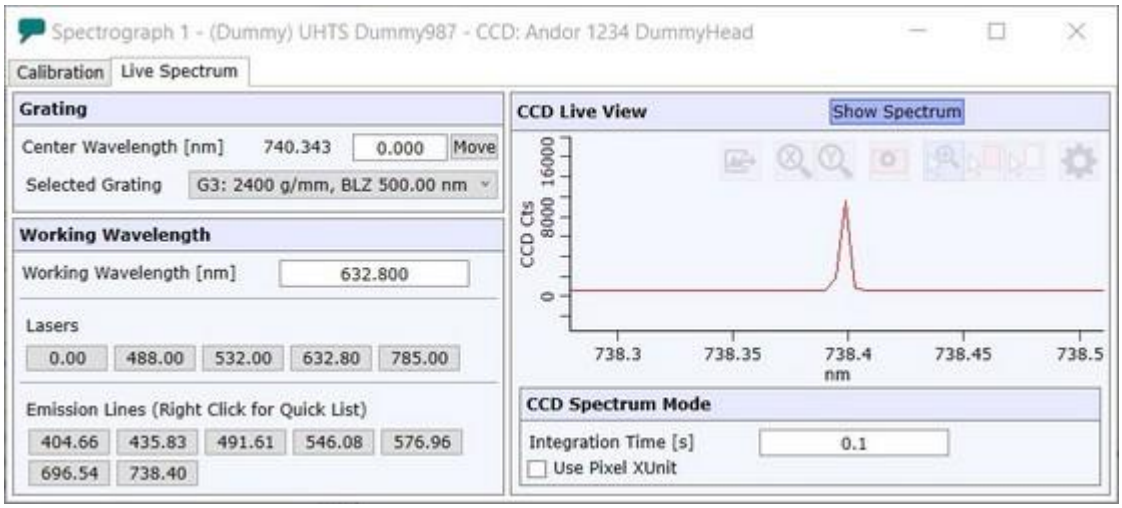
Starts the calibration or verification process.

State/Info

Each grating shows the result of the last calibration or verification: whether it was successful or not and the min/max error values in nanometer and pixels.

At the bottom area you can see the last measured spectrum with its integration time.

Live Spectrum (Tab)



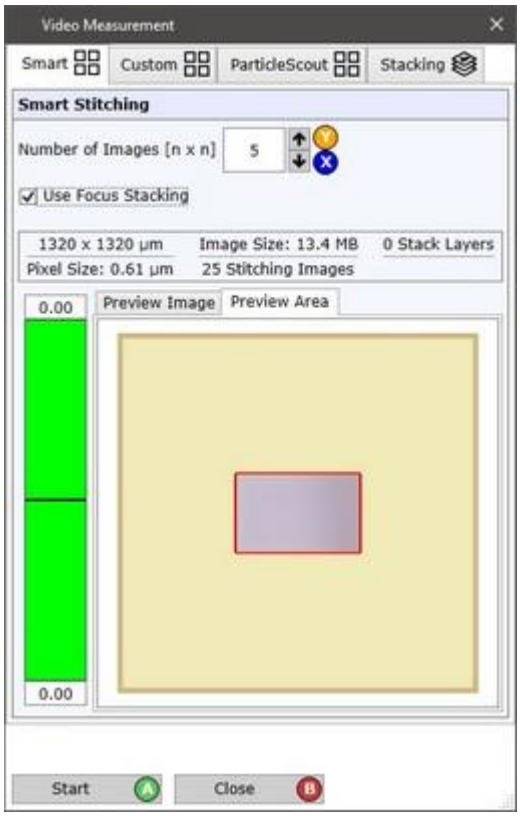
Click on Show Spectrum in order to display a live spectrum, for example to adjust the intensity of your calibration source.

You can select a grating and adjust the center wavelength by typing a number or by clicking one of the emission line buttons.

Video Measurement

Video Measurement Overview

The Video Measurement dialog can be used to perform image stitching and focus stacking.



Smart Stitching

See Smart Stitching.

Custom Stitching

See Custom Stitching.

ParticleScout Stitching

See ParticleScout Stitching.

Scan Table Stitching

For systems using a scan table and without any sample positioning device, the scan table stitching is available.
It just uses the total range of the scan table for the stitching.

Video Focus Stacking

See Video Focus Stacking.

Common Stitching User Interface

Stitching Information

1320 x 1320 µm	Image Size: 13.4 MB	10 Stack Layers
Pixel Size: 0.61 µm	25 Stitching Images	

Here you can see the current stitching information:

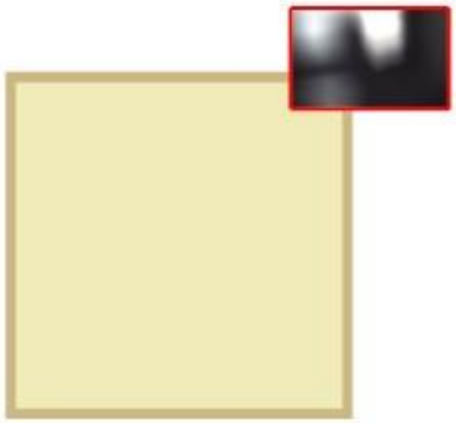
- Area in µm
- Size of a single pixel
- The total image size in megabytes/gigabytes
- The number of images
- The number of stack layers (if focus stacking is turned on)

Preview

Preview Image
During the video measurement process, this tab will show a preview image.
You can zoom into the image using the mouse wheel, pan the image using the mouse wheel button.

Export High Quality
Exports a the stitching image with a higher resolution to the current project.
The original resolution is an optimal resolution calculated using objective and camera parameters with a maximum of 64 mega pixels.
To prevent large projects, the exported standard resolution is about 1 Mega-Pixel.

Preview Area



Here you can see the current video area and the preview stitching area, positioned in absolute lateral space.
This gives you an overview about the relation of the stitching image position/size compared with the current position and video image size.

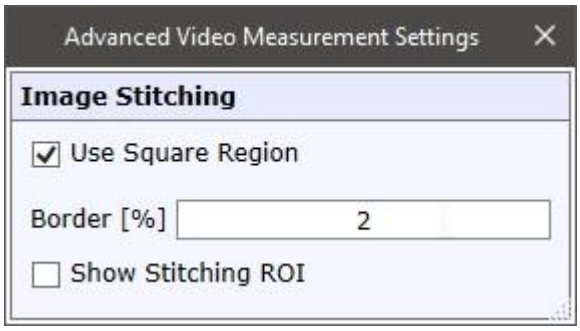
Start / Cancel / Close

Start
Starts the currently selected video measurement, depending on which tab is selected.

Cancel
Cancels the current video measurement. The preview video image is always exported to the current project (except for ParticleScout Stitching)

Close
Closes the window.

Advanced Options



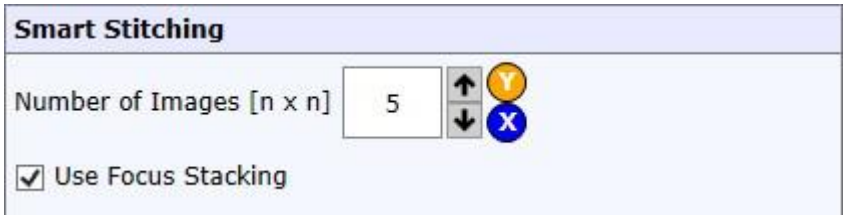
Use Square Region
If checked, a quadratic area is used. Affects the size of Smart Stitching.

Border [%]
Defines how much of a full video image is used for a single stitching image.
E.g. Video Width = 1000px, Border = 10% -> the most left 100px and the most right 100px are not used.

Show Stitching ROI
If checked, the area of a single stitching image is shown in the video image.

Smart Stitching

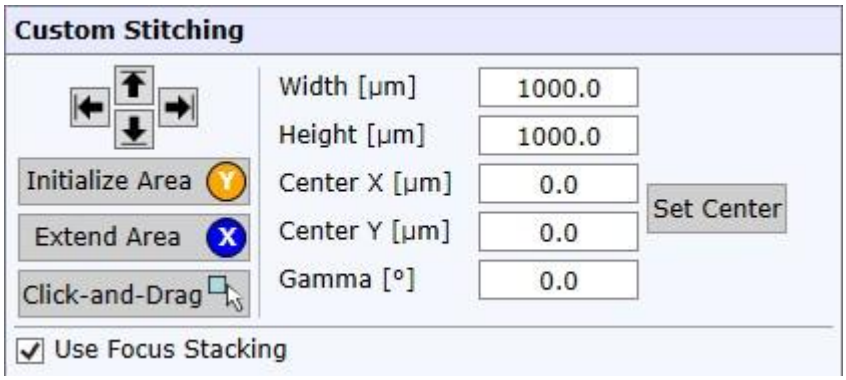
Smart Stitching can be used to measure a stitching image around the current position by simply defining a number of images.



Number of Images
Here you can set the number of images [n x n] for the stitching.
Depending on the selected objective, the number of images defines the total size of the stitching image.
You can increase or decrease the number of images using the number edit, the arrow buttons or the gamepad buttons X and Y.

Use Focus Stacking
If checked, uses the current focus stacking range in order to do a focus stacking for each single image.

Custom Stitching



Custom Stitching lets you define the stitching area exactly by setting the size and center position and a rotation.
The Area Definition Buttons will help you defining the parameters visually.

Area Definition Buttons

Arrow Buttons
Let you define the most left/top/bottom/right position for the stitching area.

Initialize Area
Sets the custom stitching area to the current video position / size.

Extend Area
Extends the current stitching area by the current position of the video image.

Click-and-Drag
Turns on the mouse listen mechanism: if turned on, you can click and drag a rectangle in an image viewer in order to define the stitching area.

Area Parameters

Width / Height
Sets the stitching width and height in microns.

Center X / Y
Sets the absolute center position of the stitching image.

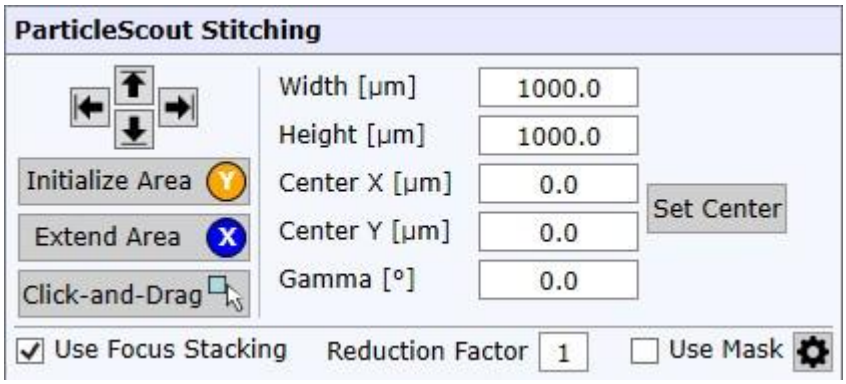
Set Center
Sets the Center X / Y parameters to the current position.

Gamma
Sets an angle in degrees for the stitching. This way you can stitch a rotated structure.

Focus Stacking

Use Focus Stacking
If checked, uses the current focus stacking range in order to do a focus stacking for each single image.
Please consider Video Focus Stacking FAQ for best results.

ParticleScout Stitching



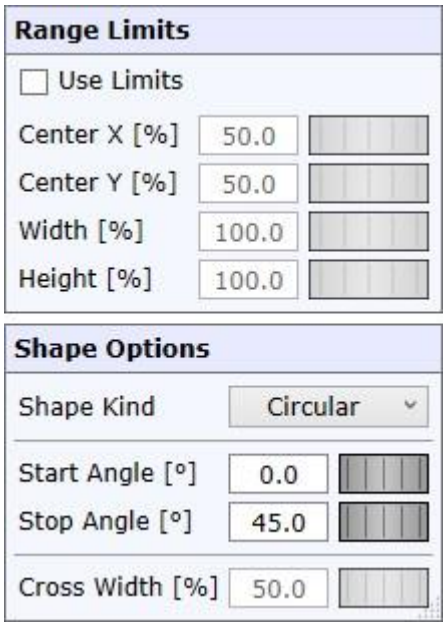
The ParticleScout Stitching will create a high resolution stitching image and open the WITec ParticleScout software after the stitching measurement has finished.

In addition to the custom stitching parameters (which define the stitching area), there are the following options:

Reduction Factor
In case of large stitching areas, the resulting stitching image may have a very large resolution and thus needs a lot of memory. If you don't need the resolution (e.g. if you only have large particles), you can reduce the image resolution by increasing the factor.
Watch the Image Size in Megabytes/Gigabytes.

Use Mask
If checked, a customizable mask will be used in order to stitch only a part of the defined stitching area, see Mask Options below.

Mask Options



Use Limits
If checked, the defined percentages for Center and Size will be used to "shrink" the mask / to use only a part of the defined stitching area.

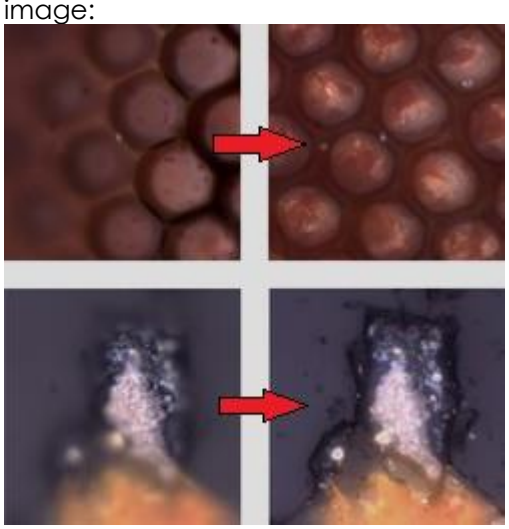
Center / Size
Lets you define a center position or size as a percentage relative to the original stitching area.

Shape Kind

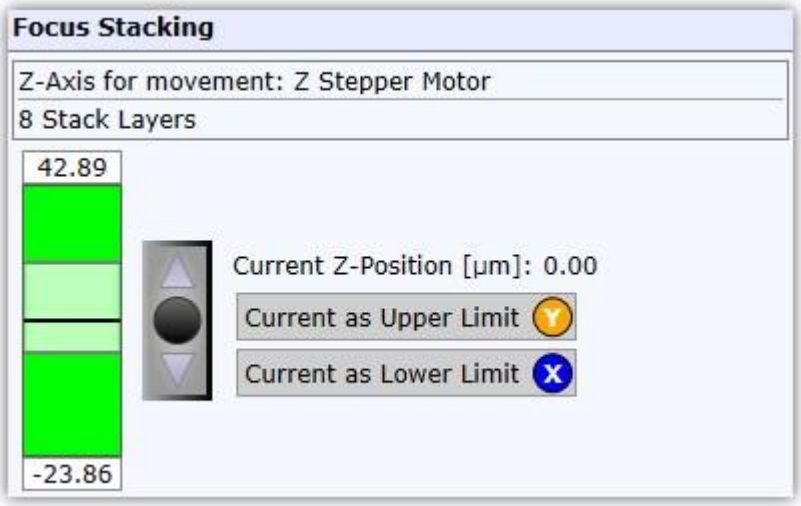
- Rectangular: just uses the range limits and defines a rectangle
- Circular: creates a circle or ellipse and uses Start/Stop Angle to define a section
- Crosswise: creates a cross using the Cross Width parameter

Video Focus Stacking

Focus stacking acquires multiple video images in different z-positions to calculate a sharp result image:



Please consider Video Focus Stacking FAQ for best results.



Green Bar

Shows the maximum Z-Range that the software is able to move by its own (Default: ±100µm, see Microscope Z Stage Range Options).

The bright area shows the Z-Range which is used for the focus stacking.
The upper edit defines the upper limit, the lower edit the lower limit.

Joystick

The joystick moves the Z-Stage. Note that the software limited z coordinates are used when opening the Video Measurement Window.

Current as Upper Limit
Uses the current Z-Position as the upper limit for the focus stacking.

Current as Lower Limit
Uses the current Z-Position as the lower limit for the focus stacking.

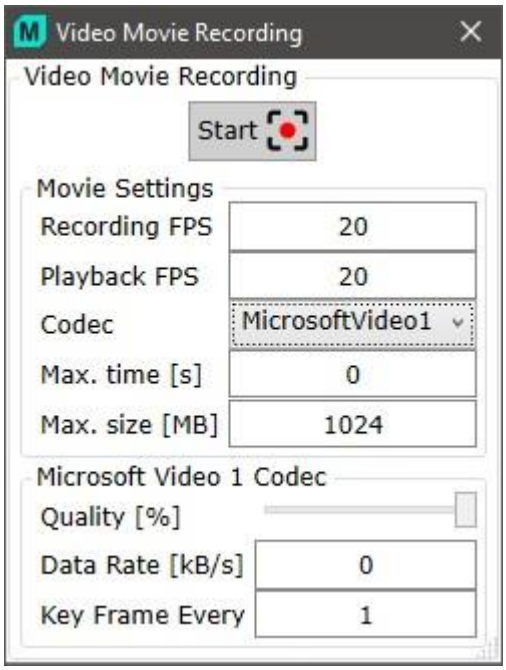
Pressing start will simple perform the focus stacking on the current position.

Video Focus Stacking FAQ

To get proper results when performing video focus stacking, please consider the following rules:

- Ensure that the aperture stop is aligned correctly
- Ensure that the image is not over saturated (too much light)
- Ensure that the used objective is NOT an AFM objective

Video Movie Recording



Start
Lets you select a file name and starts the movie recording.

Movie Settings

Recording FPS
Here you can enter a desired recording frame rate. The actual frame rate may differ because of limiting camera specifications.

Playback FPS
Here you can enter a desired playback frame rate. You can implement a slow or fast motion by setting a lower / higher playback frame rate.

Codec

Here you can select a AVI codec:

- None: Full uncompressed bitmaps will be saved. The video file will be very large.
- Microsoft Video 1: see below

Max time
Sets a maximum time for the video recording. The recording will stop automatically after this time.

Max size
Sets a maximum file size for the video file. The recording will stop automatically if the file gets larger than the max size.

Microsoft Video 1 Codec

Quality
Sets the quality of the saved video images. The lower the quality, the smaller the file size.

Data Rate
The desired data rate. A data rate of 0 sets an automatic data rate.

Key Frame Every
Defines the number of key frames.

Status

AFM Status

If your system has AFM and TrueSurface Mk3, then the AFM status view is only shown if any AFM configuration is loaded in WITec Control (AFM beam deflection laser is turned on).

If you click on the quadrant, the AFM Status opens in a separate window with freely adjustable size.

Left Bar (Z)

The left bar shows the current scan table z sensor position.

Quadrant

It shows the four quadrants of the beam deflection detector. The indicator changes depending on the operation mode:

- A spot visualizes the T-B and R-L Signal as position of the light spot on the four quadrant diode for Contact mode. The active low pass filter is indicated at the bottom.
- An arrow displays the LockInR (oscillation amplitude) as its length and LockInPhi (phase offset) as its rotation for AC mode. The note "AC coupling" at the bottom indicates that all DC voltage offsets are neglected in this representation.
- A horizontal bar represents the FMax signal (maximum force) for DPFM. The R-L Signal is not evaluated.

Right Bar (Sum)

The right bar shows the sum signal (total from the cantilever reflected light captured by the four quadrant detector).

 **Cantilever Active**

This button should be turned on when a cantilever is attached and in use.

Show Cantilever Position

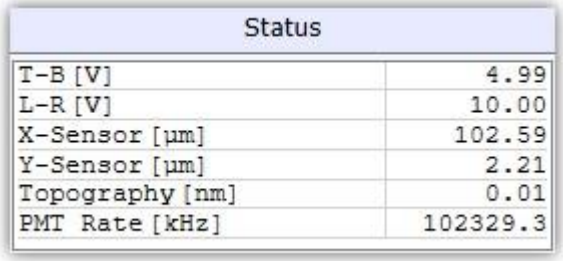
If checked, the cantilever probe position is shown in the video image instead of the laser probe position.

You can define the probe position in order to do lateral-correlated measurements.

Cantilever Positioning

Opens a little pop-up for positioning the cantilever.

Status Values



Status	
T-B [V]	4.99
L-R [V]	10.00
X-Sensor [µm]	102.59
Y-Sensor [µm]	2.21
Topography [nm]	0.01
PMT Rate [kHz]	102329.3

Shows the current status values from different hardware devices, such as scan table X/Y/Z sensors, AFM voltages, PMT rates etc. Please refer to Data Channels for further information.

State Manager

The state manager enables to save and recall predefined states that can contain the following device states:

- Laser Selection, Laser Power
- Grating and Position
- CCD Readout and Binning Settings
- Polarizer and Analyzer Positions

Behavior

When changing a configuration in WITec Control, e.g. to "Raman CCD2", then the state manager will automatically select the startup state for CCD2.
When the current output is "CCD2", then you can select all states that are configured for output "CCD2".

State Combobox

Use the state combobox to select one of the previously defined states.
Please note that the actual state of the devices can differ from the selected state, if you change the device on your own after executing the state.

Execute Selected State

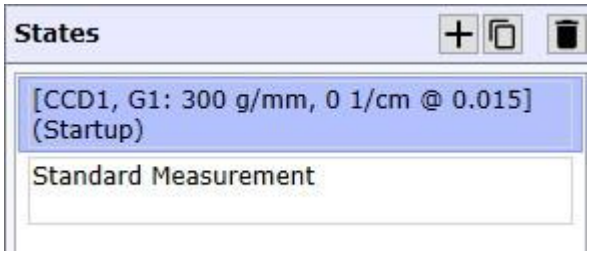
Executes the currently selected state so you can make sure that all devices are in the desired state.

Edit States
Opens the State Editor.

Open State Resetter
Opens the State Resetter:

State Editor

Add or Remove States



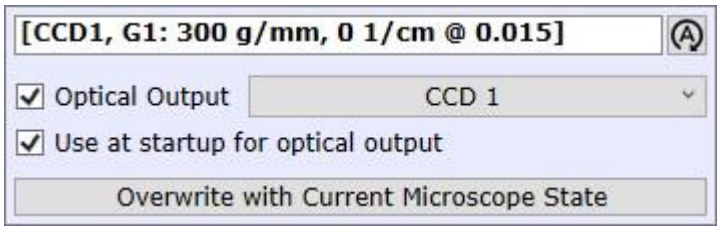
Add New State
Adds a new state to the state list.

Duplicate State
Duplicates the selected state to a new state.

Delete State
Deletes the selected state.

State List
Select a state to edit it.

Edit State



Name Edit
You can define a custom name for the state.

Automatic Name
Automatically creates a name from the current state settings.

Optical Output

If checked, an optical output will be selected when executing the state.

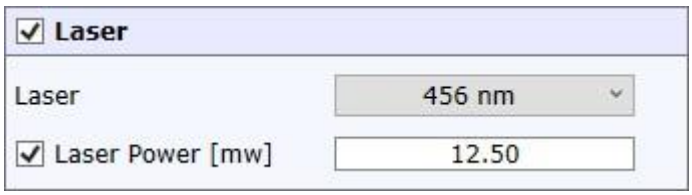
If you select CCD1/2/3, then the last selected state for this output will automatically be executed when changing the configuration in WITec Control.

Use at startup for optical output

If checked, this state will be executed when the output is selected for the first time (also on software startup).

Overwrite with Current Microscope State

Overwrites all settings with the current state of all devices.

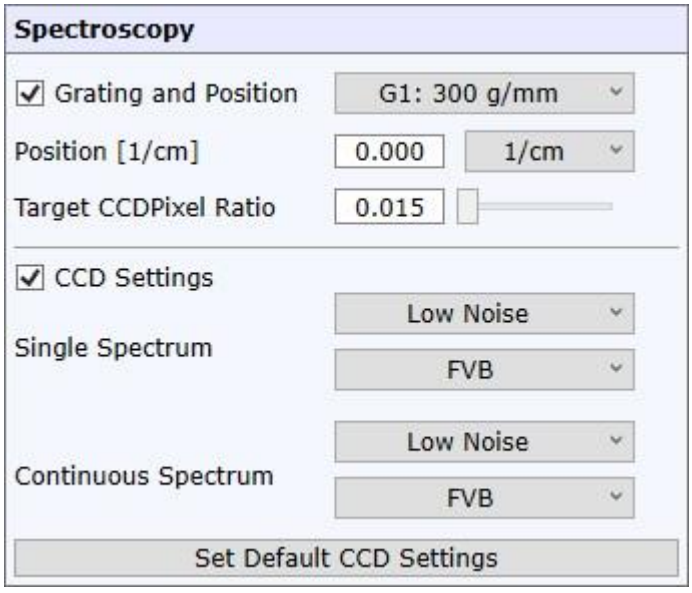


Laser

If checked, the desired laser will be selected when executing the state

Laser Power

If checked, the desired laser power will be selected when executing the state.



Grating and Position

If checked, the spectrograph will move to the desired grating and position when executing the state.

The position can be defined in wavenumbers [1/cm] or nanometer [nm].

The Target CCDPixel Ratio defines, on which pixel of the CCD you want to have the desired position to be.

E.g. Ratio = 0.0, Position = 200 -> the position 200 will be on the most left pixel of the CCD

E.g. Ratio = 0.5, Position = 200 -> the position 200 will be in the middle of the spectrum

E.g. Ratio = 1.0, Position = 200 -> the position 200 will be on the most right pixel of the spectrum

CCD Settings

If checked, the selected CCD Readout and Binning Modes will be selected when executing the state.

Set Default CCD Settings

Sets all comboboxes to the default CCD Settings defined by WITec.

Polarizer Angle

If checked, the desired polarizer angle will be selected when executing the state.

Sync Angle

If checked, the angle between polarizer and analyzer will be synchronized automatically when executing the state.

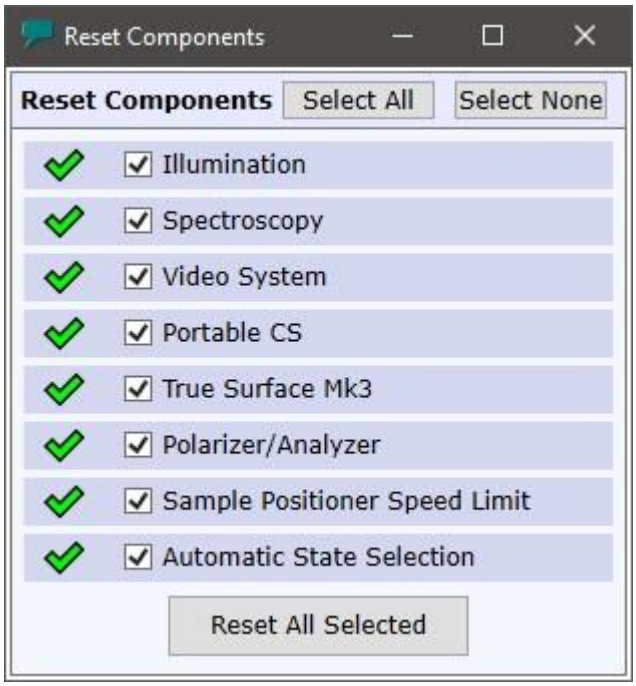
Analyzer Angle

If checked, the desired analyzer angle will be selected when executing the state.

Use Lambda/4

If checked, the lambda/4 plate will be marked as coupled and the desired position will be selected when executing the state.

State Resetter



Here you can reset the state for the following devices:

- **Illumination**
  Turns on Top Lamp
  Turns off Bottom Lamp
  Turns on all Smart Brightness features (Illumination + Video Exposure and Gain)
  Turns off all light sources (RISE)
  Turns off darkfield mode
  Sets Field/Aperture-Stops to default values

- **Spectroscopy**
  Uses the default CCD Camera Settings for all CCD Cameras (Readout and Binning Mode)
  Turns off "Calibration Lamp permanently on"

- **Video System**
  Selects the Top Camera, Disables Vignetting Correction

- **Portable CS**
  Deactivates the current learned portable coordinate system

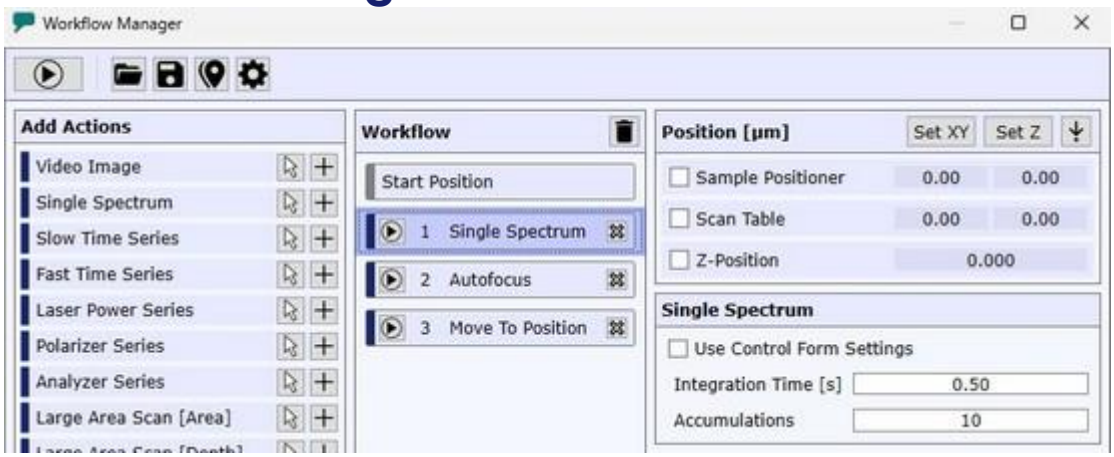
- **TrueSurface Mk3**
  Deactivates TrueSurface Mk3

- **Polarizer / Analyzer**
  Sets both to 0°, couples out analyzer, turns off sync
  If Lambda/4 coupled: sets to Linear 0°

- **Sample Positioner Speed Limit**
  Turns off XY Speed Limit and resets value

- **Automatic State Selection**
  Resets the automatic state selection on configuration/output change
  (Startup States and Last used state for each output)

Workflow Manager



The WITec Workflow Manager can be used to define a sequence of measurements and other actions in order to perform multiple measurements at different positions, each having customizable settings.

Top Buttons

Start / Stop Workflow
Starts or stops the workflow.
The progress is displayed on the Video Window.

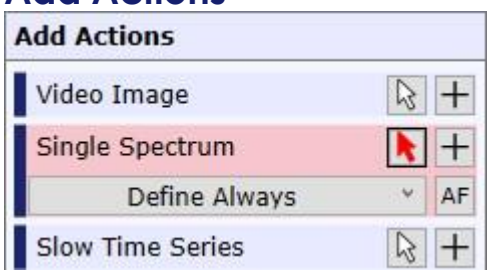
Load / Save Workflow
Here you can load or save a workflow from/to the hard drive.

Adjust Positions
If checked, you can go through all actions that define a position and adjust it:

- Press "Apply & Next" to apply the current position for the selected workflow action and move to the next action.
- Press "Return to Initial" to move to the position of the current action (reset changes)

Options
- Stays open during Workflow Run: if checked, the window stays open while workflow is running.

Add Actions

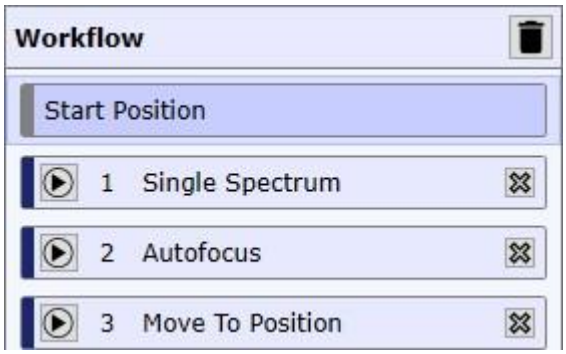


Add Action
Adds the desired action to the current workflow.

Add Action using the Listen Cursor Mechanism
If checked, you can click (or drag a line/rectangle, depending on the measurement type) in any viewer or the video window to add a workflow action at a certain position:

- Define Always: For each added action, the measurement parameters will be defined by the workflow manager
- Define Once: For the first added action, the measurement parameters will be defined by the workflow manager. All subsequent added measurements will use the parameters of the first action.
- Use Current Parameters: The measurement uses the parameters that were used in the last measurement of the same type (or: the parameters defined in the Control Window)
- AF: If checked, adds an autofocus action before adding the action.

Workflow List



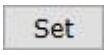
Shows all actions in the current workflow.
You can use the mouse drag drop feature to reorder the actions.

Remove All Actions
Removes all actions from the current workflow.

Run Action
Starts the desired action. For testing purposes.

Remove Action
Removes the action from the list

Start Position
If this element is selected, you can define a start position. It is used when a workflow is based on relative position definitions.
The first workflow action that defines an absolute position will override the start position.



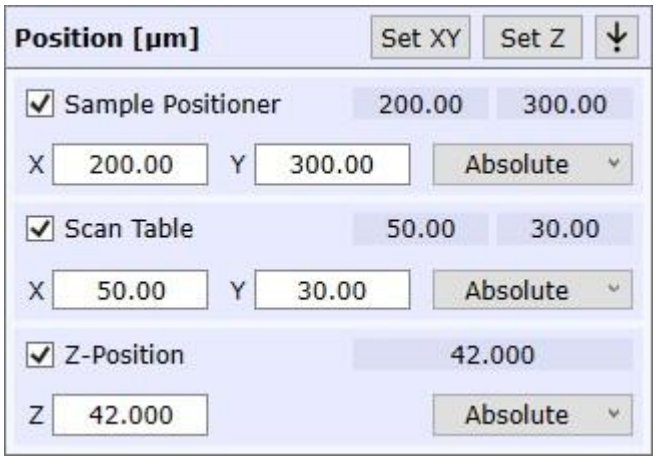
Set Start Position

Sets the current position (Sample Positioner, Scan Table, Microscope Z-Stage) as the start position.

Move to Start Position
Moves the sample positioner, scan table and microscope Z to the start position.

Position Parameters

Shows the position parameters of the currently selected action.



Set XY
Sets the sample positioner and scan table position parameters to the current position.

Set Z
Sets the microscope Z position parameter to the current position.

Move to Action Position
Moves the sample positioner, scan table and microscope Z to the center of the selected action.

Sample Positioner (Checkbox)
If checked, the sample positioner will be moved to a desired position before the action is executed.

Scan Table (Checkbox)
If checked, the scan table will be moved to a desired position before the action is executed.

Z-Position (Checkbox)
If checked, the microscope Z stage will be moved to a desired position before the action is executed.
Note that the current software limited range is allowed only.

X/Y/Z (Number Edits)
Here you can define the desired positions.

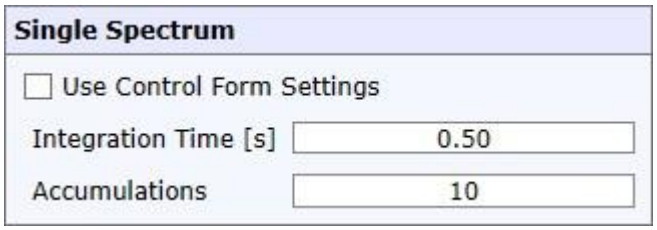
Absolute / Relative (Combobox)

- Absolute: defines an absolute position for the selected positioning system.
- Relative: defines a relative position. The values are added to the position of the previous action. The resulting absolute position can be calculated before running the workflow.

- Rel. AF/TSM (Z-Position only): defines a relative position, which is added to the resulting Z position of a previously performed autofocus or true surface Mk3 action.
  The absolute Z position is unknown before running the workflow.

Preview Position
Shows the preview position of the selected action.
If the checkbox is not checked or a relative position is defined for this action, then the position is calculated using positions of previous actions (and from the position defined in the "Start Position", if there are no actions with absolute positions defined).

Measurement Parameters



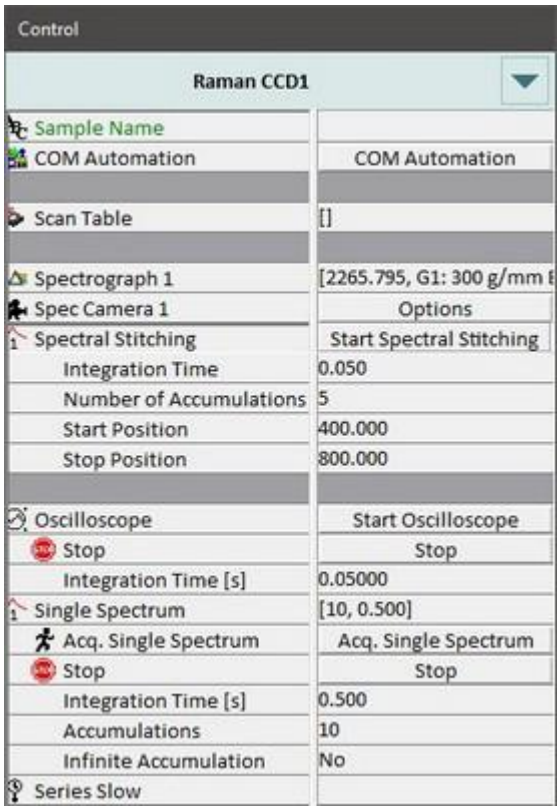
Here you can define all measurement parameters of the selected workflow action.
See Control Window for individual explanations of all measurement parameters.

Use Control Form Settings (Checkbox)
If checked, the action uses the current parameters defined in the Control Window (instead of the parameter settings in the workflow manager).
The control window holds the parameters of the last measurement. This way, you can also define the settings once for the first action, and check the checkbox in all subsequent measurements of the same type to reuse the same settings.

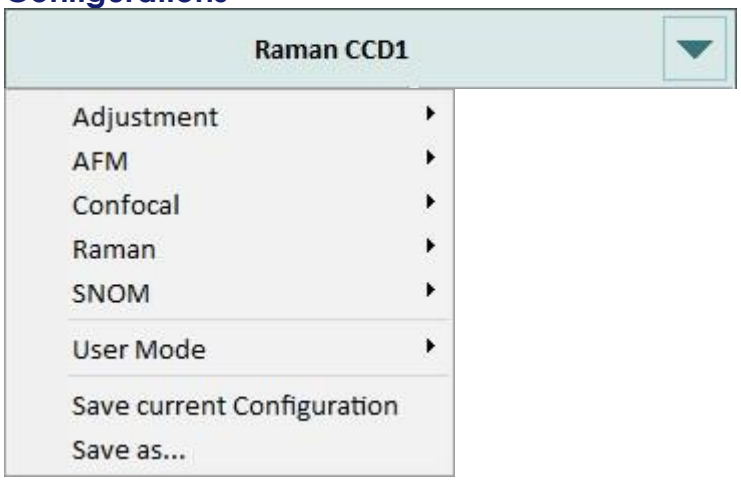
Control Window

Control Window Overview

Depending on the selected configuration and the installed hardware, the Control Window provides access to most of the hardware and measurement parameters:



Configurations



This area shows the currently selected measurement configuration.
You can click on the label or on the icon in order to select a configuration for a certain measurement, e.g. Raman, AFM, etc.
All parameters in the Control Window are stored in the configuration.
Further information about the usage of predefined configurations can be found in the operation guide.

User Mode

Here you can set the User Mode depending on the level of knowledge. This will hide or show certain parameters in the Control Window.

Beginner
Only basic parameters and functions of the configuration are available.

Expert
All parameters and functions of the configuration are available. Recommended setting.

Super User
All parameters are available like in the Default tree. Only use it if you know what you do.

Save current Configuration
Saves and overwrites the current configuration.

Save as...
Saves the current configuration into a new file.

> A configuration consists of two files. The style defines the appearance of the Control window, the other file contains all parameters.
>
> The shown configurations are stored in `%userprofile%\WITec\WITec Suite X.X\WITec Control\UserConfigurations`. Upon start WITec Control copies all configurations from `%allusersprofile%\WITec\WITec Suite X.X\Configs\WITec Control\UserDefaults\UserConfigurations` if not existing.

Navigation

Double-Click on a label on the left side or click on the little +/- sign to expand/collapse a sub tree or use the right/left arrow keys. It is possible to navigate through the parameters using the up/down arrow keys.

Edit values

Simply click on a row in order to edit a parameter. The value is changed by holding the Ctrl key and pressing the up/down key to increase/decrease the value. For larger increments hold Ctrl + Shift and use the up/down arrow keys.

If parameter names are shown as a red label, one of the parameters might be wrong, are out of range or a combination of parameters does not work.

Stop button

All Stop buttons within the Control tree will stop the currently running sequencer no matter in which subsection it is located. This applies also to the Stop button in the Main window.

Listen functions

The Listen functions within the Control tree enables to determine certain parameters like positions in a Graph viewer, Image viewer or in the video window by mouse.

An active Listen function is indicated by the text color of the axis position turning to red in the status bar of the Graph viewer or Image viewer (left) and by a symbol in the video window (right). Clicking on the red text or on the symbol in the video window turns off the Listen function.

Never

The Listen function is switched off.

Once

The Listen function is active. It is turned off automatically after a value is selected by mouse.

Multiple

The Listen function is active until it is switched off by the user by either setting it to Never or clicking on a red line in a status bar of the Graph viewer or Image viewer.

Context menu

It can be opened by clicking the right mouse button anywhere in the Control tree.

Create Default Tree

The Default tree shows all available parameters no matter which configuration is selected.

Use Configuration Layout

Changes back from the Default tree and shows parameters as defined in the style of the selected configuration.

Collapse

Closes all sub-trees.

Auto Collapse

If this is selected, open sub-trees are closed as soon as a new sub-tree is opened.

Style Editor

The Style editor enables to change the style of a configuration using a graphical user interface. This is for WITec-internal use only!

Parameter groups

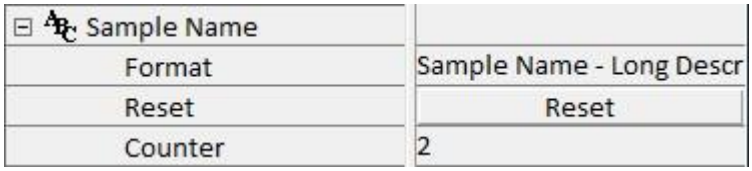
Here you can find information about specific parameter groups. If the parameter description is gray, the parameter is only available in the default tree.

- Sample Name
- COM Automation
- Heating
- Tip Approach
- Scan Table
- Detection
- Photon Detector Amplifier
- Topography Correction
- Optical Distance Sensor
- Spectrograph
- Spec Camera
- Oscilloscope
- Time Series (Fast)
- Single Spectrum
- Distance Curve
- Line Scan
- Image Scan
- Image Scan (Multi Pass)
- Large Area Scan
- Series Slow
- Sample Raster
    - Process Script

- Spectral Stitching
- Adjustment
- Frequency Sweep
- Feedback settings
- PFM Control
- EFM Control
- Kelvin Probe Control
- Piezoresponse Control
- Time Spectrograph
    - Point Viewer
- Spectral Auto Focus
- Signal Stabilization
- Lithography
    - Lithography Commands
- Auto Save
- Data Channels

Sample Name

The Sample Name parameter group allows to specify a sample name, which is then used in the name of new created data objects. The following configuration independent parameters are available:



Sample Name

Shows the X position of the scan table and can be increased or decreased up to the limits of the scan table. If an out of range value is entered, the scan table will move to its maximum position which will then also be displayed as the parameter value.

Format

Different predefined formats are available:

Sample Name - Long Description

A combination of the sample name and a long version of the used measurement mode is used as name for new data objects.

Sample Name - Short Description

A combination of the sample name and a short version of the used measurement mode is used as name for new data objects.

Sample Name

Only the sample name is used as name for new data objects.

Reset

The button resets the counter.

Counter

Value of the counter used as number for the next created data object.

Format

Defines the complete name format for new data objects.

Delimiter

Defines the delimiter between the name parts.

COM Automation

This parameter group controls for the remote access over the COM interface of WITec Control.

Further information:

- COM Automation

COM Automation	COM Automation

COM Automation

This button allows exclusive write access to a remote application. A window opens which blocks WITec Control user entries and enables to revoke remote access by a button.

Allow Remote Read Access

It is possible to deny remote read access using this parameter.

Allow Remote Access

This button has the same function like the COM Automation button.

Revoke Remote Access

This button can revoke the remote access.

Heating

The Heating parameter group controls the WITec heating stage which is driven through the alphaControl controller.

Upon start of the software, the heating stage is calibrated using the PT100 element within the stage. Information about this process is displayed in the message window. The state of the heating stage (enabled, disabled, stabilized,...) as well as the current temperature while heating is also displayed in the message window.

If the heating stage is present, the temperature determined at the start and the end of a measurement (such as an image scan) is saved in the automatically created text object describing the scan.

The following parameters allow control over the the heating stage:

Heating	[20]
Enable Temp. Control	Yes
Target Temp. [°C]	20
Temperature Ramp	
End Temp. [°C]	20
Temp. Gradient [°C/	5
Start Gradient	Start Gradient
Stop Gradient	Stop Gradient
Calibrate	Calibrate

Enable Temp. Control

Using this parameter the temperature control can be enabled (Yes) or disabled (No). If the temperature control is disabled, all other parameters in this group will be inactive and the target temperature is set automatically 20 °C.

Target Temp. [°C]

Here the target temperature of the stage can be entered. If it is changed, the stage will try to reach the new target temperature using the P, I and D parameters for the Proportional(P) Integral(I) Differential(D) regulating loop.

Temperature Ramp

Using this group it is possible to ramp the temperature linear to a certain target temperature. The following parameters allow this control:

End Temp. [°C]

The temperature the stage should reach at the end of the ramp.

Temp. Gradient [°C/min]

The slope of the temperature ramp in °C per minute.

Start Gradient

Starts the temperature ramp.

Stop Gradient

Stops the temperature ramp.

Calibrate

This button triggers the automatic calibration of the heating stage using the PT100 element inside the stage. (This procedure is automatically executed upon starting WITec Control.)

P-Gain

The P-gain for the PID regulating loop.

I-Gain

The I-gain for the PID regulating loop.

D-Gain

The D-gain for the PID regulating loop.

Get Temperature Interval [s]

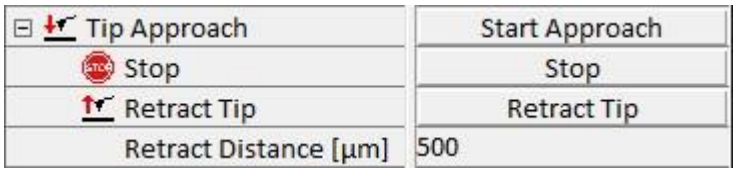
This parameter determines in which time intervals the temperature is measured.

Tip Approach

This sequencer is used for the tip approach for AFM and SNOM measurements with the alphaControl. The following parameters are used to control and perform a tip approach.

Further information (Operation Guide):

- AFM Overview
- AFM Procedure



Start Approach

This button starts the tip approach procedure.

The automatic approach may fail if one of the following problems occur:

- The approach is interrupted by the user clicking on a Stop-button.
- Instability of the sum signal. If the sum-signal increases by 10 % or decreases by 20 %.
- No contact was registered after the microscope moved 2 mm down.
- The setpoint has been altered by the user during the approach.
- The setpoint has been reduced by 50 % during an AC approach.

Retract Distance [µm]

This parameter defines the upward travel distance of the microscope z stage during tip retraction.

Retract Tip

This button retracts the tip using the microscope z stage by the defined retract distance.

Out of Contact Speed [µm/s]

This parameter defines the speed of the microscope Z stage during an approach before the feedback setpoint is reached (see Section 3.4.5).

In Contact Speed [µm/s]

This parameter defines the speed of the microscope Z stage during an approach after the feedback setpoint is reached (see Section 3.4.5). As the scan table (Section 3.4.3) is completely extended in the Z-direction, the microscope Z stage travels at the in contact speed downwards, while the feedback loop retracts the scan table to its final Z position (see below).

Final Position Z [µm]

This parameter sets the z-position of the scan table at which the approach is completed.

Approach Method

The tip approach depends on the selected feedback settings. This function allows the selection of the proper approach method for the following modes:

Contact Mode

During the approach in contact mode, the tip is in contact with the surface as soon as the setpoint is reached. At this point the cantilever is bent and a force, defined by the setpoint and the spring constant of the cantilever, is acting between tip and sample. Following this the scan table retracts to its middle position while the microscope Z stage simultaneously compensated for this movement in order to keep the bending of the cantilever constant.

AC Mode

During the approach in AC mode, the cantilever is oscillating at a defined free amplitude before the sample is reached. As the tip reaches contact with the sample this amplitude is damped and the tip is in its designated position once the setpoint defined by the feedback has been reached. Due to long range forces however, the amplitude damping can begin before the tip is in physical contact with the sample. Therefore, the indication that the tip is in contact during AC approach is the change of phase shift φ when the setpoint is decreased slightly. To determine the phase

shift reliably, φ is sampled continuously during the tip approach. As soon as the final Z position is reached, the setpoint is decreased further and the standard deviation σ(φ) is determined. If σ(φ) < σmax (the maximum deviation of φ) the setpoint is decreased further while the final Z position is corrected using the microscope Z stage. The tip approach is stopped when the standard deviation σ(φ) ≥ σmax.

For safety reasons, the AC mode tip approach is also stopped if the current setpoint is smaller than half of the initial setpoint.

PFM Mode

The approach in PFM mode is similar to the approach in contact mode with the difference the the signal used for regulation is not the T-B signal (ie the signal of the constant bending of the cantilever). The signal used here is the Fmax signal determined from the PFM curve. This ensures a constant maximum force on the cantilever.

> The approach in PFM mode will only be successful if the Fmax window is selected properly.

Tip Regulated Contact Mode

In this mode, the tip positioning in Z direction is regulated by the positioning piezo located in the cantilever arm instead of the Z axis of the scan table. Therefore the retracting of the tip is done to the middle position of this piezo and not of the scan table.

PhiStdDevAccums

To calculate the standard deviation σ(φ), n samples of φ are utilized. The parameter PhiStdDevAccums defines the number n of the φ samples.

Stop@PhiStdDev

This parameter defines the maximum deviation σmax at which the AC approach is stopped.

Scan Table

The Scan Table parameter group is for controlling the position of the piezo stage.

⊟ Scan Table	[0.000, 0.000]
Listen Position	Never
Position (X) [µm]	0.000
Position (Y) [µm]	0.000
Position (Z) [µm]	0.000
Position (Z, Microscope	0.000

Position(X/Y/Z)[µm]

Shows the X position of the scan table and can be increased or decreased up to the limits of the scan table. If an out of range value is entered, the scan table will move to its maximum position which will then also be displayed as the parameter value.

Position(Z, Microscope)[µm]

This has the same function like the Move to Z-Position button in the Video Control Window.

Listen Position

The listen position parameter allows the selection of coordinates for the positioning of the scan table and/or the microscope Z stage using any graph or image with position information. The X and Y coordinates can be selected using any image captured in the X-Y plane such as a 2D scan, video

image or a bitmap and also graphs from cross sections or line scans. The Z axis can be altered by clicking on a depth scan for example.

Move mode

Using this drop down menu, the behavior of the Z axis can be controlled with the following options:

I Z for Feedback

This mode is typically used for SNOM or AFM measurements. Here the Z axis of the scan table is controlled through the PI controller using the feedback signal as a reference. If this mode is selected, the position of the Z axis of the scan table cannot be changed manually.

I Z by Microscope

This is the typical mode of operation when performing confocal or confocal Raman measurements, in which the microscope Z stage is used as the Z axis of the internal coordinate system. This allows depth scans greater than the piezo z range.

I Z by Scan Table

Here the Z axis of the scan table is the Z axis of the internal coordinate system. Additionally, using the Z axis of the scan table only allows for depth scans within the piezo z range.

I No Z Movement

This is selected, if no z movements are possible during a measurement.

Detection

The Detection parameter group is for controlling photon counting devices and the inverted beampath.

Further information (Operation Guide):

- Confocal
- SNOM
- StrobeLock

Flip Mirror 1

This parameter controls the flip mirror in the inverted beampath (for systems build until 2015). It is possible to switch between Video Camera and Detection. This is done automatically when a measurement is started or stopped.

PMT/APD/PMT2 On

Pressing this button turns on the respective photon counting device. This is done automatically when a measurement is started.

If the count rate exceeds 4500 kHz, the photon counting device will be turned off automatically for protection. In this case the button can be used to turn on (reset) the photon counting device after reducing the light intensity.

It is the responsibility of the user to avoid exposing too high light intensity to the photon counting device.

Photon Detector Amplifier

This parameter group controls an amplifier e.g. for an analog PMT. The parameters are:

Photon Detector Amplifier	
Controlled Amplifier	PrimaryAmplifier
Gain [V/A]	10^5 (High Speed)
Bandwidth [kHz]	500.0
Coupling	DC
Control Voltage [V]	0.50
Offset [%]	0.10

Controlled Amplifier

The amplifier can be selected. In normal case only one amplifier will be available.

Gain [V/A]

This parameter defines the amplification. It is also possible to choose between low noise and high speed.

Bandwidth [kHz]

Read-only parameter, which shows the bandwidth in kHz dependent on the selected Gain.

Coupling

AC or DC can be selected here dependent on the type of voltage that should be amplified. DC should be selected for the analog PMT.

Offset [%]

This parameter is for compensation of the offset. This is factory defined and should only be changed if really necessary.

Control Voltage [V]

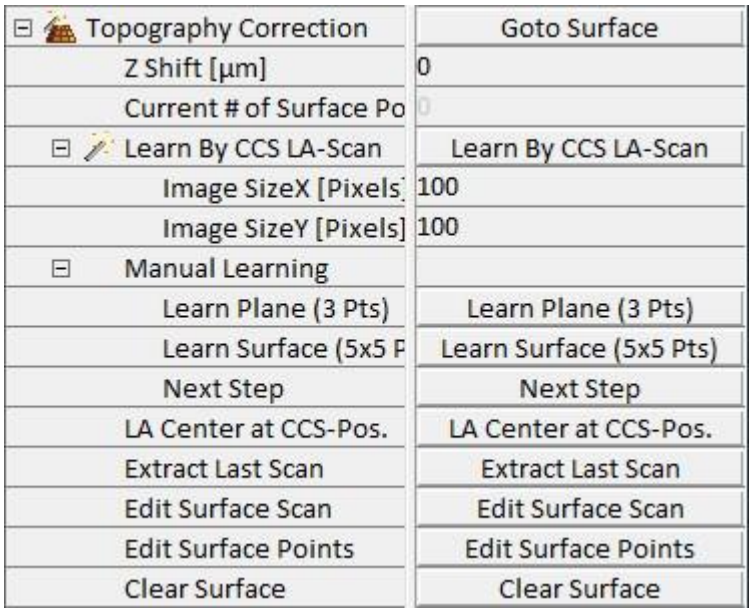
The Control voltage of the PMT changes its sensitivity. It can be adjusted between 0 V and 1.2 V.

Topography Correction

The Topography Correction parameter group is for learning a topography either manual or by using a Confocal Chromatic Sensor (CCS) for use with a Large Area Scan.

Further information (Operation Guide):

- Manual Topography Correction
- TrueSurface



Goto Surface

This will move the z-stage into the learned focus position, if the current position exists in the current surface and a z-value can be interpolated.

> If the z-stage is moved by the user in while the user-z is selected in microscope z, an offset to the learned focus will be added and this button will not go to the correct focus position anymore.

Z Shift [µm]

This parameter can be used to vary define an offset to the learned focus position. This can also be done while a surface corrected large area scan is performed.

Current # of Surface Points

This will just show the number of points that define the current surface.

Learn By CCS LA-Scan

This will scan the defined large area scan geometry using the CCS sensor.

Image SizeX/Y [Pixels]

This will define the image pixel size of the large area scan for the CCS learning.

Manual Learning

This group of parameters enables to learn simple surface without CCS. The progress is shown in the messages window.

Learn Plane (3 Pts)

This will start a sequencer which will move the sample to 3 positions defined by the large area scan geometry for a tilt correction.

Learn Surface (5x5 Pts)

This will start a sequencer which will move the sample to 25 positions defined by the large area scan geometry for a simple surface correction.

Next Step

Press this button after focusing the current point to move to the next point or to finish the learning.

LA Center at CCS-Pos.

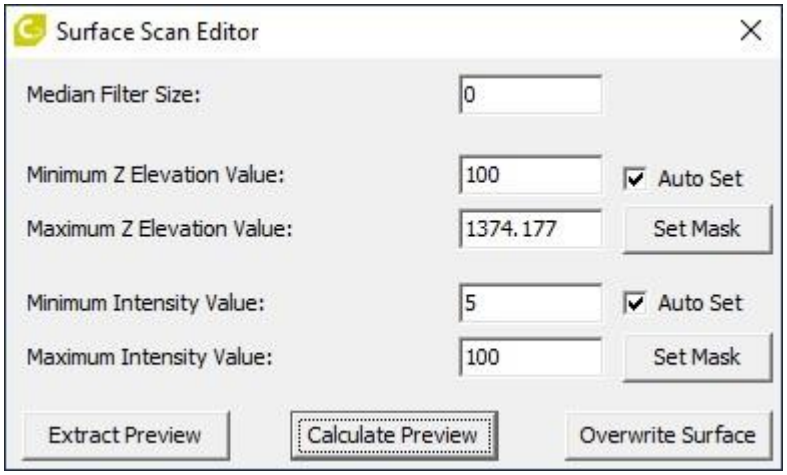
This will set the large area scan geometry center to the current x-y-position and uses the currently measured CCS-Elevation for setting the z-position.

Extract Last Scan

This button enables to extract the Elevation and Intensity data of the last scan to the Project Manager.

Edit Surface Scan

When a surface was learned using the CCS, this button opens a dialog to change the current surface, e.g. mask out wrong elevation values. For this the last measured Elevation and Intensity image are also opened.



Median Filter Size

Defines the size of a median filter, which is applied on the surface.

Minimum Z Elevation/Intensity Value

Minimum allowed Z elevation/intensity value. Lower values will be added to the mask.

Maximum Z Elevation/Intensity Value

Maximum allowed Z elevation/intensity value. Higher values will be added to the mask.

Auto Set

If set, the mask in the Elevation or Intensity image will automatically update when a value is changed.

Set Mask

This button manually updates the mask in the Elevation or Intensity image.

Extract Preview

This button extracts the preview to the Project Manager.

Calculate Preview

This button recalculated the preview applying the currently selected masks and filter size.

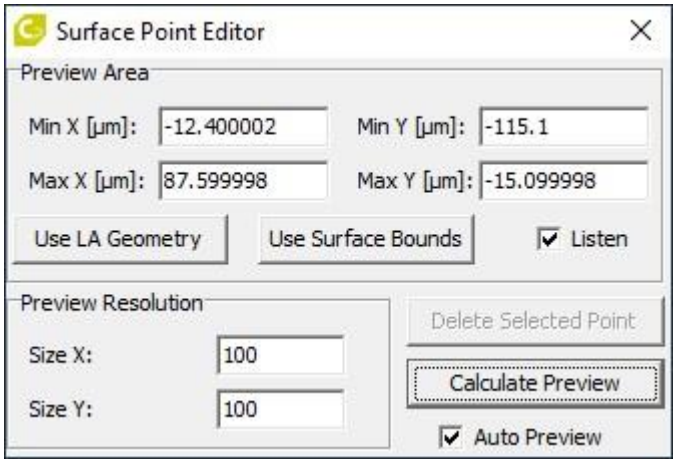
Overwrite Surface

Pressing this button overwrites the surface by the current version with selected masks and filter applied.

Edit Surface Points

This will open a dialog to see what the surface (learned manually or by CCS) would look like when a

different area is selected.



Min/Max X/Y [µm]

Bounds of the current preview.

Use LA Geometry

The bounds of the Large Area Scan are used.

Use Surface Bounds

The bound of the current surface are used.

Listen

If this is activated, the center position of the current area can be changed by clicking in an image or in the video window.

Size X/Y

Changes the resolution of the preview image by changing the number of pixels in X or Y direction.

Calculate Preview

This button will update the preview using the current values.

Auto Preview

If this is activated, the preview is updated on any change.

Clear Surface

This will clear the current surface.

Optical Distance Sensor

The Optical Distance Sensor parameter group contains parameters to control the Confocal Chromatic Sensor (CCS).

Further information (Operation Guide):

- TrueSurface
- Profilometer

Optical Distance Sensor	[100, 10000, 1]
Sensor Optic	Nr.1: 2000µm
LED Intensity [%]	100
Sampling Rate [Hz]	10000
Averaging Factor	1
Peak Detection Mode	First Peak
Elevation @ Focus-Z [µ	1000.000

Senor Optic

Using this parameter the used senor can be selected and thereby its calibration is loaded. In normal case only one sensor is installed. The sensor range is shown here.

LED Intensity [%]

Dependent on hardware a read-only value. It shows the LED Intensity of the MicroEpsilon-Controller-intern LED. Always adjust this value for the respective sample. The status section will show the current CCS intensity, this should be above 1 % and below 100 %.

Sampling Rate [Hz]

This controls the integration time of the CCS CCD chip. The highest possible sampling rate is always recommended (due to Dark Calibration errors on lower sampling rates/higher integration times).

Averaging Factor

Elevation values will just be averaged.

Peak Detection Mode

Dependent on hardware a read-only value. It defines how the sensor will calculate the Elevation from the spectrum.

Highest Peak

Used for opaque samples.

First Peak

Used for transparent samples. Be careful when using this mode, since the change is much higher to get a wrong value (because e.g. sometimes noise is interpreted as the first peak)

Elevation @ Focus-Z [µm]

Read-only parameter, which should show half of the sensor range.

Calibration

Parameters for the sensor calibration.

Dark Calibration

This button starts the dark calibration. Make sure that the light of the CCS is blocked before starting.

Spectrograph

For each spectrometer connected to the alphaControl one Spectrograph X parameter group will be available.

Most of the spectrograph settings are defined under Spectroscopy Settings.

Spectrograph 1	[2039.069, G1: 600 g/mm BLZ
Spectral Unit	rel. 1/cm

Spectral Unit

Changing the spectral unit will change the X axis and thus the display of the hardware spectrum currently recorded. Any measurement started after changing this parameter will also use the unit set with this parameter. Additionally, it defines the unit for the spectral ranges of spectral stitching and spectral autofocus. The units available are:

- nm
- µm
- 1/cm
- rel. 1/cm
- eV
- meV
- rel. eV
- rel. meV

Only available on special systems:

Grating

The grating parameter allows the selection of the gating used for spectrometry. The gratings listed in the the pull down menu depend on the individual configuration but generally have the form

[Grating number: Groove density Blaze wavelength] and an example typical for the UHTS 300 would be G1: 600 g/mm BLZ=500nm.

Laser Wavelength [nm]

This parameter should contain the wavelength of the laser used for the spectral measurements.

Center Wavelength [nm]

The wavelength hitting the center of the CCD chip can be adjusted using this parameter. This wavelength will then also be the central wavelength in the spectra displayed in the software. To change the central wavelength, the software calculates the necessary rotation of the grating selected.

Spectral Center

The spectral center is identical to the center wavelength. The only difference is that the units this central position is described by are variable and adjustable using the Spectral Unit parameter (see below).

Listen

The Listen parameter allows the selection of a new spectral center using any spectrum. The grating will be moved so that the position clicked on in the spectrum will be the new central wavelength.

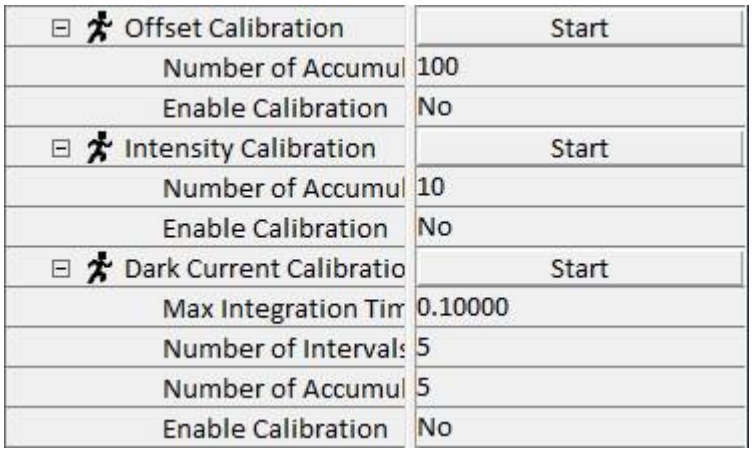
Spec Camera

For each spectral camera connected to the computer, one Spec Camera X parameter group will be available.

Further information:

- Service Monitor (CCD temperature and status)
- Spectroscopy Settings (CCD readout and binning)
- InGaAs camera (Operation guide)

The following parameters are available for InGaAs cameras only.



Offset Calibration

The calibration routine measures a series of spectra (accumulations adjustable by the user) with 0 s integration time and no light falling on the detector. If enabled, all measured spectra are now subtracted by the average spectrum of the acquired series. In order to get only positive values an offset of 2000 is added. Existing Intensity and dark current calibration are now invalid and therefore disabled automatically.

Intensity Calibration

In order to calibrate the gain of each pixel, a spectrum with a smooth distribution is needed. The gain of each pixel is calculated by the average intensity of its neighbor pixels. At the beginning of the algorithm an integration time is searched that gives a maximum signal of 50000. Now at 10 different integration times a series of spectra (accumulations adjustable by the user) is acquired. From this data a gain lookup table is calculated. An existing dark current calibration is now invalid and therefore disabled by the software.

Dark Current Calibration

The dark current calibration routine acquires a sequence of time series at different integration times. The user can change the maximum integration time, the number of time series and the number of accumulations. From this data the dark signal rate is calculated for each pixel. Depending on the current integration time the dark signal is subtracted from the measured spectra. For best results the proposed integration times used for the measurements need to be covered by this calibration.

Start

This button starts the calibration. Only possible for Intensity and Dark Current Calibration, if the offset calibration is valid and on. Make sure no light falls on the camera for Offset and Dark Calibration. The recorded calibration is saved in the internal settings folder.

Number of Accumulations

This parameter describes how many spectra will be accumulated.

Enable Calibration

This parameter turns the respective calibration on and off. It is set automatically to Yes, if the Calibration was successful.

Number of Intervals

The Number of Intervals determines how many different integration time steps are taken between zero and the maximum integration time.

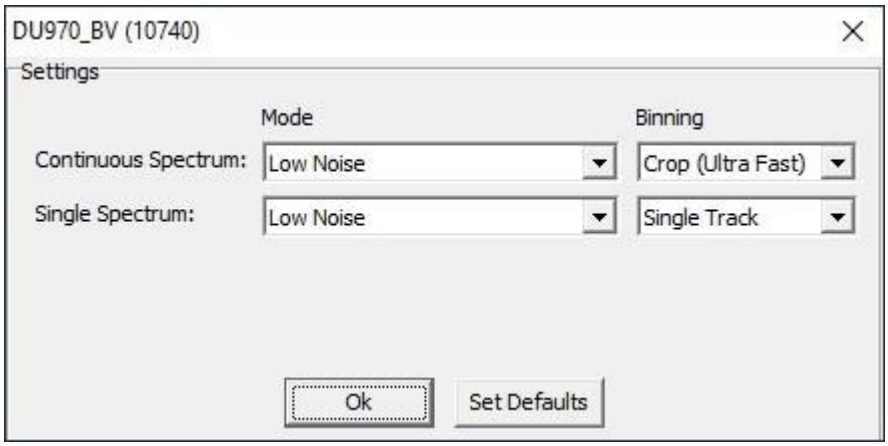
Max Integration Time

This parameter defines the maximum integration for the Dark Calibration.

Only available on special systems:

Options

Pressing this button, opens the spectral camera options dialog. The available options depend on the camera model, so maybe not all options are available in your case.



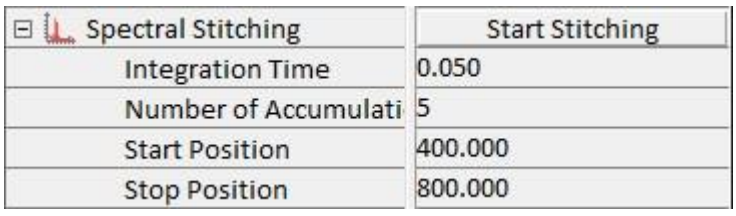
For explanations of all options please refer to Spectroscopy Settings.

Spectral Stitching

This parameter group allows recording of single spectra with an extended spectral range. Therefore the grating is moved to different spectral positions to cover the defined range. The data is combined to one spectrum.

Further information (Operation Guide):

- Raman



Start Stitching

Pressing this button, will start the acquisition using the current parameters.

Integration Time

This parameter defines the integration time at each spectral position.

Number of Accumulations

This parameter describes how many spectra will be accumulated at each spectral position.

Start Position

Defines the spectral start position of the spectral range for the resulting spectrum in the spectral unit defined in the Spectrograph section.

Stop Position

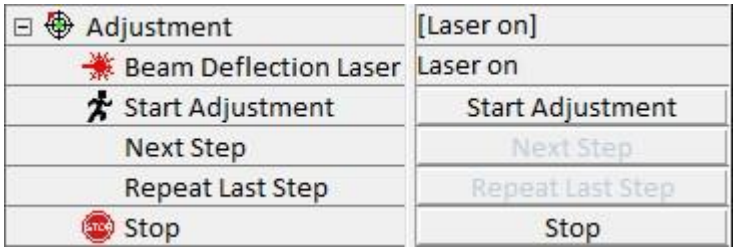
Defines the spectral end position of the spectral range for the resulting spectrum in the spectral unit defined in the Spectrograph section.

Adjustment

The adjust sequencer offers software assisted step by step alignment procedures for certain measurement modes. It automatically changes the parameters required for the adjustments. If necessary, the sequencer requests user inputs and/or manual adjustments from the user via the messages window, where information about the progress of the adjustment is also displayed. The adjustment can be stopped at any time by pressing any stop button within the software.

Further information (Operation Guide):

- AFM Overview
- AFM Procedure



Beam Deflection Laser

This parameter controls whether the laser is On or Off.

Start Adjustment

Pressing this button will start the adjustment procedure using the current set of parameters.

Next Step

When executing an adjustment this button will become active. After finishing a task given to the user by the sequencer via the message window, this button should be pressed to continue the adjustment.

Repeat Last Step

This button goes back one step and allows repetition of the last task performed during the adjustment.

Adjust Method

This parameter defines for which method the adjustment will be performed.

Frequency Sweep

The frequency sweep varies the frequency of the output signal of the lock-in amplifier in order to determine the response in amplitude and phase of the detected signal as a function of the excitation frequency. E.g. in AC mode this is used to find the resonance frequency of the cantilever. Amplitude and phase are represented in a graph viewer.

Further information (Operation Guide):

- AFM AC mode
- AFM AC parameters

Start Sweep

This parameter starts the frequency sweep.

Listen Range

From the graph which displays the frequency sweep, a frequency range can be selected and the corresponding values will automatically be entered as the initial and final frequency.

Initial Frequency [Hz]

This parameter defines the initial frequency of the frequency sweep.

Final Frequency [Hz]

This parameter defines the end frequency of the frequency sweep.

Divisions

This parameter defines the number of steps between the initial and final frequency.

Create Data

This parameter allows the user to either only view the frequency sweep or to save the frequency sweep as a graph data object in the project manager.

Auto Resonance

This button starts the auto resonance procedure. It automatically determines the resonance frequency of the cantilever for AFM AC Mode. Similar to the frequency sweep, the frequency of the output signal of the lock-in amplifier is varied and the amplitude of the cantilever is measured at every frequency. In this procedure a coarse frequency sweep is performed over a large frequency range (typically from 10 kHz up to 500 kHz). As the maximum amplitude of the cantilever is approached, a second fine frequency sweep is performed to determine the exact resonance frequency.

The graph represented in the graph viewer displays the amplitude and phase variations close to the resonance frequency. The resonance frequency is automatically set as the driving frequency and the phase offset is adjusted to zero.

Initial Start Frequency [Hz]

This parameter sets the initial start frequency for the auto resonance procedure.

Initial End Frequency [Hz]

This parameter sets the initial end frequency for the auto resonance procedure.

Driving Amp. pk-pk [V]

This parameter is used to set the output of the internal oscillator of the lock-in amplifier. This amplitude can be varied from 0–20 V and is used as the drive amplitude.

Listen Frequency

With the frequency cursor, the driving frequency can be set by clicking on any graph viewer linked to a frequency.

Driving Frequency [Hz]

This parameter is used to set the driving frequency of the reference oscillator.

Filter Frequency [Hz]

This parameter sets the low pass filter for the PSD outputs of the lock-in amplifier. This filter is implemented as a 3rd order IIR filter.

For AFM AC Mode measurements the default setting of this filter is 10 % of the driving frequency.

Phase Offset [°]

The measured phase can be shifted with this parameter.

Auto Phase

This button sets the phase offset in a way that the current measured phase becomes zero.

Feedback settings

The feedback settings allow access to the parameters used in conjunction with the PI control of the scan table Z axis.

Further information (Operation Guide):

- AFM Overview
- AFM Procedure

- PI Controller

Feedback Settings	[1.5, 5, 5]
Setpoint [V]	1.5
P-Gain [%]	5
I-Gain [%]	5
PI Controlled Channel	LockIn R

Setpoint [V]

The setpoint voltage can be adjusted from -10V to +10V and is compared to the voltage measured from the PI Controlled Channel (see below).

P-Gain [%]

This parameter allows the increase or decrease of the proportional gain of the PI controller from 0 % to 100 %. It might be necessary to adjust this value to avoid oscillations due to the natural frequency of the cantilever or oscillations induced by the sample.

I-Gain [%]

This parameter allows the increase or decrease of the integral gain of the PI controller from 0 % to 100 %. It might be necessary to adjust this value to avoid oscillations due to the natural frequency of the cantilever or oscillations induced by the sample.

PI Controlled Channel

With this parameter, the channel which is compared to the setpoint can be selected. The following options are available for selection:

Top-Bottom

This signal is the difference between the electrical signal received from the top and the bottom halves of the four quadrant diode. This is the typical setting for AFM and SNOM contact measurements.

LockIn R

LockIn R is the amplitude recorded through the Lock-In amplifier. This channel is typically selected if the measurement mode is AFM AC.

Fmax

In this selection the maximum force, as determined from the peak of the pulsed force curve in DPFM, is used as the controlled variable.

off

No channel is used as an input and the PI controller is off.

Aux1

The signal read from the Aux1 input is used as the controlled variable.

Aux2

The signal read from the Aux2 input is used as the controlled variable.

Z-sensor

Using this signal, the capacitive sensor in Z direction of the scan table is used as an input and the system then acts as an active feedback controller for the Z position of the scan table.

Inverted

This variable allows the inversion of the control deviation signal. This is due to the fact that in AFM contact mode the scan table should retract if the signal is too high whereas it should move up in AFM AC mode.

HV Amp Active

During AFM measurements, the regulation of the bending of the cantilever can be achieved in two different ways. Either the Z axis of the scan table is used or a cantilever arm with a piezo positioning element. If such a cantilever arm is used, a high voltage signal needs to be provided to the piezo. The HV Amp Active can therefore be activated (setting YES) or not (setting NO). The signal is then automatically amplified and directed to the respective hardware.

> If the regulation using the cantilever with the positioning element is used, the topography readout of the scan table will not show the topography since the Z position of the table will be held constant during those measurements. Instead the feedback signal recorded can be used to derive the topography from it.

Output Limitation Active

This parameter is generally set to Yes to protect the controlling card of the scan table. This is necessary because the output of the alphaControl is ±10V which exceeds the maximum range of the controlling card of the scan table.

Output Limitation Range

Here a multiplicative factor is entered (typically 0.65) which if multiplied with the voltage output range of the alphaControl (20V) results in the correct range of the controlling card of the scan table (13V). The minimum and maximum values are 0 and 1 respectively.

Output Limitation Offset

A multiplicative factor for the offset of the signal in the range from -1 to +1 can be entered as the output limitation offset. Multiplying this factor with the positive voltage range of the controller (+10V) results in the offset necessary for the electronics of the scan table.

PFM Control

The PFM control parameter group contains the additional control parameter required for the acquisition of DPFM images.

Further information (Operation Guide):

- AFM DPFM
- AFM DPFM parameters

PFM Control	[Off, 1000]
Data Sampling	[Both]
Bandwidth Limited	Both
Max. MB/s	2.00
Max. pts/period	1000
Modulation	Off
Driving Amp. pk-pk [V]	0
Driving Frequency [Hz]	1000
Excitation Phase [°]	0
Reference Modulation	0
Listen (F max)	Never
Fmax Window Start	0
Fmax Window Widt	0
Listen (Adhesion)	Never
Adhesion Window S	0
Adhesion Window V	0
Listen (Stiffness)	Never
Stiffness Window St	0
Stiffness Window W	0

Data Sampling

This section controls the data transmission from the AlphaControl. A higher sampling rate could improve determination of adhesion,

Bandwidth Limited

Defines which value should be used as limit.

Max. MB/s

Limited by data size per second.

Max. pts/period

Limited by data points per period.

Both

Limited by both values.

Max. MB/s

Maximum data rate depending on the data size per second.

Max. pts/period

Maximum data rate depending on the number of data points per period.

Modulation

This binary variable either turns the modulation of the cantilever On or Off.

Driving Amp. pk-pk [V]

The peak to peak voltage of the sinusoidal modulation of the cantilever can be entered as a value between 0 and 20 V.

Driving Frequency [Hz]

The frequency of the cantilever oscillation can be entered as a value between 1 and 10000 Hz.

Excitation Phase [°]

The excitation phase can be adjusted in order to ensure that the pulsed force curve will be displayed in the graph window in the standard way with the snap-in near the left hand side of the window, then the force increasing to the maximum force, followed by the decrease of the force up to the maximum adhesion and the free oscillation after the snap-out as shown in Fig. 1

.

Reference Modulation Phase [°]

The reference modulation phase can be adjusted to ensure that the sinusoidal reference modulation signal displays a minimum where the DPFM curve displays a maximum. This is necessary for the proper representation of the pulsed force curves as force-distance curves.

Listen (Fmax/Adhesion/Stiffness)

The listen parameter allows the selection of the angular range for the determination of the maximum force/adhesion/stiffness from the displayed DPFM curve by mouse.

Fmax/Adhesion/Stiffness Window Start [°]

The start of the region in the DPFM curve where the hardware will search for the maximum force or determine the adhesion/stiffness can be defined from 0 to 360° using this parameter.

Fmax/Adhesion/Stiffness Window Width [°]

The width of the region in the DPFM curve where the hardware will search for the maximum force or determine the adhesion/stiffness can be defined from 0 to 360° using this parameter.

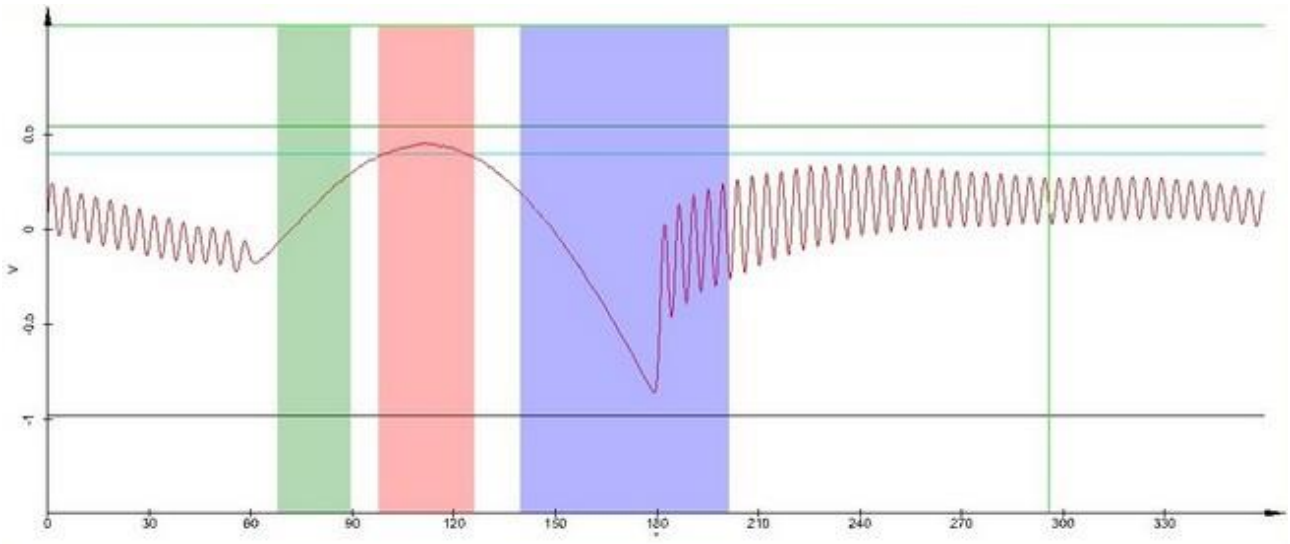


Fig. 1: The typical PFM curve with the search regions marked. (Green = Stiffness; Red = Fmax; Blue = Adhesion)

EFM Control

The EFM control parameter group enables to provide a voltage on a output of the alphaControl. This can be used for Electric Force Microscopy (EFM), to supply the cantilever tip with a voltage, or for other purpose.

Further information (Operation Guide):

- EFM Overview

EFM Control	
EFM Output	Disabled
DC Component [V]	0.000
Signal to Output	DC Only
Output DAC	Dither DAC

EFM Output

Enables or disables the output.

DC Component [V]

Adjusts the voltage supplied at the output, from -10 V to 10 V.

Signal to Output

Defines the type of voltage.

DC Only

A DC voltage is supplied. This is used in normal case.

AC Only

An AC voltage is supplied.

Output DAC

This parameter defines which output should be used.

Dither DAC

The Dither Ouput is in normal case used for the oscillation of the cantilever in AC mode.

Aux1 DAC

This is an axillary output which is used i.e. for EFM.

Kelvin Probe Control

The Kelvin Probe Control parameter group contains the advanced parameters for Kelvin Probe Force Microscopy (KPFM). The following values are initialized with default values when starting the measurement, but can be adjusted during the measurement:

Further information (Operation Guide):

- KPFM Overview

Kelvin Probe Control	[0, 0.000, 0]
VDC Driving Amp. pk-p	0
VDC Offset	0.000
VDC Driving Frequency	100
Listen (T-Bmin)	Never
T-Bmin Window Start [	120
T-Bmin Window Width	180
VAC Driving Amp. pk-p	0
Filter Frequency [Hz]	99.999832

VDC Driving Amp. pk-pk [V]

The modulation amplitude of V_{DC} in V, which defines the voltage range for the minimum search.

VDC Offset

An Offset for V_{DC} in V, which shifts the voltage range for the minimum search.

VDC Driving Frequency [Hz]

The Modulation frequency of V_{DC} in Hz, which defines the search speed. The time per pixel should be adjusted dependent on this value, i.e. 10 ms/pixel for 100 Hz.

Listen (T-Bmin)

Enables the definition of the T-Bmin search window by mouse. The T-Bmin search window defines the search range for the minimum T-B signal along the x-axis.

T-Bmin Window Start [°]

Defines the starting point of the T-Bmin search window.

T-Bmin Window Width [°]

Defines the width of the T-Bmin search window.

VAC Driving Amp. pk-pk [V]

The modulation amplitude of V_{AC} in V. The sum of VAC Driving Amp., VDC Driving Amp. and the absolute value of VDC Offset must be smaller or equal to 20 V.

Filter Frequency [Hz]

This parameter defines the amount of averaging of the T-B amplitude for the minimum search. A smaller value results in a smoother curve, but also in a greater offset between the real position of the minimum and the measured position of the minimum.

Piezoresponse Control

The Piezoresponse Control parameter group contains the parameters required for the Piezoresponse Force microscopy (PRFM).

Further information (Operation Guide):

- PRFM Overview

Piezoresponse Control	[0, 999.99829]
Driving Amp. pk-pk [V]	0
Driving Frequency [Hz]	999.99829
Filter Frequency [Hz]	3
Phase Offset [°]	0

Driving Amp. pk-pk [V]

The modulation amplitude applied to the tip in V.

Driving Frequency [Hz]

The modulation frequency applied to the tip in Hz.

Filter Frequency [Hz]

This parameter defines the amount of averaging on the measured signal. A smaller value delivers smoother results, but also makes edges less sharp.

Phase Offset [°]

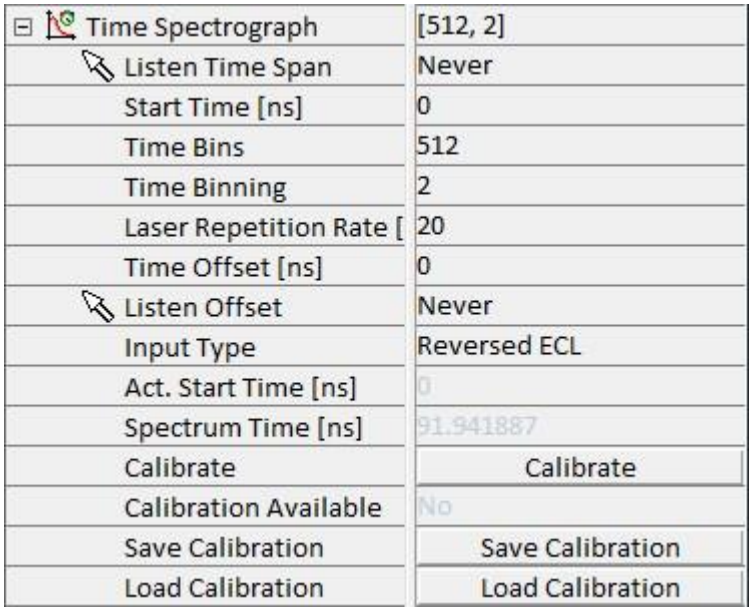
This parameter defines the phase offset.

Time Spectrograph

The Time Spectrograph parameter group contains the parameters required for the time-resolved spectroscopy.

Further information (Operation Guide):

- StrobeLock



Listen Time Span

If this is activated, the time span can be selected by mouse in a time spectrum.

Start Time [ns]

This parameter shifts the time spectrum to the left.

Time Bins

This parameter defines how many time bins are recorded per spectrum. One bin is the smallest time difference the controller can detect, which is slightly below 30 ps.

Time Binning

This parameter is used to combine selected number of bins into one pixel on the time axis. Higher values improve the signal-to-noise ratio, but reduce the resolution.

Laser Repetition Rate [MHz]

The Laser Repetition Rate parameter has to exactly match the repetition rate of the excitation laser.

Time Offset [ns]

Time Offset can be used to shift the x-axis until the time 0 corresponds to the real starting time marked by the steep increase at the beginning of a typical time-spectrum.

Listen Offset

If this is activated, the offset can be selected by mouse in a time spectrum.

Input Type

ECL

Not recommended. Uses the NIM inputs of the TDC card. The laser pulse acts as Start signal, the APD acts as Stop signal.

TTL

Only for testing. Uses the TTL inputs of the TDC card. The laser pulse acts as Start signal, the APD acts as Stop signal.

Reversed ECL

This is the default setting. Uses the NIM inputs of the TDC card. The APD acts as Start signal and the laser pulse as Stop signal.

Reversed TTL

Only for testing. Uses the TTL inputs of the TDC card. The APD acts as Start signal and the laser pulse as Stop signal.

Act. Start Time [ns]

Shows the currently used Start Time.

Spectrum Time [ns]

Shows the current timespan of one spectrum.

Calibrate

Starts the calibration process..

Calibration Available

Shows whether a calibration is available.

Save Calibration

Saves the current calibration to a file.

Load Calibration

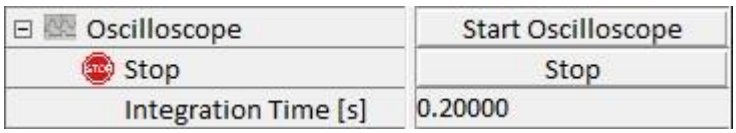
Loads a previously recorded calibration.

Oscilloscope

The oscilloscope sequencer allows the user to display measured values in a similar way as is done through an oscilloscope. The measured values which are displayed are set by the configurations.

If single value signals such as the counter output of a photomultiplier are displayed, the signal will be integrated for the set time and the recorded value will then be displayed. The currently displayed

values will shift to the left in the oscilloscope window with each new value recorded and the total time displayed is adjustable as well. If the recorded signal is a spectrum, the CCD camera will integrate the signal for the set amount of time and then refresh the entire oscilloscope window using the new spectrum. The following parameters are adjustable within the oscilloscope parameter group.



Start Oscilloscope

Pressing this button will start the oscilloscope using the current set of parameters.

Displayed Time [s]

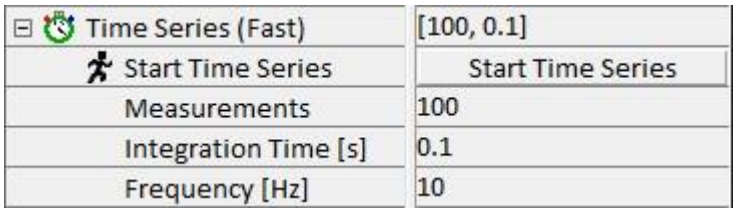
This parameter sets the total time displayed within the oscilloscope window.

Integration Time [s]

This parameter sets the integration time per point measured. This is the counting time for a photon counting device or the integration time in the cases where spectra are recorded.

Time Series (Fast)

The time series (fast) sequencer allows the acquisition of data sources (e.g. spectra or count rates) at a fixed position as a function of time. In this mode changes to the sample over time can for example be observed. The fast time series was developed for maximum speed and therefore does not allow pauses between the measurements. The following parameters can be adjusted to control the time series:



Start Time Series

Pressing this button will start the time series using the current set of parameters.

Measurements

This parameter determines how many measurements will be performed. It can be adjusted between 1 and 217 (=131072).

Integration Time [s]

Using this parameter, the integration time per measurement can be set between 0.1 ms and 10 s. Changes to this parameter will automatically also change the frequency parameter. If the integration time entered is shorter than the fastest possible by the device (e.g. the CCD camera), the time will automatically set to the fastest possible by the device.

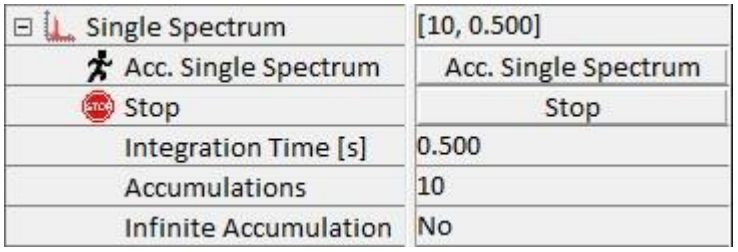
Frequency [Hz]

Using this parameter, the frequency of the measurements can be set between 0.1 Hz and 10 kHz.

Changes to this parameter will automatically also change the integration time parameter. If the frequency entered is higher than the highest possible by the device (e.g. the CCD camera), the frequency will automatically set to the highest possible by the device.

Single Spectrum

This parameter group allows recording of single spectra using a CCD camera connected to the system. A single spectrum acquisition stores only one spectrum. This spectrum can be an accumulation of several spectra, which can either be added or the average spectrum can be calculated. The single spectrum acquisition is controlled by the following parameters:



Acc. Single Spectrum

Pressing this button, will start the acquisition using the current set of parameters.

Accumulations

This parameter describes how many spectra will be accumulated using the integration time for each of them.

Integration Time [s]

This parameter defines the integration time for one spectrum. The minimum and maximum integration times depend on the CCD camera used.

Infinite Accumulation

If this option is selected (Yes) the CCD camera will continuously record spectra using the integration time and the software will accumulate them until the measurement is interrupted by the user by pressing any of the stop trigger buttons.

Accumulation Mode

Here the mode of accumulation can be switched between accumulate and average. If average is selected, only the average spectra will be displayed whereas if accumulate is selected, the spectra are added and displayed as the sum of all spectra recorded.

Distance Curve

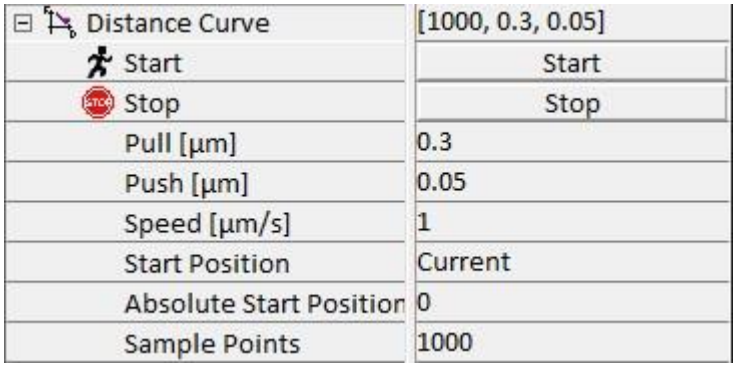
Distance curves are used to study phenomena which occur at a defined sample position if a probe is approached to and retracted from the sample by using the z-scan of the scan table. The probe can be for instance a cantilever tip in AFM and SNOM measurements, where the tip-sample interactions are examined. In these experiments the cantilever tip is approached to and retracted from the sample. The recorded signals as a function of distance are:

- the T-B signal of the beam deflection system if the AFM is operated in contact mode. The resulting curve displayed in the graph viewer is a force-distance curve.
- the variation of the amplitude if the AFM is operated in AC mode. The resulting curve displayed in the graph viewer is an amplitude-distance curve.

Typically, distance curves are recorded when the tip is already in contact with the sample and its position is controlled by the feedback loop. In addition to studies of tip-sample interactions, the probe can also be a light source, in which case the light intensity is recorded as a function of distance from the sample. The parameters required to measure a force-distance curve are listed below.

Further information (Operation Guide):

- AFM Overview



Start

This parameter starts the distance curve.

Sample Points

This parameter defines the number of points recorded in a distance curve.

Pull [µm]

This parameter defines the distance the probe will be lifted off the sample before and after recording a distance curve.

Push [µm]

This parameter defines the distance the probe will be moved downwards into the sample. As reference for the downwards movement is the start position (see below).

Speed [µm/s]

This parameter defines the speed of movement in the z-direction of the scan table during the complete distance curve.

Start Position

This parameter defines the reference point within the internal coordinate system for the pull and push parameters described above. This parameter can either be set to current or absolute. If absolute is chosen, the Absolute Start Position parameter (see below) is used as the start position.

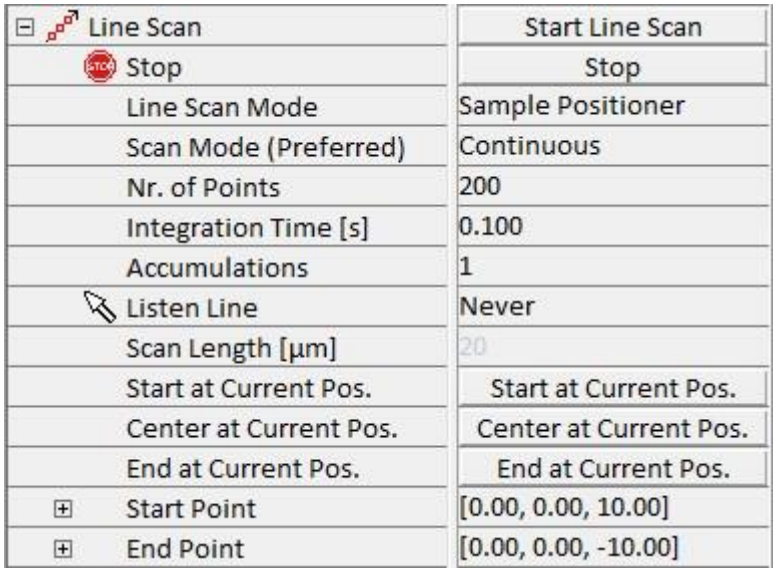
Absolute Start Position [µm]

This parameter defines the start position in terms of the absolute position along the z-axis of the scan

table.

Line Scan

The line scan parameter group allows the adjustment of all parameters necessary for the collection of data along a definable straight line. It allows the collection of spectra or distance curves (adjust the parameters in the respective section) along the line. The behavior of the line scan can be defined using the following parameters.



Start Line Scan

Pressing this button starts the line scan and the data acquisition using the parameters currently entered.

Line Scan Mode

Scan Table
This mode uses the piezo stage for the movement.
Sample Positioner
This mode uses the motorized stage for the movement.
Sample Pos. + Topo. Cor.
This mode uses the motorized stage for the movement in combination with a recorded topography for the z position. Refer to Topography Correction.

Scan Mode (Preferred)

The movement of the line scan can be continuously or stepwise. If a continuous movement is not possible the measurement will automatically switch to stepwise. This is the case, if the scan table is used for moving the sample or force distance curve will be acquired. In continuous mode the number of accumulations are always equal 1.

Nr. of Points

This parameter defines the number of equidistant points at which measurements will be performed along the line.

Integration Time [s]

This parameter defines the integration time for spectral acquisitions.

Accumulations

This parameter defines the number of accumulations. This parameter will be used for spectral acquisitions in stepwise mode, only.

Listen Line

With this function the start position, the end position or the center position can be defined by clicking or drawing in any acquire image.

Scan Length [µm]

Read only value, shows the length of the line to be measured.

Start at Current Pos.

The current position is used as start point.

Center at Current Pos.

The current position is used as center point.

End at Current Pos.

The current position is used as end point.

Start Point

This parameter group contains the parameters for the three coordinates X, Y and Z (in µm) of the starting point for the line scan. Note that the scan table performs the line scan.

End Point

This parameter group contains the parameters for the three coordinates X, Y and Z (in µm) of the end point for the line scan.

At Every Point

Here the user can define which actions should be performed at every point. Distance Curve and Single Spectrum can be selected (Yes or No selection). If any of these are selected, the parameter values set in the corresponding parameter groups are used for the respective task. If both of the tasks are selected they will be performed sequentially.

Image Scan

In the Image Scan parameter group, all parameters necessary for the capture of a two or three dimensional data set can be defined. Parameters such as the geometry of the scan or the scan speed can be adjusted in this parameter group.

Further information (Operation Guide):

- AFM Overview
- Raman Image scan
- SNOM Overview

Image Scan	[150, 150, 25, 25]
Points per Line	150
Lines per Image	150
Layers per Scan	1
Geometry	
Listen Position/Area	Never
Width [µm]	25
Height [µm]	25
Depth [µm]	0.01
Center at Current Pc	Center at Current Pos.
Center (X) [µm]	0
Center (Y) [µm]	0
Center (Z) [µm]	0
Gamma [°]	0
Signal Stabilization	Start Stabilization
Scan Details	[Create New Object, Area
Scan Mode	Area
Change Scan Directi	Keep Direction
Slow Scan Direction	Top->Bottom
Overwrite Previous	Create New Object
Acquire Data	Forward
Start Scan	Start Scan
Restart	Restart
Stop	Stop
Act. Int. Time (Trace) [s	0.01
Int. Time (Trace) [s]	0.01
Time / Line (Trace) [s]	1.5
Min. Time Retrace [s]	0.5

Start Scan

Pressing this button starts the scan and the data acquisition using the parameters currently entered.

Restart

This trigger button causes the scan to restart using the parameters currently entered.

> WITec Control allows most parameters within the image scan parameter group to be changed during the scan. This allows optimization of the scan parameters while scanning.

The data of the currently collected data set will be lost.

Points per Line

This parameter allows the user to change the number of pixels, data points or spectra per line.

Lines per Image

Lines per image allows the user to select the number of lines scanned inside the selected scan area (see below).

Layers per Scan

This parameter defines how many layers are recorded in a stack scan (confocal mode only). It does not have an effect on other modes.

Geometry

The geometry parameter group allows the selection of the desired scan area where the coordinates are given in the internal coordinate system (see Fig. 3.2). Since the scan table performs the movement, the selectable geometry is limited by the scan range of the scan table. The following parameters can be adjusted:

Listen Position/Area

Upon clicking onto a position in an image, the position clicked will be marked as the new center position for the scan or it allows the selection of the desired scan area or a line for a depth scan.

Width [µm]

The width sets the size of the box scan. It is always the size of the fast scan direction (length of one line).

Height [µm]

The height is always the size of the slow scan direction (height of the rectangular) of the box scan. This parameter will not have any effect if a depth scan is performed.

Depth [µm]

The depth parameter is used not only in conjunction with the width for depth scans, but also in conjunction with both width and height for stack scans. In depth scans, it is the dimension of the slow scan direction and in stack scans, the distance from the uppermost to the lowest stack scanned.

Center at Current Pos.

Clicking this button causes the current coordinates within the internal coordinate system to be copied into the respective fields for the center position of the scan.

Center (X/Y/Z) [µm]

The X, Y and Z coordinate of the central position about which the scan will be performed.

Alpha [°]

Alpha identifies the rotation of the scan area about the X axis and is measured relative to the Y axis.

Beta [°]

Beta identifies the rotation of the scan area about the Y axis and is measured relative to the X axis.

Gamma [°]

Gamma identifies the rotation of the scan area about the Z axis and is measured relative to the X axis.

Entering a positive value for one of the angles will rotate the scan in the mathematically positive direction. Since, however, the recording device (e.g. AFM tip) remains stationary, the resulting image will appear rotated in the mathematically negative direction. For the determination of the position of the scan area when using a rotated scan, the rotation about the X axis is applied first. Then the rotation about the Y axis is performed relative to the rotated plane. Last, the rotation about Z is applied which is again performed within the rotated/tilted plane.

Signal Stabilization

Please refer to the Signal Stabilization section.

Scan Mode

Using this parameter, the scan mode can be selected from the following options.

Area

Using this selection a single scan is performed on the plane defined through the angles Alpha, Beta and Gamma (typically the X-Y plane if Alpha = Beta = 0◦) using the width and height entered. The scan table will remain in the final position of the scan upon completion.

Area Loop

In this mode the scan will be performed as in the single setting. However, it will automatically be repeated upon completion using the exact same settings. Change Scan Direction and Overwrite Previous (see below) are additional parameters defining the behavior of this scan mode.

Depth

Using this selection, a single depth scan is performed perpendicular to the plane defined through the angles Alpha, Beta and Gamma (typically the X-Z plane if Alpha = Gamma = 0◦) using the width and depth entered. The scan table will remain in the final position of the scan upon completion.

Depth Loop

In this mode the scan will be performed as in the depth setting. However, it will automatically be repeated upon completion using the exact same settings. Change Scan Direction and Overwrite Previous (see below) are additional parameters defining the behavior of this scan mode.

Stack

A stack scan is a collection of single scans which are performed at different depths. The number of scans performed depends on the Layers per Scan parameter and the different depth levels on the combination of Layers per Scan and the Depth parameters. The individual scans are labeled automatically with incremented numbers.

Change Scan Direction

This parameter takes effect for Loop or Stack scans and has the following options:

Keep Direction

In this setting each scan will start in the direction defined by the Slow Scan Direction.

Alternate

This setting will cause the Slow Scan Direction to alternate with each scan. Therefore if a scan, which had started at the upper left hand corner of the scan area, has finished, the next scan will start at the bottom left hand corner.

Slow Scan Direction

This parameter sets the scan direction.

Overwrite Previous

This parameter takes effect for continuous or stack scans and can be set to Create New Object and Overwrite. If Create New Object is selected a new data object will be created for each scan. If the option Overwrite is selected the second scan will overwrite the first one.

Speed Defined By

This parameter can be set to Integration Time or Time per Line. Both of these parameters can be entered and are described as

$$[\text{Time per Line}] = [\text{Integration Time}] * [\text{Points per Line}]$$

Speed Defined By determines which variable will remain unchanged if the Points per Line parameter is changed.

Act. Int. Time (Trace) [s]

Read only value, which shows the adjusted cycle time (integration time + readout time) of the CCD camera after the measurement started.

Int. Time (Trace) [s]

The integration time in the forward scan direction defines the time for one pixel, data point or spectrum. Changing this time will automatically change the Time / Line (Trace) [s] parameter.

Int. Time (Retrace) [s]

The integration time in the backward scan direction also defines the time for one pixel, data point or spectrum. If no data is recorded in this scan direction this time basically defines the backward motion speed of the scan table. Changing this time will automatically change the Time / Line (Retrace) [s] parameter.

Time / Line (Trace) [s]

The time required for one line of the scan in the forward direction can be altered here. Changing this parameter will automatically change the Int. Time (Trace) [s] parameter.

Time / Line (Retrace) [s]

The time required for one line of the scan in the backward direction can be altered here. Changing this parameter will automatically change the Int. Time (Retrace) [s] parameter.

If no data is recorded in the backward direction this time can be shortened significantly. However, if the time is selected too short, the sample might move due to the impact upon turnaround if the sample is not fixed properly.

Min. Time Retrace [s]

This parameter defines the time for one line in the backward direction, if no data is recorded and if its value is smaller than the needed time per line in the forward direction.

Acquire Data

This parameter determines whether or not data is recorded in only the Forward scan direction or in both the Forward and Backward scan directions.

Store PFM Data

If this is activated the DPFM curves (T-B signal) will be saved to a separate file. The software will prompt for the file location after clicking Start Scan.

PFM File Name

Shows the path for saving the DPFM curves.

Pre/Post Pixels

This parameter defines how many pixel triggers will be output before and after each line. This might be necessary if certain external devices are triggered through the alphaControl.

Slow Turnaround [%]

The slow turnaround reduces the impact caused by the change of direction of the scan table. If the slow turnaround is activated the scan table will be accelerated to the final scan speed and slowed down from the final scan speed upon turnaround. The distance taken for this acceleration can be altered with this parameter. The reference used with this percentage is the scan width and half of this distance will be used on each side of the line scanned. WITec Control sets this parameter automatically and it is dependent on the mode of measurement.

Linear Overscan [%]

The linear overscan is an extension of the scan width which is used in combination with the TrueScan mode implemented within the alphaControl. The reference used with this percentage is the scan width and half of this distance will be used on each side of the line scanned. WITec Control sets this parameter automatically and it is dependent on the mode of measurement.

Signal Routing

Trig 1/2/3 Out

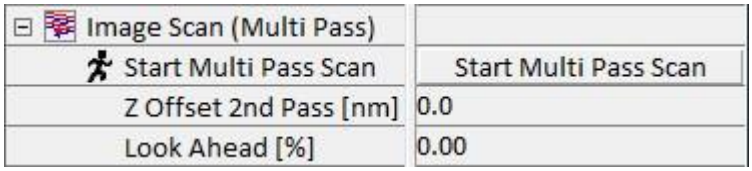
The Trigger Outputs can be set to Pixel Clock or Line Clock.

Image Scan (Multi Pass)

In the Image Scan (Multi Pass) parameter group, parameters necessary for a two pass lift mode scan can be defined. Parameters such as the geometry of the scan or the scan speed can be adjusted in the Image Scan parameter group. Further information can be found in the Lift Mode and KPFM section of the Operation guide.

Further information (Operation Guide):

- AFM AC Lift mode
- KPFM



Start Multi Pass Scan

Pressing this button starts the multi pass scan and the data acquisition using the parameters currently entered here and in the Image Scan section.

Z Offset 2nd Pass [nm]

This parameter defines the height of the second pass relative to the topography recorded in the first pass.

Look Ahead [%]

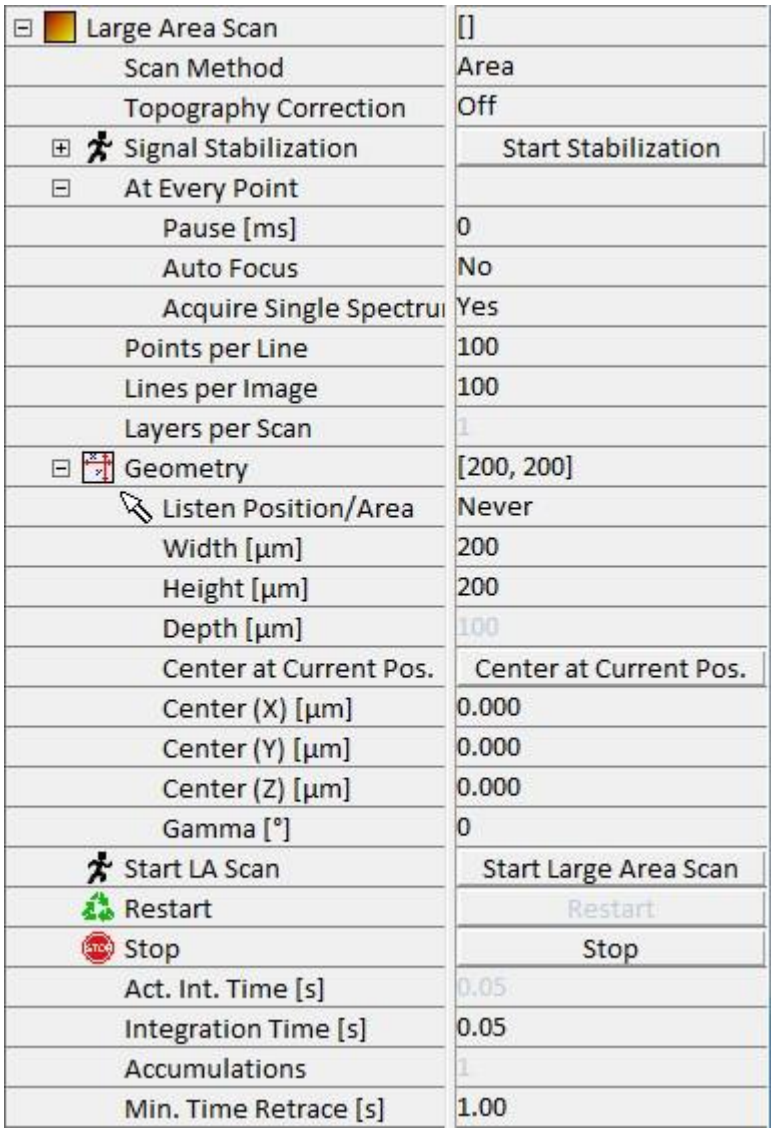
Defines an offset in % between first and second pass along the moving direction.

Large Area Scan

In the Large Area scan parameter group, all parameters necessary for the capture of a two or three dimensional data set can be defined. Parameters such as the geometry of the scan or the scan speed can be adjusted in this parameter group.

Further information (Operation Guide):

- Raman Large Area Scan



Start Large Area Scan

Pressing this button starts the scan and the data acquisition using the parameters currently entered.

Restart

This trigger button causes the scan to restart using the parameters currently entered.

The data of the currently collected data set will be lost.

Points per Line

This parameter allows the user to change the number of pixels, data points or spectra per line.

Lines per Image

Lines per image allows the user to select the number of lines scanned inside the selected scan area (see below).

Layers per Scan

This parameter defines how many layers are recorded in a stack scan (confocal mode only). It does not have an effect on other modes.

Geometry

The geometry parameter group allows the selection of the desired scan area where the coordinates are given in the internal coordinate system (see Fig. 3.2). Since the scan table performs the movement, the selectable geometry is limited by the scan range of the scan table. The following parameters can be adjusted:

Listen Position/Area

Upon clicking onto a position in a image, the position clicked will be marked as the new center position for the scan or it allows the selection of the desired scan area or a line for a depth scan.

Width [µm]

The width sets the size of the box scan. It is always the size of the fast scan direction (length of one line).

Height [µm]

The height is always the size of the slow scan direction (height of the rectangular) of the box scan. This parameter will not have any effect if a depth scan is performed.

Depth [µm]

The depth parameter is used not only in conjunction with the width for depth scans, but also in conjunction with both width and height for stack scans. In depth scans, it is the dimension of the slow scan direction and in stack scans, the distance from the uppermost to the lowest stack scanned.

Center at Current Pos.

Clicking this button causes the current coordinates within the internal coordinate system to be copied into the respective fields for the center position of the scan.

Center (X/Y/Z) [µm]

The X, Y and Z coordinate of the central position about which the scan will be performed.

Gamma [°]

Gamma identifies the rotation of the scan area about the Z axis and is measured relative to the X axis.

Entering a positive value for the angles will rotate the scan in the mathematically positive direction. The resulting image will appear rotated in the mathematically negative direction.

Scan Mode

Using this parameter, the scan mode can be selected from the following options.

Stepwise Raster

This mode is an Intermittent area scan. The movement will stop before performing the tasks defined in At Every Point parameter group (see below) and will go to next point afterwards.

Area

Using this selection a scan is performed on the plane defined through the angle Gamma and the width and height entered (typically the X-Y plane). The sample positioner will remain in the final position of the scan upon completion.

Depth

Using this selection, a single depth scan is performed perpendicular to the plane defined through the angle Gamma and the width and depth entered (typically the X-Z plane). The sample

positioner will remain in the final position of the scan upon completion.

Stack

A stack scan is a collection of single scans which are performed at different depths. The number of scans performed depends on the Layers per Scan parameter and the different depth levels on the combination of Layers per Scan and the Depth parameters. The individual scans are labeled automatically with incremented numbers.

At Every Point

This parameter group defines the actions performed at every point in the stepwise raster mode.

Pause [ms]

A pause can be defined before performing the next tasks at each point.

Auto Focus

A spectral auto focus can be performed at each point. The parameters can be defined in Auto Focus.

Single Spectrum

If this parameter is set to Yes a single spectrum is performed at each point.

Topography Correction

Set this to On to follow a recorded surface in z direction during the scan. The surface can be defined in Topography correction.

Signal Stabilization

Please refer to the Signal Stabilization section.

Act. Int. Time (Trace) [s]

Read only value, which shows the recalculated time per pixel after the measurement started. This can be due to the minimum cycle time (integration time + readout time) of the CCD camera or the topography correction. (Only for continuous modes, not for Stepwise Raster)

Integration Time [s]

The integration time defines the time for one pixel, data point or spectrum.

Accumulations

The number of accumulations can be used in Stepwise Raster mode only.

Min. Time Retrace [s]

This parameter defines the time for one line in the backward direction, if its value is smaller than the needed time per line in the forward direction.

Signal Routing

Trig 1/2/3 Out

The Trigger Outputs can be set to Sampling Clock or Stop & Go Clock.

T1/2/3 Duty Cycle

Sets the duty cycle in percent.

T1/2/3 Idle State

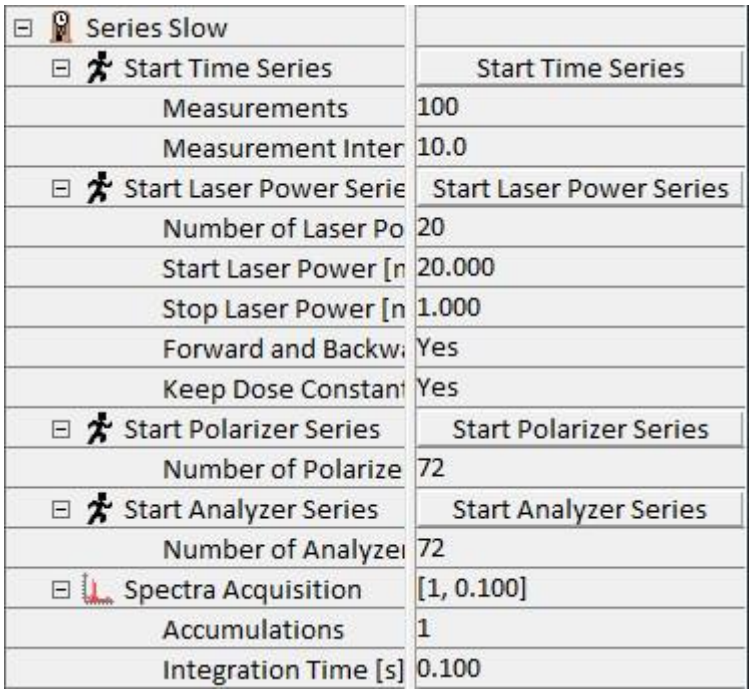
The idle state can be set to Don't Care, Low or High.

Series Slow

The slow series allows intermittent (slow) time series, laser power series, polarizer and analyzer series and externally triggered series including to record user data channels.

Further information (Operation Guide):

- Raman Slow series



Start Time Series

Starts a slow time series.

Measurements

This parameter determines how many measurements will be performed.

Measurements Interval [s]

The measurement interval defines the time between two data points.

Start Laser Power Series

Starts a laser power series. This is only available for TruePower lasers.

Number of Laser Power Steps

This parameter determines how many measurements will be performed.

Start Laser Power [mW]

Defines the laser power of the first data point.

Stop Laser Power [mW]

Defines the laser power of the last data point.

Forward and Backward

If yes is selected, the series will run to the stop laser power and then back to the start laser power.

Keep Dose Constant

If yes is selected, the integration time is adjusted to compensate the intensity change of the spectrum due to the change of the laser power. The integration time parameter is defined for the highest laser power and is therefore the maximum integration time.

Start Polarizer/Analyzer Series

Starts a Polarizer/Analyzer series. This only available for an automated Polarizer/Analyzer.

Number of Polarizer/Analyzer Steps

This parameter determines how many measurements will be performed along a full rotation of the laser polarization or analyzer.

Spectra Acquisition

The spectral acquisition parameters for all kinds of series.

Accumulations

This parameter describes how many spectra will be accumulated.

Integration Time [s]

This parameter defines the integration time.

Measurement Mode

This parameter determines which mode will be used for the slow time series.

Manual

Each next measurement point is triggered by the Next Measurement Button. This can be used for an external or manual triggered series.

Timed

The series is triggered Measurement Intervall parameter.

As Fast As Possible

The measurement points are processed subsequently without pause.

Timing Mode

This parameter defines the whether the measurement interval is measured between two Starts or between the Stop and the Start which takes also the time of the measurement itself into account.

Microscope State after Single measurement

This defines which beam path configuration should be used after a single measurement. By default it is empty and the microscope stays in the measurement beam path configuration.

Put in BeamPath|SetStateVideo to change to video mode between single measurements or put in Laser|Selected|Shutter:SetValue:False to close the laser shutter only.

(Only one command is allowed, no combinations of two or more commands.)

User Data Channels

Enables the definition and data input of data channels defined by the user. (The values in the dialog are not refreshed when data is changed over the COM interface.)

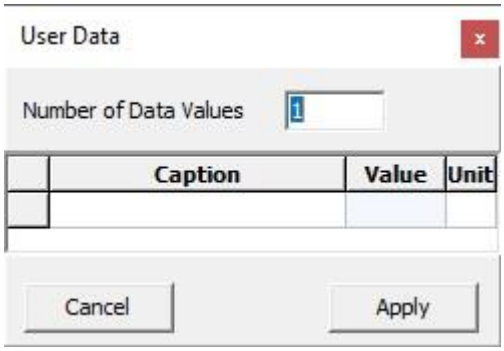
Include User Data

If this is activated, the user data is stored in the project.

Define Data Channels

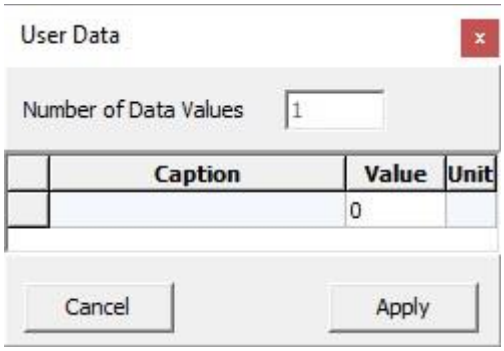
Clicking this button opens a new window for definition of number of user data channels and their

captions and units.



Enter User Data

Clicking this button opens a new window for entering of the values for each data channel for the next measurement.



Sub-Sequence

A subsequence allows to insert an action (i.e. autofocus) before each measurement in the time series.

Sub Sequencer

Enables the use of the subsequencer and determines which kind of subsequencer. Only none (default) and Process Script are available. The parameters of the Process Script need to be set in the regarding section.

Continue if Failed

If Stop is selected the time series will stop in case the subsequence is failed, otherwise it will continue.

Sub-Sequence Done

Turns to yes once the subsequence was finished.

Start Sub Sequence

Triggers the subsequence in the Manual measurement mode.

Next Measurement

Triggers the next data point, if Manual is the selected measurement mode.

Index of next Measurement

Read-only parameter which shows the index of the following measurement.

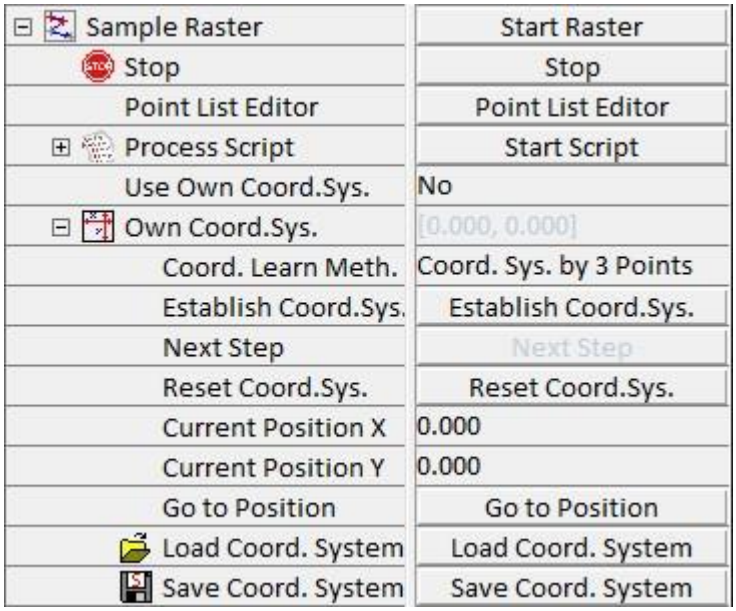
Sample Raster

This parameter group is used in combination with the Point Viewer. In the Point Viewer up to

thousand points on the sample can be defined where automated measurements can be performed. The Sample Raster parameter group is used to define the script that should be executed on the defined points. Also, the execution of the raster is started from this parameter group and the Point Viewer can be opened.

Further information (Operation Guide):

- Raman Sample Raster



Start Raster

This trigger button starts the execution of the currently defined raster (= the point list as defined in the Point Viewer). The scan table goes to zero position each time before the script is executed.

Point List Editor

This button opens the Point Viewer window. In this window the points of which the raster consists can be defined.

Process Script

Please refer to the Process script section.

Use Own Cood.Sys.

Set this to On to enable the use of the user-defined coordinate system.

Own Cood.Sys.

Enables to use a user-defined coordinate system for the raster.

Process Script

The Process Script parameter group allows the definition of script based command lines for the automatic execution of several consecutive tasks. Each task that can be included in the script is shown in this parameter group. Tasks, which are fully defined in their respective parameter groups (such as Image Scan or Auto Focus) use the parameters set in their respective parameter groups.

These tasks will nevertheless be shown in the Process Script parameter group, but without any parameters. Some additional tasks are included in the Process Script parameter group and their parameters can be adjusted herein. Table 1 shows the tasks available and their parameters.

Table 1: The tasks usable in the Process Script sequencer. New parameters are described and the location of the parameters defined in other parameter groups are indicated.

Task Name	Command Line	Parameter	Parameter Description
Pause	`pause`	Duration [ms]	The duration of the pause at this point
Move Z Microscope	`movezmicroscope`	Distance [µm]	The distance the microscope Z stage should move (+ = increase; - = decrease in objective- sample distance)
		Speed [µm/s]	The speed of the microscope Z stage during the movement
Auto-Focus	`autofocus`	Uses the parameters in Auto Focus.	
Tip-Approach	`tipapproach`	Setpoint for A.	The feedback setpoint used for the Approach
		P-Gain for A.	Feedback gains for the Approach
		I-Gain for A.	
Single Spectrum	`singlespectrum`	Uses the parameters in Single Spectrum.	
Image Scan	`imagescan`	Uses the parameters in Image Scan, but with the current scan table position as center position.	
Save Project	`saveproject`	Uses the parameters in the program options.	
Store Position	`storeposition`	Stores the current scan table position (Piezo-Scanner only)	
Goto Position	`gotoposition`	Moves to the stored scan table position	

During the execution of one scrip, the parameters remain constant. If, for example, several Move Z Microscope commands are included in the script, all of them will be executed with the same speed and distance.

The script itself can contain the various commands separated by semicolons. An example for a script is given below:

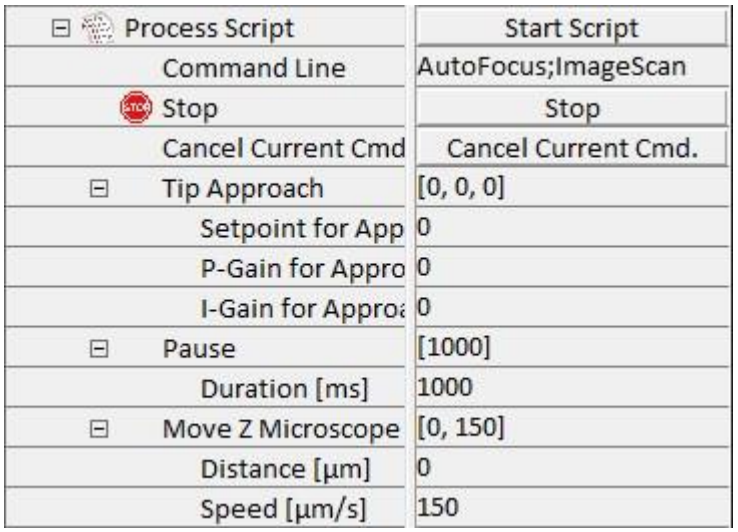
```
pause ; autofocus ; singlespectrum ; imagescan ; saveproject ;
movezmicroscope
```

In this example, the microscope will first pause for the defined duration before performing the auto focus function. Then a single spectrum will be recorded before an image scan is performed. Following the completion of this image scan, the project will be saved and the microscope will move the defined distance in Z.

Scripts can be executed by pressing the Start Script button (see below) or through a raster as defined in the point editor.

Once the execution of a script is triggered, it is first checked for syntax errors. Should the script contain any error, the execution will not be started and a corresponding message identifying the number of the command where the error occurs will be displayed in the message window. The erroneous command will also be displayed.

Apart from the executable commands the following entries can be found in the Process Script parameter group:



Start Script

Using this button the execution of the script can be started.

Cancel Current Cmd.

This trigger button cancels the current command. The next command in the command line will automatically be executed. In the example of the command line shown above, pressing the Cancel Current Cmd. button while the image scan is executed will cancel this execution and the saveproject function will automatically be executed.

Command Line

The command for the script function as shown in an example above can be entered here.

Command Parameters

This group contains all tasks which can be executed through the script and which have been explained in Table 1.

> Using the Save As function in the Configurations menu, the current script as well as all parameters set in the individual parameter groups can be saved for later usage. This would essentially then represent another configuration, based on a standard one, but with the predefined parameters for the script execution.

Auto-Focus

Additional parameter for the autofocus.

Second Chance

Enables a second run in case the aufofocus was not successful.

Continue if Failed

If Stop is selected the process script will stop in case the autofocus is failed, otherwise it will continue.

Point Viewer

The Point Viewer (see Fig. 1) is used to define the points for the use with the sample raster. At the points defined in the point list, the process script sequencer can be executed before the system automatically moves the sample to the next point in the list. The points can be edited manually or imported from .CSV files or SP1 KLARF 1.2 files. Please contact WITec should you require import functions for different file formats.

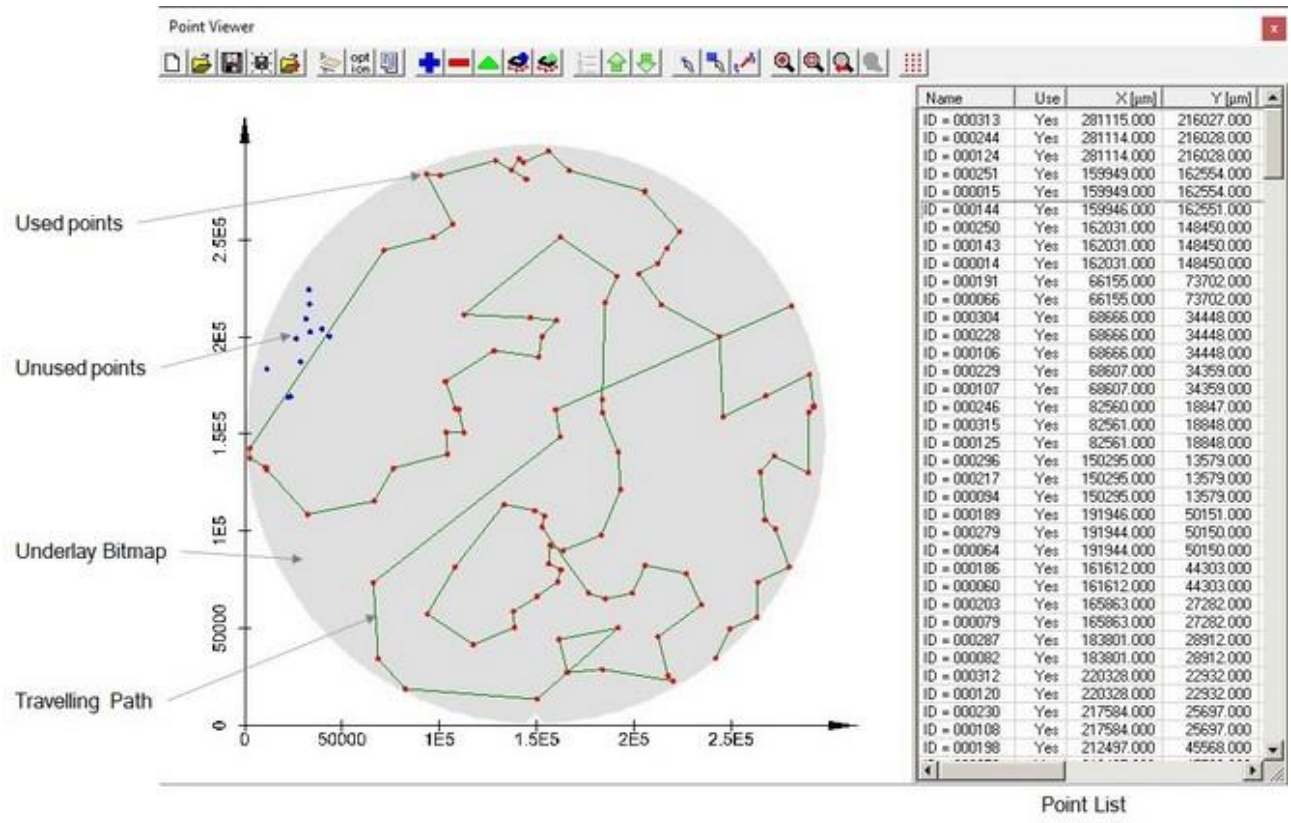


Fig. 1: The Point Viewer window showing the menu bar, the sample area and the point list. Some functions of the window are labeled.

In the following, the menu bar and the functions of the speed buttons will be described before the sample area is outlined. The point list and the context menu will then be described.

Speed Buttons

The following speed buttons are available in the menu bar of the Point Viewer window:

New point list

By pressing this speed button a new point list is created. The existing point list will be deleted.

Open Point List

This function starts the Windows R standard open-dialog for WITec Point List files (*.wpt). Using this dialog, a saved point list can be opened. The saved point list file also contains the bitmap underlay and the sample and bitmap coordinates. Previously opened point lists will be closed.

Save Point List

This menu function allows a Point List to be saved. If it is a new list, the save file as-dialog will be opened.

Save Point List As

This function is similar to the save point list function, while offering the possibility to change the filename.

Import SP1 KLARF 1.2 File

This function allows the import of data saved in the SP1 KLARF 1.2 file format. Previously opened point lists will be closed. The bitmap underlay as well as the sample and bitmap size are defined internally and set automatically for the import filter.

Define Sample Size and Underlay Bitmap

This speed button opens the sample size and bitmap underlay dialogs as shown in Fig. 2.

This dialog can be used to load a bitmap (in .BMP format) which is used as a background in the sample area of the Point Viewer window. The dialog allows the definition of the sample area and the size and position of the bitmap therein. In Fig. 2, a sample area of 200 µm x 200 µm was defined and the bitmap was defined in the upper left-hand corner of the sample area as can be seen in the point viewer in the background of Fig. 2.

The bitmaps should, if possible, be located in the directory C:\ProgramData\WITec\WITec Suite X.X\Configs\WITec Control\PointViewerBackgrounds because this is the standard directory where the bitmaps are searched for.

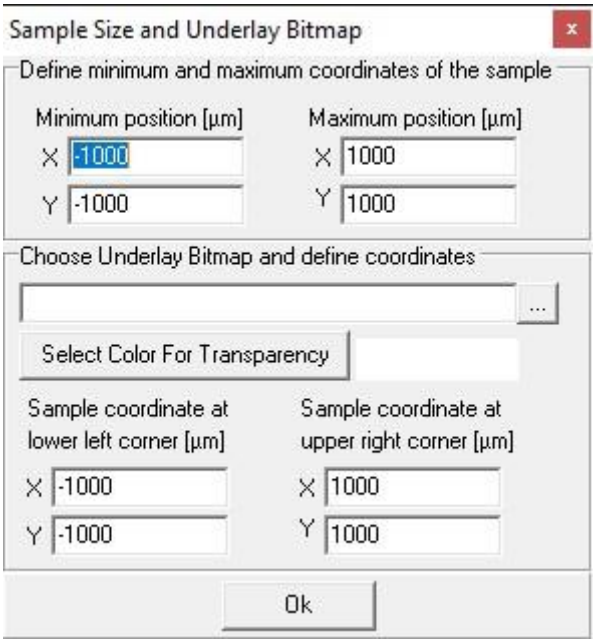


Fig. 2: The Sample Size and Bitmap Underlay dialog window

The following entries can be modified in this window:

Minimum Position [µm] X/Y

The minimum position of the sample within the coordinate system should be entered here. Following a change to the values entered here, the corresponding fields for the bitmap coordinates in the lower left hand corner are changed to the same value.

Maximum Position [µm] X/Y

The maximum position of the sample within the coordinate system should be entered here. Following a change to the values entered here, the corresponding fields for the bitmap coordinates in the upper right hand corner are changed to the same value.

Underlay Bitmap

The file containing the underlying bitmap should be entered here or selected by the ... button.

Select Color for Transparency

This trigger button opens the color selection dialog from which the transparency color for the bitmap can be selected. This color will not be displayed in the sample area. The currently selected color is shown to the right of the trigger button.

Sample coordinate at lower left corner [µm] X/Y

These fields are automatically filled in following a change to the minimum sample position field, but can also be changed manually. The coordinates of the bitmap at the lower left corner can be entered here. See Fig. 2 for an illustration of the effects of the coordinates entered. Here the underlying bitmap is shown at the coordinates as entered in the Define Sample Size and Underlay Bitmap dialog shown.

Sample coordinate at upper right corner [µm] X/Y

These fields are automatically filled in following a change to the maximum sample position field, but can also be changed manually. The coordinates of the bitmap at the upper right corner can be entered here. See Fig. 2 for an illustration of the effects of the coordinates entered. Here the underlying bitmap is shown at the coordinates as entered in the Define Sample Size and Underlay Bitmap dialog shown.

Options

In the options dialog, which is shown in Fig. 3, the export parameters for the export and graphic representation of the point list, as well as for the ASCII export of the list itself, are given.

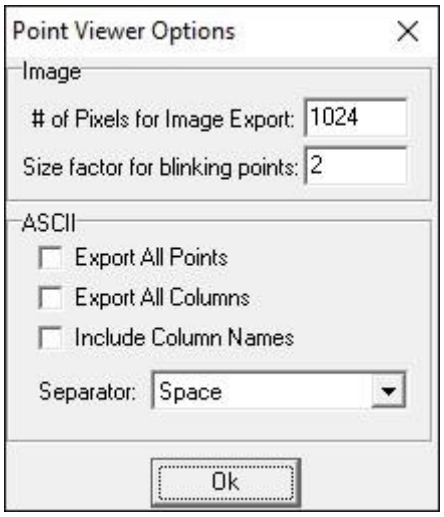


Fig. 3: The options dialog for the point viewer.

For the export of an image representing the points as shown in the sample area, the number of pixels in the larger dimension of the bitmap can be entered. The dimension of the smaller side of the exported image will be determined by the aspect ratio of the current view.

> The actual number of pixels may vary due to an automatic crop function, which cuts off excess white borders.

The Size factor for blinking points is the factor by which the points are enlarged and then reduced again when blinking. The blinking in the sample area indicates that the points are selected.

For the export of the point list in ASCII format, the following options are available:

Export All Points

If this check box is selected, all points will be exported regardless of their Usage status (see below). If it is unchecked, only points with the usage set to Yes or Used will be exported.

Export All Colmns

If this checkbox is selected all columns will be exported. Otherwise only those selected through the context menu (see below) are included in the export.

Include Column Names

This selection allows the column names to be included with the exported ASCII file.

Separator

The following separators for the ASCII export are selectable:

- Space
- Tab
- Semicolon

 **Add Point**

This speed button is only active if the point list is not imported from a KLARF file but is defined manually as a four column table. If active, it opens the edit point dialog as shown in Fig. 4.

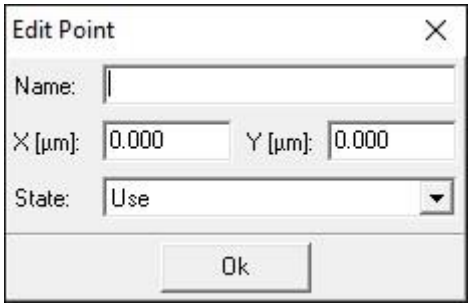


Fig. 4: The edit point dialog.

The name for the point, the X and Y coordinates in µm and the state can be selected here. The states are explained in the context menu section.

 **Delete Point [Del]**

This button is only active if points are selected (highlighted in the point list). Pressing this speed button deletes the selected points.

 **Edit Point**

This button is only active if a single point, which has been defined manually, is selected. Upon activating this speed button, the system opens the edit point dialog as shown in Fig. 4 and described with the Add Point speed button above.

Add Current position to point list

Takes the current position of the sample positioner as new point.

Replace Point by current postition

Replaces the position of the selected point by the current position of the sample positioner.

 **Sort by order**

This button sorts the points in the order that they to be are executed. The display order may be changed by, for example, displaying them sorted by their name, usage or coordinates.

> Upon defining the points, or upon opening or importing a point list, the order of the points is defined. Resorting the points by their name, coordinates or usage will only change the appearance of the points and NOT the order in which they are executed. This speed button displays the points again in the order of execution.

 **Move Selection Up [Ctrl + U]**

This button causes the selected points to be moved up in the list. It is only active if the list is displayed in the way it is processed.

> This changes the order of execution.

 **Move Selection Down [Ctrl + D]**

This button causes the selected points to be moved down in the list. It is only active if the list is displayed in the way it is processed.

> This changes the order of execution.

 **Select Single Point**

If this button is activated, single points from the point list can be selected by clicking on them in the sample area. The selected point will blink in the sample area and will be highlighted in the point list. Holding Ctrl allows the selection of multiple points. Holding Shift causes this point to be deselected. This function will remain active until de-activated (though clicking onto the button again) or until a different mouse function is activated.

 **Select Multiple Points**

If this button is selected, multiple points from the point list can be selected by drawing a rectangle around them in the sample area. The selected points will blink in the sample area and be highlighted in the point list. Holding Ctrl allows the selection of multiple points. Holding Shift causes this point to be deselected. This function will remain active until deactivated (by clicking onto the button again) or until a different mouse function is activated.

 **Immediately move to selected Point**

This button allows the immediate movement of the sample to the selected point. This point will only be active if the sample positioning system is present. Once activated, the system will move the sample to the point selected by the mouse. The point can either be selected by clicking onto the graphic representation in the sample area or by selecting it from the point list. This function will remain active until deactivated (by clicking onto the button again) or until a different mouse function is activated.

 **Zoom in**

If this button is selected an area for zooming-in can be selected from the sample area. This function will remain active until de-activated (by clicking onto the button again) or until a different mouse function is activated.

 **Zoom to fit all**

Pressing this button causes the entire sample area to be displayed. This is the standard view when defining the underlay bitmap and the sample size as well as after loading new point lists.

 **Zoom to previous**

This button returns to the last zoomed view.

Zoom to next

This button moves one step forward (if available) in the list of zoomed views.

Create Point Array

With this dialog a periodic point distribution can be created.

Create Point Array

Number of Points X	5
Number of Points Y	5
Distance of Points X	200
Distance of Points Y	200
Start Position X	0
Start Position Y	0
Start Position Anchor	Middle - Middle
Horizontal Direction	From left to right
Vertical Direction	From top to bottom
Meander	No
Walking Direction	Horizontal
Overwrite current list	No
Create on Parameter change	No
Create Array	Create Array

Sample Area

The sample area can be seen at the left in Fig. 1. Here the bitmap underlay is shown as well as the points. Additionally, the traveling path of the microscope can be displayed through the context menu (see below).

The visible parts in the sample area are:

Coordinate System

The coordinate system is scaled according to the sample size as defined by the Define Sample Size and Underlay Bitmap dialog (see Fig. 2).

Underlay Bitmap

The bitmap chosen through the Define Sample Size and Underlay Bitmap dialog (see Fig. 2) is displayed at the corresponding coordinates.

Points

The points displayed are either red if set to Yes or Used or blue if set to No.

Selected points blink in the sample area.

Points defined outside the sample area are not displayed.

Traveling Path

The traveling path of the instrument is shown in green, connecting the points in the order they will be processed in. Only points set to Yes are connected.

> The traveling path can be optimized through the context menu (see below).

Current Position

The current position of the instrument is indicated by green crosshairs.

Point List

The point list set up is similar to a spreadsheet, with each row representing a point. The number of columns may vary depending on the import filter used. However, manually defined point lists as well as point lists imported from .CSV files have only four columns:

Name | Use | X [µm] | Y [µm]

These columns are also the first four in other imported point lists. The columns displayed can be selected through the context menu (see at the bottom). By clicking on the column headers, the list is sorted by these columns (i.e. by name or usage).

> This will not change the order in which the points are handled, but only their representation in the list. Use the Move Selection Up or Move Selection Down speed buttons to change the order in which the points are processed.

If points are selected, they will be marked along the entire row.

Context Menu

The context menu, which can be seen in Fig. 5, can be opened by a right click within the point viewer window.

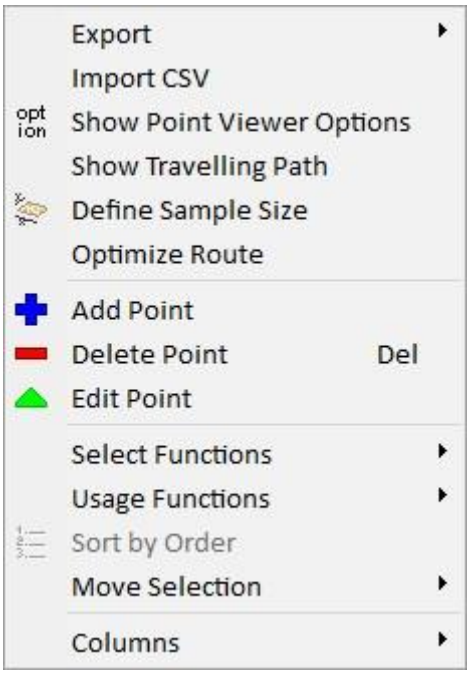


Fig. 5: The Point Viewer context menu.

The commands available through the context menu are:

Export

The export menu point allows the export of the sample area as a bitmap as well as the ASCII export of the point list. The exported bitmap will be copied to the Graphic Export window. The exported ASCII data will be copied to the clipboard, from which it can then be pasted into the desired document. The export functions use the settings set in the options dialog (see above).

Import CSV

Using this menu, comma separated value data (.CSV) can be imported. The data must be a three

column CSV file with the first column containing the name of the point, the second the X coordinate in µm and the third the Y coordinate in µm. (The format of the name has to be alphanumerical without spaces and special characters. The X and Y coordinates may have a + or - as a leading sign, a dot as the decimal separator and an optional 'e' or 'E' indicating an exponent followed by a signed integer. The strings may not have a thousands separator.)

Example:

```
Pointname,10.4,-100
```

The usage state for all points imported is initially set to Yes.

Show point viewer options

This menu point opens the same dialog as the options speed button (see above).

Show Traveling Path

Using this menu entry, the display of the traveling path can be turned on and off.

Define Sample Size

This menu entry opens the Define Sample Size and Underlay Bitmap dialog (see above).

Optimize Route

This menu entry automatically optimizes the traveling route in order to minimize the travel length and thus the traveling time. A window will inform the user about the change in traveling distance due to the reordering of the points.

This procedure is not recommended while performing a measurement because it can require substantial processing power. If the point list contains more than 2000 points the software prompts the user for a confirmation of the task due to the considerable amount of time it might take.

Add Point

This menu point has the same functionality as the Add Point speed button (see above).

Delete Point

This menu point has the same functionality as the Delete Point speed button (see above).

Edit Point

This menu point has the same functionality as the Edit Point speed button (see above).

Select Functions

This menu point allows the selection of all points as well as the inversion of the current selection.

Usage Function

The usage of the currently selected points can be edited here. The possible choices are:

Use

At this point the process script sequencer is executed once the point list is carried out.

Do not use

The system will, upon execution of the raster, ignore this point and the system will not drive to it.

Used

The system automatically sets points with the label Yes to Used once the process script has been executed at this position.

Used -> Use

This function changes all points marked Used in the current selection to Yes.

Points marked No in the current selection are not changed.

Sort by Order

This menu point has the same functionality as the Sort by Order speed button (see above).

Move Selection

This menu entry contains the two entries Move Selection Up and Move Selection Down, which are identical to the corresponding speed buttons (see above).

Columns

This menu entry contains all columns of the point list. Activating them in this menu entry will show them in the point list editor.

Spectral Auto Focus

The Spectral Auto Focus component automatically finds the z-axis position with the best Raman signal. This is especially useful for automated measurements like in a Process script or in the ParticleScout.

Auto Focus	Start Auto Focus
Mode	Find Peak
Max. Search Range [µm]	100
Center [µm]	0
Step Size Multiplier	1.00000
Min. Integration Time [s]	0.0099999998
Listen Mask	Never
Mask	250;4000

Mode

- Find Peak: Searches for the maximum total signal doing multiple passes (high accuracy).
- Find Raman Signal: Additional background subtraction and searches for the maximum Raman signal in one pass (fast).

Max. Search Range (Z-Axis Range)

The maximum search range for the autofocus.

Center

The autofocus is performed around the center position using half of the Max. Search Range up and down.

Step Size Multiplier

- Only used in autofocus mode "Find Raman Signal".
- Defines the accuracy and speed for this autofocus mode
- Typical range: 0.5 - 2.0
- Larger values: larger steps -> less accuracy -> faster.
- The actual step size depends on the objective magnification.

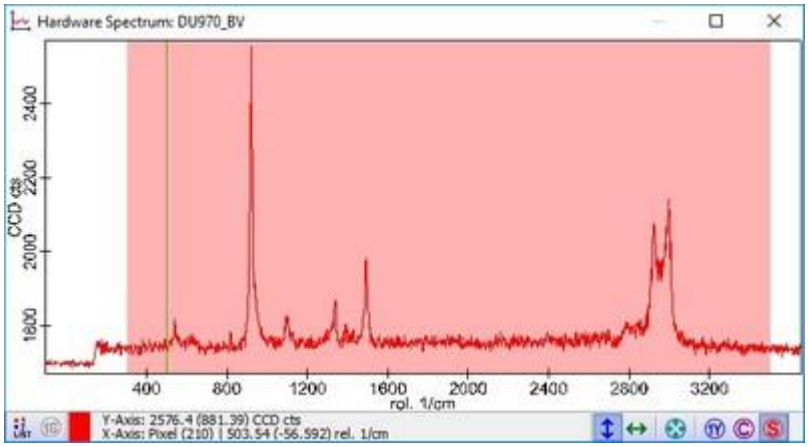
Min. Integration Time

The minimum integration time for each spectrum that is acquired for finding the best focus position. (Can be larger if z-stage is slower.)

Listen Mask

The mask determines which spectral region is used to search the maximum signal.

It is defined using value-pairs separated by semicolon: "250;300;500;550" (The mask is set from 250 to 300 and from 500 to 550.)



The defined mask is visible in the hardware spectrum window of the used CCD camera. If listen mask mode is activated, you can also edit the mask in this window.

Post Focus Move [µm]

Moves the defined amount of microns after the autofocus.

Signal Stabilization

The Signal Stabilization is a feature to compensate thermal drift in the z-direction by defining a reference point, which is checked after each line of the measurement. This can be used either in the Image Scan or the Large Area Scan.

Further information (Operation Guide):

- Signal Stabilization (Raman)

Signal Stabilization	Start Stabilization
Stabilization Enable	No
Stabilization Mode	Peak
Actuator for Compe	Microscope Stage
Step Size Multiplier	1.00000
Number Of Accumu	3
Listen Stabilization	Never
Position (X) [µm]	0.000
Position (Y) [µm]	0.000
Position (Z) [µm]	0.000
Listen Mask	Never
Mask	

Start Stabilization

This button starts the signal stabilization to test the parameters.

Stabilization Enable

This parameter turns the signal stabilization on and off also during the measurement. It is set automatically to Yes if Start Stabilization was successful.

Stabilization Mode

The Stabilization Mode defines the type of surface.

Peak

Used for a sample which only delivers intensity directly at the surface.

Positive Edge

This is for using the upper surface of a volume sample, which delivers signal also below the surface.

Negative Edge

This is for using the lower surface of a volume sample, which delivers signal also above the surface.

Actuator for Compensation

Microscope Stage or Scan Table can be selected here to compensate the drift in the z-direction. The Scan table should be preferred because of the better linearity.

Step Size Multiplier

This parameter can be used to adjust if the intensity peak delivered from the surface, is broader or narrower than normal.

Number of Accumulations

This parameter defines how many spectra will be accumulated at each step.

Listen Stabilization

If this is activated, the position of the reference point for the stabilization can be defined by clicking into an image or the video window.

Position (X/Y/Z) [µm]

These parameters define the position of the reference point for the stabilization. For an Image Scan the values have to be within the scan table range.

Listen Mask

If this is activated, the mask determining the spectral region can be selected by mouse.

Mask

The mask determines which spectral region is used for the compensation. Refer to Spectral Auto Focus.

Lithography

In the Lithography parameter group, a Lithography file can be selected, previewed and processed. Samples can be found in the default lithography folder which opens by clicking the Choose File button. All commands are described under Lithography commands.

Further information (Operation Guide):

- Lithography

Start Lithography

Pressing this button starts the lithography process using the selected file.

File Name

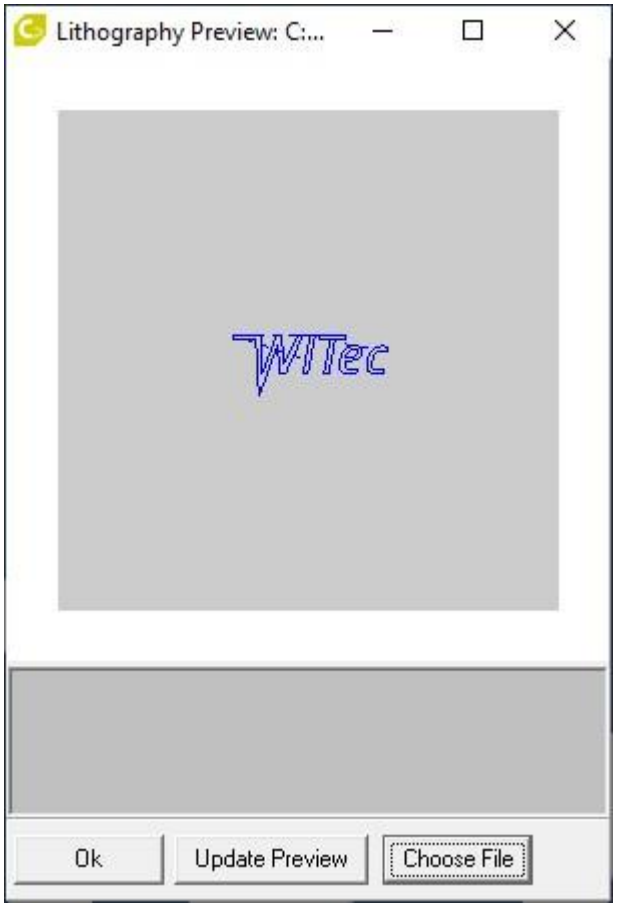
Shows the path of the currently selected file.

Choose File

Opens a dialog to select the file.

Preview

Pressing this button opens a preview of the selected file shown below.



Lithography Commands

With the setup of the software several example scripts are delivered. It is highly recommended to look at these scripts in order to learn how the following commands can be used in combination.

General

Syntax

Each command must have brackets and end with a semicolon.

Example: `TestCommand ();`

Long and Short Command Names

All commands have a long and a short name. The command MovingSpeed(10) is equal to MS(10). In the following description the short names are shown in square brackets after the long name.

The commands are not case sensitive.

Comments

For commenting a single line out, "//" can be used at line start. For a complete block the signs "/*" and "*/" can be used to mark the beginning and the ending of a text block.

Include

With the directive #include a sub script could be added to the script. This helps to reuse parts in other scripts.

Commands

MovingSpeed [ms] (v)

Sets the speed for subsequent moves. The value of the speed v must be set in the unit [µm/s]. The speed must be in the range from 0.1 to 1000.

Example: `MovingSpeed (20);`

Sleep [sl] (t)

The processing of the lithography script is paused for t milliseconds. The parameter may be omitted, in this case the sleep duration is 100ms.

Example: `Sleep (500);`

MoveZMicroscope [mz] (z)

The stepper motor is driven that way, that the distance between objective and sample increases by the value of z. The distance must be set in the unit [µm]. If the distance between objective and sample would exceeds the software range the script will not start.

Example: `MoveZMicroscope (-10);`

SetTrigger [st] (T1, T2, T3)

Using this command you can set, reset and toggle up to three independent trigger signals. T1, T2 and T3 can be one of: DontChange, Off, On or Invert. The output connector of the trigger signals can be set using the signal routing device. The trigger signal defined with T1 is output on Trig 2 Out by default. All three parameters may be omitted.

Example: `SetTrigger (On);`

DisplayMessage [dm] (MessageText)

Displays the message specified in MessageText in the Message Window.

Example: `DisplayMessage ("I'm drawing now");`

Setpoint [sp] (S)

Sets the setpoint of the P-I controller subsystem to the value of S [V]. If the P-I controller is not included in the system this command is not available.

Example: `Setpoint (0.5);`

Snapshot [snap] (FileBaseName, C)

Makes a snapshot with the video camera and stores the image to a Bitmap file. The file name is named FileBaseName-xxxx.bmp, where xxxx is a counter value, starting from 0000, how many files in the current script have been stored. To specify if you want color or B/W images, set parameter C to 1 for color, 0 for monochrome.

Example: `Snapshot("ImageName", 0);`

SetLaserShutter [sls] (State)

Using this command you can open or close the shutter of the current used Laser. The laser must be a True Power Laser equipped with a Laser shutter. State can be "Open" or "Close".

Example: `SetLaserShutter (Open);`

Commands relating to the Piezo Scan Stage

These commands are only available if the microscope is equipped with a piezo scan stage.

PushTransformation [pusht] ()

Every coordinate P(x,y,z) you specify (for the piezo stage) gets transformed with a so called transformation–matrix M before being sent to the scan table.

$$T = M * P$$

To 'save' a copy of the current transformation–matrix, you use the PushTransformation() command. Transformations get stored on a stack one after another, so that you can store multiple transformations, and recall them in reverse order.

PopTransformation [popt] ()

To recall the last 'pushed' transformation from the stack. Calling PopTransformation() more often than

having called PushTransformation() results in an error.

Rotate [rot] (Alpha, Beta, Gamma)

The current transformation matrix is multiplied with a rotation matrix resulting out of the three angles Alpha, Beta and Gamma. Alpha rotates the coordinates about the X–Axis, Beta rotates coordinates about the Y–Axis, Gamma rotates coordinates about the Z–Axis. All parameters may be omitted.

Scale [sc] (Sx , Sy , Sz)

The current transformation matrix is multplied with a scaling matrix resulting out of the three scaling factors Sx, Sy and Sz. All parameters may be omitted.

SetOrigin [so] (Ox , Oy , Oz)

The current transformation matrix is multiplied with a translation matrix resulting out of the three origin coordinates Ox, Oy and Oz. All parameters may be omitted, in this case the current position of the scan table is used as (0,0,0) position within the current transformation.

MoveRelative [mr] (Dx , Dy , Dz)

Moves the scan table to the new position

$$T = T + M * D$$

If the new position is outside of the scan range, the script will not start. All parameters may be omitted.

MoveAbsolute [ma] (Px, Py, Pz)

Moves the scan table to the new position

$$T = M * P$$

If the new position is outside of the scan range, the script will not start. All parameters may be omitted.

JumpRelative [jr] (Dx, Dy, Dz)

Same as MoveRelative except that the new position is driven to at a (nominal) speed of 1000 µm/s.

JumpAbsolute [ja] (Px , Py, Pz)

Same as MoveAbsolute except that the new position is driven to at a (nominal) speed of 1000 µm/s.

WaitForStablePosition [wpos] ()

The processing of the lithography script is paused as long as the position of the scan table is not stabilised. If the position has not stabilised after 2000 times querying it, the script will continue anyway.

Commands relating to the stepper motor driven Sample Positioner

These commands are only available,

- if the microscope is equipped with a sample positioner driven by stepper motors
- if the alphaControl is of generation Marvin 3a (serial number 120-1200-XXX) (with Firmware 1.016) or newer

SetOriginSamplePos [so_sp] ()

The current position of the sample positioner is treated as the origin of the sample-positioning coordinate system (0/0). Absolute positioning commands for the sample positioner are interpreted relative to this origin.

This command is automatically executed once when the script is started.

MoveRelativeSamplePos [mr_sp] (Dx, Dy)

Moves the sample positioner relative to the current position. All parameters may be omitted.

MoveAbsoluteSamplePos [ma_sp] (Px , Py)

Moves the sample positioner to the absolute position (relative to the last set origin). All parameters may be omitted.

JumpRelativeSamplePos [jr_sp] (Dx , Dy)

Same as MoveRelativeSamplePos except that the new position is driven to as fast as possible. The X- and Y-axis may be at the final position at different times.

JumpAbsoluteSamplePos [ja_sp] (Px, Py)

Same as MoveAbsoluteSamplePos except that the new position is driven to as fast as possible. The X- and Y-axis may be at the final position at different times.

Auto Save

Deprecated! The new Autosave settings are located in the Program options.

This auto save parameter group is still available for use with the COM interface e.g. LabView. The autosave can be triggered through the Store Project button. The parameters available for the autosave function are described below:

Auto Save	
Store Project	Store Project
Start Directory	c:\users\demouser\WITec
Extra Directory	Data
File Name	Sample
File Number	1
Next File	c:\users\demouser\WITec
Directory Mode	Extra Directory
Store Mode	Store and Clear
Overwrite Mode	Add Extra Suffix

If directories defined for the auto saving procedure do not exist, they will be created by the software upon saving.

Store Project

This button stores the project using the current settings.

Start Directory

In this field the starting directory can be defined. The full path where the file will be stored will be explained below.

Extra Directory

An additional directory for saving the project can be defined using this field. The full path where the file will be stored will be explained below.

File Name

The first part of the file name can be defined using this field. The composition of the full file name is described in the next point.

File Number

Here a number for the file saved can be assigned. This will automatically be incremented with each saving cycle and the number is automatically expanded to four digits. If for example the number entered is 2 the number used will be 0002.

The total file name will then be:

[File Name] [File Number].WIP

Next File

This field is for information purposes only and displays the path and filename of the next file that will be saved.

Directory Mode

Various modes can be selected here. Below the resulting file path for all methods will be shown.

No Extra Directory

[Start Directory]\

Extra Directory

[Start Directory]\[Extra Directory]\

Date

[Start Directory]\YYYYMMDD\

Here YYYY identifies the year, MM the month and DD the day.

Extra Directory + Date

[Start Directory]\[Extra Directory]\YYYYMMDD\

Date + Extra Directory

[Start Directory]\YYYYMMDD\[Extra Directory]\

Store Mode

Store and Store and Clear can be selected for this parameter. The difference is that Store and Clear will clear the project after saving it.

Overwrite Mode

This parameter will only be of importance if the file which is about to be saved exists already. If Overwrite is chosen, the existing file will be overwritten. If Add Extra Suffix is selected the file name shown above will be changed to:

[File Name] [File Number] YYYYMMDD HHMMSSmmm.WIP

Here YYYY denotes the year, MM the month, DD the day, HH the hour, MM the minute, SS the second and mmm the milliseconds of the moment when the file is saved.

Calibrate Scan Table

This sequencer is executed upon each startup of WITec Control but generally remains hidden from the user.
Only advanced users should alter the settings herein.

When executed, the scan table will be driven to two different positions per axis at which the output of the capacitive sensors are measured. Using these values a lead and an offset error are calculated for each axis. These values are then used to correct the stationary positioning of the scan table during its use. A text data object named Calibration Information is added to the project tree for information purposes.

> This file should also be checked if the calibration of the scan table fails. This may occur if the offset potentiometers for the scan table on the alphaControl are not set to their minimum positions.

The following parameters are available to set the calibration routine:

Start Calibration

This trigger button starts the calibration sequencer.

CalibrateXAxis (CalibrateYAxis, CalibrateZAxis)

This parameter determines if the X (Y,Z) axis is calibrated (Yes) or not (No).

XAxisPos1/2 (YAxisPos1/2 ZAxisPos1/2) [µm]

Using these parameters the two target positions for the calibration of the X (Y,Z) axis can be entered. Entering an out of range value will cause the software to automatically enter the maximum/minimum value possible.

Calibrate Relative To Current Position

If this option is selected (Yes), the scan table will move not to the position entered in e.g. XAxisPos1 but to ([Current X-Axis Position] + XAxisPos1). Similarly it will move to ([Current X-Axis Position] + XAxisPos2) instead of XAxisPos2.

Data Channels

The following output channels are available depending on the system configuration.

X/Y-Sensor

The X/Y-sensor output is the signal collected from the capacitive sensor measuring the X/Y-axis position of the scan table. Its unit is µm. This signal is used to determine the precise position of the scan table.

Z-Sensor (Topography)

The Z-sensor output is the signal collected from the capacitive sensor measuring the Z-axis position of the scan table. Its unit is typically µm. This signal is used to represent the topography of a surface during AFM and SNOM measurements. For confocal measurements the z-sensor output reads the precise z position of the scan table.

Feedback

The feedback output is the setpoint position of the z-axis of the scan table. Its unit is typically µm* (proportional to µm), but without the linear corrections induced by the sensor. This signal is used in AFM and SNOM measurements especially if the surface topography varies by less than 1 nm.

T-B

The T-B output is the difference in the electrical signals collected from the top and the bottom halves of the four quadrant photo diode of the beam deflection system. Its unit is V. This output is used to monitor the feedback during contact mode measurements.

L-R

The L-R output is the difference in the electrical signals collected from the left and right halves of the four quadrant photo diode of the beam deflection system. Its unit is V. This output is used to monitor the torsion of the cantilever during contact mode measurements.

Sum

The Sum output is the total electrical signal collected from the four quadrant photo diode of the beam deflection system. Its unit is V.

Lock-In R

The Lock-In R output signal of the lock-in amplifier is the signal amplitude. Its unit is V and it is used to monitor the feedback during AC mode measurements.

Lock-In Phi

The Lock-In Phi output signal of the lock-in amplifier is the phase φ. Its unit is ° and it is used to monitor the phase shift during AC mode measurements.

F max.

The F max. output is the maximum of the PFM curve as evaluated by the controller hardware. Its unit is V and it is mainly used as the PI controlled channel in PFM measurements.

Adhesion

The adhesion is measured in V and is determined by the controller based on the adhesion search window from the PFM curve. Variations in the adhesion of a sample can be determined using this signal.

Stiffness

The stiffness is measured in V and is evaluated by the controller based on the stiffness search window from the PFM curve. Variations in the stiffness of a sample can be determined using this signal.

Aux 1 and Aux 2

Aux 1 and Aux 2 are the auxiliary channels of the alphaControl system. The unit of these signals is V.

Ext. AD 1/2/3/4

Ext. 1 to Ext. 4 are the auxiliary channels of the alphaControl system. The unit of these signals is V.

PMT, APD and other photon counting devices

Several photon counting devices can be added to an alpha system. Typically, the first photon counter is a PMT and the second counter an APD. The output of the counters is the actual counter reading of how many photons were counted during the last pixel. Its unit is cts (counts).

Count Rate of photon counting devices

The Count Rate output is the counter reading divided by the time required to record the pixel. Its unit is Hz (counts /second).

Sample Pos. X/Y

The Sample Pos. X/Y is the X/Y position of the sample positioning stage in the currently active coordinate system. Its unit is µm.

Microscope Z

The microscope Z output is the relative position of the microscope z-stage. Its unit is µm.

Inv. Mic. X/Y/Z

The inverted microscope X/Y/Z output is the relative position of the inverted microscope in the X/Y/Z-direction. Its unit is µm.

CCS Elevation

The CCS Elevation measures the height at the current position within range of the CCS in µm.

CCS Intensity

The CCS Intensity measures intensity of the CCS signal in %.

Heating Stage Temperature

The heating stage temperature contains the temperature as determined through the calibrated PT100 element in the heating stage.

Fast Stream Channels

Fast stream channels can be transferred with higher speed (5 MHz) from the controller to the PC. There is a limited number of channels available for this fast transfer (T-B, L-R, Aux1 and Aux2). The T-B in fast streaming mode is used e.g. in DPFM measurements.

Spectral Channels

This category of data sources contains the outputs of the spectral cameras connected to the system. Up to three spectral cameras can be added. In this case, one measuring point describes a set of measured data (e.g. a full spectrum).

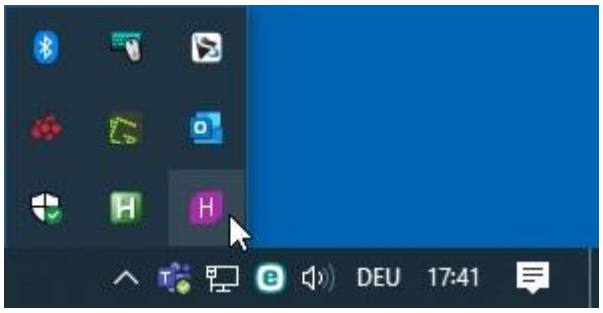
WITec Service Monitor

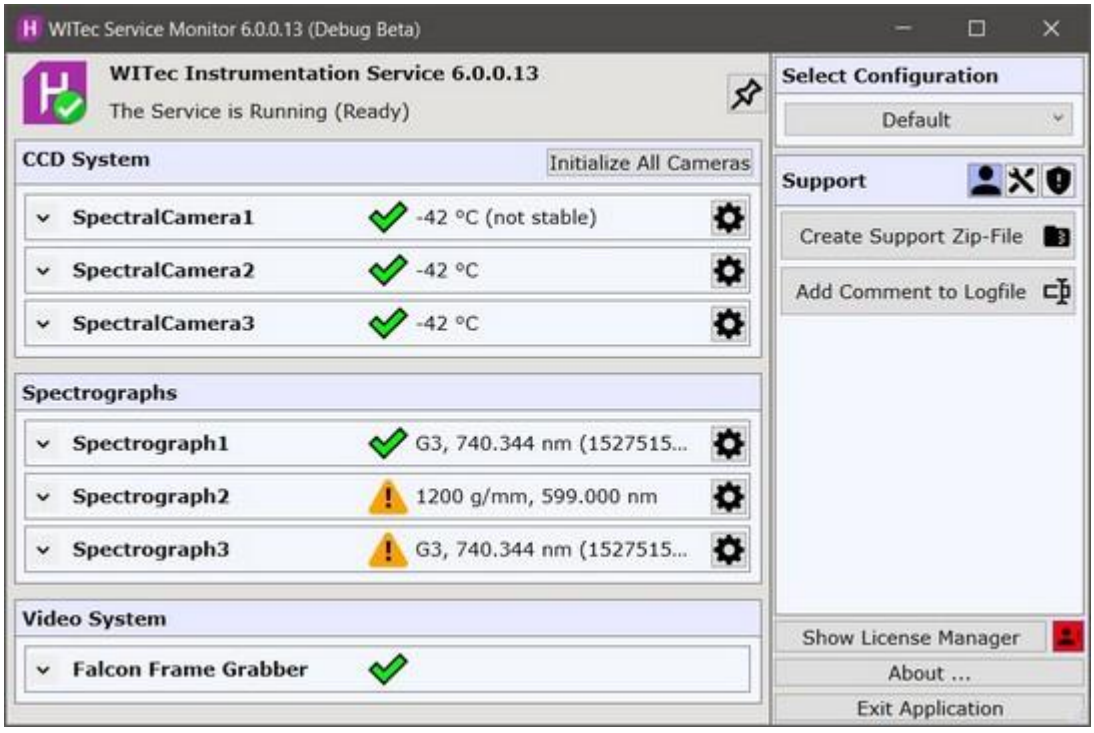
WITec Service Monitor Overview

The WITec Service Monitor is automatically installed with the WITec Suite Complete Setup and automatically starts upon logging on.

It shows the status of the instrumentation service, the microscope controller, the Spectroscopy System - including CCD Cameras and Spectrographs - and all Video Cameras. Additionally, it provides several configuration capabilities for the WITec Support Team.

You can open the Service Monitor in the windows task icon bar in the bottom right corner of the main screen by clicking on the H Icon:





Device List

A green check mark indicates that the device is connected and working as expected.
You can click on a device name to extend the view and see more information.

Microscope Controller

This will show the connected microscope controller unit with its serial number and firmware version.

CCD System

Shows all configured spectral cameras and the current temperature of all connected/powered cameras.

Initialize All Cameras

Disconnects and (re-)initializes ALL spectral cameras.

This allows to use cameras that were plugged in or powered *after* the WITec Software started.

Please do not click this button during any measurement!

 **Configure CCD Camera**

See CCD Camera Configuration.

Spectrographs

Shows all configured spectrographs and the current grating with position.
If a warning icon is displayed, the spectrograph might not be calibrated.
See Spectrograph Calibration.

Video System

Shows all configured video cameras (or for older systems: the frame grabber).

Menu

Select Configuration

Lets you select between different microscope configurations.

This feature is only used for special microscopes having multiple optic / sensor combinations.

Create Support ZIP-File

Opens the Create Support ZIP-File Dialog.

See How to Create Support ZIP File.

Add Comment to Logfile

Allows to add comments in case of errors or bad behavior of the software, so WITec can analyze the log files and know at which time something happened.

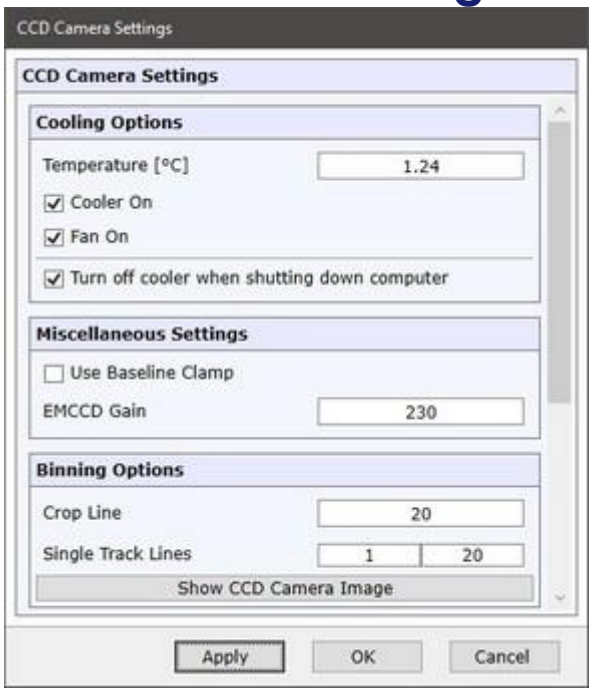
Show License Manager

Opens the WITec License Manager Application

Support

Those tools are intended to be used only by WITec Support members.
The True Power Laser Tool allows you to perform a Laser Fiber Adjustment.

CCD Camera Configuration



Cooling Options

Temperature
Sets the temperature of the CCD chip cooling unit.

Cooler On
Tuns the cooling unit on or off.

Fan On
Turns the cooler fan on or off.

Turn off cooler when shutting down computer
If checked, the cooler is turned off upon shutting down the computer.
Otherwise the camera keeps cooling and can be used immediately after a computer start.

Miscellaneous Settings

Use Baseline Clamp
Only for cameras supporting baseline clamp.
If checked, the A/D converter offset will be stabilized. The maximum possible number of spectra per second will be reduced.

EMCCD Gain
Only for cameras supporting EMCCD amplification.
Sets the gain for the EMCCD mode.

Binning options

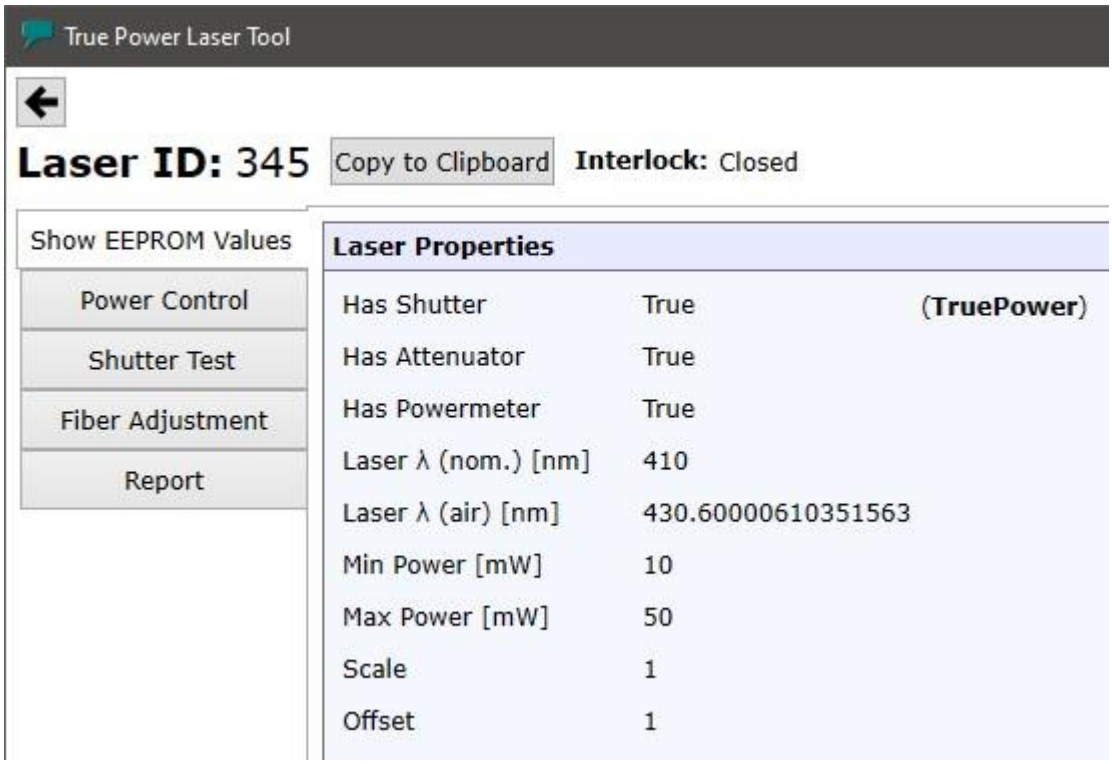
The binning options are used for the crop and single track measurement modes.
Please set the parameters in a way that for all used gratings and spectral positions, the signal is on the configured crop line or within the range of the configured single track lines. Use a calibration light source and the CCD Camera Image to check the parameters.

Crop Line
Defines which CCD line should be used in crop measurement mode.

Single Track Lines
Defines which lines should be used in single track measurement mode.

Show CCD Camera Image
Shows the CCD camera image, intended to set up binning options and to check the position of the signal on the camera.
Intended only for a system responsible person.

True Power Laser Tool



Show EEPROM Values
Shows the current laser properties.

Power Control
Allows to change the laser power and to close the attenuator.

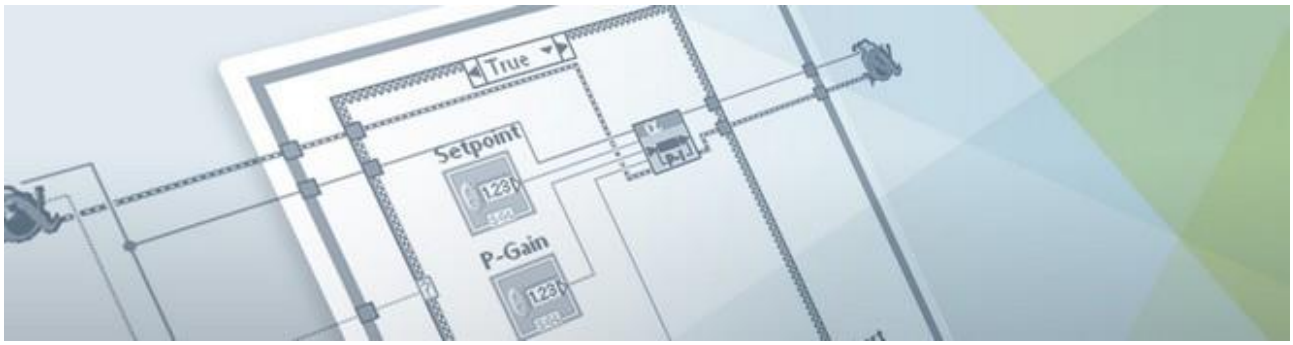
Shutter Test
Here you can check whether the laser shutter is working correctly.

Fiber Adjustment
Shows the current laser power to adjust the laser fiber.

Report
Only for support team:
Runs a self-test and creates an automatic report.

COM Automation

COM Automation Overview



The COM Automation feature enables remote control of WITec Control.

- It is possible to read and write all parameters and to press buttons in the Control window.
- Also many feature in the Video window are accessible. (Excluded: Image Stitching and Focus Stacking)
- Other software (Project, TrueMatch/ParticleScout) is not accessible.

License requirements:

- COM Automation

  (Please contact WITec if you would like a quotation for your system.)

What is COM?

Component Object Model (COM) is a technique for inter-process communication developed by Microsoft. It can be used in a large range of programming languages.

What is LabVIEW?

Laboratory Virtual Instrument Engineering Workbench (LabVIEW) is a visual programming language developed by National Instruments. It is commonly used for data acquisition, instrument control and automation.

What is offered by WITec?

The COM Automation bundle includes:

- Read and write access to the COM interface of WITec Control accessible by various programming languages
- LabVIEW driver including some documented examples
- Python and C# projects to enable easy use

The COM interface can be accessed by various programming languages, WITec offers documented examples for LabVIEW. For Python and C# projects with ready to use classes are supplied with WITec Control. General information about the structure of the COM interface are documented in the interface section.

COM Server and Classes	Describes the COM server and its available classes.
COM Interfaces	Describes the available interfaces.
COM Subsystems	Describes the different types of parameters and how to find them.
Remote access	Describes how to access the COM interface remotely by a computer which is not the one of the microscope.
LabView	Explains how to install the LabVIEW driver.
Python	Explains how to install and use the WITec Python package
C#	Basic example code for C# which is a good starting point also for other programming languages.
MATLAB	Some remarks about using MATLAB and why it is not recommended.

Allow write access to WITec Control

Read access to all parameters is granted by default. To set parameters or trigger actions write access to WITec Control has to be allowed. Click COM Automation in the COM Automation section of the control tree. This opens a window which blocks user entries in WITec Control until Revoke is clicked.

The correct functionality of the WITec Control COM Interface can be checked by starting WITec Control and running the test program "WITec Control COM Connection Tester" which is installed with WITec Control.

> Please note, that WITec will of course assist you with errors or problems caused by WITec Control or the WITec LabVIEW driver. However, WITec will not test or correct userwritten code. The correct functionality of these is solely the user's responsibility.

COM Server and Classes

COM Server

The COM server consist of a DLL and a type library. Both is by default installed with WITec Control.

It can be used as local or out-process server over its class ID (clsid) **C45E77CE-3D66-489A-B5E2-159F443BD1AA**. There is no program ID (progid) defined. For the use as remote server refer to remote access. The use as in-process server is not possible.

The name of the type library is BasicUniversalCOMServerLib.tlb. The help string (shown in OLE/COM viewer or LabVIEW) is **Basic Universal COM Server Library**.

Implemented Classes

This section lists all available classes that are offered by the DLL. Each class has implemented one or more interfaces.

The naming of all classes starts with **CBUCS** (**C**lass **B**asic **U**niversal **C**OM **S**erver).

CBUCSCore

Interfaces: IBUCSCore, IBUCSAccess

Each client should create one entity of this class to create all subsequent objects with the IBUCSCore interface functions.

CBUCSSubSystemList

Interfaces: IBUCSSubSystemList

This object is created by the GetSubSystemsList() function of the IBUCSCore interface. The object contains the available subsystems and their respective interface GUIDs. Objects of this class are no subsystem itself.

CBUCSSubSystem

Interfaces: IBUCSAccess, IBUCSSubSystemInfo

All subsystem classes are derived from this class. This class is not created by the client.

CBUCSSingleValue

Interfaces: IBUCSSingleValue (+ interfaces of CBUCSSubSystem)

All classes for single values are derived from this class. This class is not created by the client.

CBUCSIntSingleValueMinMax

Interfaces: IBUCSInt, IBUCSSingleValueHasLimits, IBCUSIntMinMaxValues (+ interfaces of CBUCSSingleValue)

This class implements all interfaces necessary to modify an integer value. The default interface is IBUCSInt. All other interfaces of this class can be cast to this interface.

CBUCSFloatSingleValueMinMax

Interfaces: IBUCSFloat, IBUCSSingleValueHasLimits, IBUCSFloatMinMaxValues (+ interfaces of CBUCSSingleValue)

This class implements all interfaces necessary to modify an floating point value. The default interface is IBUCSFloat. All other interfaces of this class can be cast to this interface.

CBUCSStringSingleValue

Interfaces: IBUCSString (+ interfaces of CBUCSSingleValue)

This class is for modifying an string values of any desired length. The default interface is IBUCSString.

CBUCSBoolSingleValue

Interfaces: IBUCSBool (+ interfaces of CBUCSSingleValue)

This class is for modifying a boolean value. The default interface is IBUCSBool.

CBUCSEnumSingleValue

Interfaces: IBUCSEnum (+ interfaces of CBUCSSingleValue)

This class is for modifying a list or enumeration value. The default interface is IBUCSEnum.

CBUCSTrigger

Interfaces: IBUCSTrigger (+ interfaces of CBUCSSubSystem)

This class is to trigger an action. The default interface is IBUCSTrigger.

CBUCSStatusContainer

Interfaces: IBUCSStatusContainer, IBUCSFillStatusContainer (+ interfaces of CBUCSSubSystem)

This class is for reading status data. The default interface is IBUCSStatusContainer.

COM Interfaces

This sections lists all available interfaces and their respective methods. The classes implementing theses interfaces are described in the classes section.

The naming of all interfaces starts with **IBUCS** (**I**nterface **B**asic **U**niversal **C**OM **S**erver). All interfaces derive from IDispatch.

IBUCSCore

Reads available subsystem objects in their hierachic structure.

GetSubSystemsList(in Name : BSTR, in SubSystemDepth : int) : IBUCSSubSystemList

Description List all subsystems starting from a defined subsystem and returns them as list.

Parameters *Name:* Path to the starting subsystem. Use an empty string to start at the root.

SubSystemsDepth: Defines the number of levels that should be listed. If it is 0 only one level is listed (the subsystems of the defined subsystem).

Return Creates a CBUCSSubSystemsList object and returns the IBUCSSubSystemsList pointer.

GetSubSystem(in Name : BSTR, in refid : IID) : IUnknown

Description Creates a subsystem object and uses the specified interface.

Parameters *Name:* Path to the subsystem.

refid: GUID of the interface that should be used for the subsystem. It should be implemented for the requested subsystem.

Return If the path to the subsystem is valid and the interface is implemented, it returns the defined interface as IUnknown*. It needs to be cast to the interface according to the specified IId.

GetSubSystemEx(in Name : BSTR, in InterfaceName : BSTR) : IUnknown

Description Creates a subsystem object but accepts the interface GUID as string.

Parameters *Name:* Path to the subsystem.

InterfaceName: GUID of the interface that should be used for the subsystem as string (format like in the table at the end). It should be implemented for the requested subsystem.

Return If the path to the subsystem is valid and the interface is implemented, it returns the defined interface as IUnknown*. It needs to be cast to the interface according to the specified IId.

GetSubSystemDefaultInterface(in Name : BSTR) : IUnknown

Description Creates a subsystem object and uses the default interface.

Parameters *Name:* Path to the subsystem.

Return If the path to the subsystem is valid, it returns the default interface (according to the IDL definition) as IUnknown*. It needs to be cast to the default interface.

IBUCSSubSystemsList

The interface for CBUCSSubSystemsList which contains a list of subsystems.

GetNumberOfSystems() : int

Description Gives the number of elements in the list.

Parameters -

Return Number of subsystems in the list.

GetBaseName() : BSTR

Description Retrieves the base name of all subsystems.

Parameters -

Return Path of all subsystems in the list. (The path was used to create the list.)

GetSubSystemName(in SystemIndex : int) : BSTR

Description Returns the name of the subsystem specified by SystemIndex.

Parameters *SystemIndex:* Index of the subsystem. Must be >= 0 and < than the number of subsystems (GetNumberOfSystems() - 1)

Return String containing the name of the subsystem.

GetSystemSubSystemsList(in SystemIndex : int) : IBUCSSubSystemList

Description Creates a list of subsystems below the subsystem specified by SystemIndex.

Parameters *SystemIndex:* Index of the subsystem. Must be >= 0 and < than the number of subsystems (GetNumberOfSystems() - 1)

Return Creates a CBUCSSubSystemsList object containing the subsystems below the specified subsystem and returns the IBUCSSubSystemsList pointer to it.

GetSubSystemNameAndIId(in SystemIndex : int, out SystemName : BSTR, out DefaultInterfaceId : BSTR) : HRESULT

Description Returns the name and the GUID string of the subsystem specified by SystemIndex.

Parameters *SystemIndex:* Index of the subsystem. Must be >= 0 and < than the number of subsystems (GetNumberOfSystems() - 1)

SystemName: A string pointer which is filled with name of the subsystem

DefaultInterfaceId: A string pointer which is filled with GUID of the default of the subsystem

Return Returns true if it was successful.

IBUCSAccess

This interface controls the access of the client(s) to the server.

HasReadAccess() : bool

Description Determines whether the client has read access.

Parameters -

Return Returns true if the object has read access on the server.

HasWriteAccess() : bool

Description Determines whether the client has write access.

Parameters -

Return Returns true if the object has write access on the server. If one object of an application has write access on the server, all objects creates by this application have write access.

RequestWriteAccess(in WantWriteAccess : bool) : HRESULT

Description Requests write access for the client.

Parameters *WantWriteAccess:* IndexTrue if the client wants to gain write access and false if the client wants to drop the write access. The server takes care that only one application has write access.

Return Returns true if the object has write access on the server after the call.

IBUCSSubSystemInfo

This interface retrieves information about a subsystem.

GetName() : BSTR

Parameters -

Return Complete path including the name of the subsystem.

GetEnabled() : bool

Parameters -

Return Returns true if the subsystem is enabled and can be used.

IBUCSSingleValue

Can be used to retrieve information about a changeable value (parameter). Only implement this interface for parameters which value can be converted to a string.

GetDisplayName() : BSTR

Parameters -

Return Returns the caption (label) of the parameter in the host application (WITec Control).

This description may contain white spaces and special characters.

GetValueAsString() : BSTR

Parameters -

Return Current value of the parameter as string.

IBUCSSingleValueHasLimits

This interface can request whether the parameter has a upper or lower limit different from the one given by its data type. It can not be used to retrieve the values of the upper or lower limit.

HasMinimum() : bool

Parameters -

Return True if the parameter has programmatic lower limit.

HasMaximum() : bool

Parameters -

Return True if the parameter has programmatic upper limit.

IBUCSInt/IBUCSFloat/IBUCSBool/IBUCSString

These interfaces allow access to a parameter with regard to its type.

GetValue() : int/float/bool/BSTR

Parameters -

Return The value of the parameter as respective type.

SetValue(in Value : int/float/bool/BSTR) : HRESULT

Parameters *Value:* The new value of the parameter as respective type. Only possible if write access on the server is granted. The host application needs to take care that the value is within the possible range. The server dll will not check for it.

Return Returns true if it was successful.

IBUCSIntMinMaxValues/IBUCSFloatMinMaxValues

These interfaces retrieve the upper or lower limit of a parameter due to its type.

GetMinimum() : int/float

Parameters -

Return The lower limit of the parameter due to its type.

GetMaximum() : int/float

Parameters -

Return The upper limit of the parameter due to its type.

IBUCSEnum

This interface can be used to change or read a list or enumeration parameter. List parameters have a respective string for each numerical value.

GetAvailableValues(out NumericalValues : SAFEARRAY, out StringValues : SAFEARRAY) : int

Parameters *NumericalValues:* Array pointer, which is filled with the numerical values of this parameter.

String Values: Array pointer, which is filled the strings for the respective numerical values.

Return Returns the number of available values.

`C/C++/C#`: After using this function it is necessary to call `SafeArrayDestroy()` for NumericalValues and StringValues!

GetValue(out ValueString : BSTR) : int

Parameters *ValueString:* A string pointer which is filled with the respective string of the current selected value by the server.

Return The current numerical value of the parameter.

SetValueNumeric(in Value : int) : HRESULT

Parameters *Value:* The numerical value that should be set for the parameter. The host application needs to take care that the value is within the possible range. The server dll will not check for it.

Return Returns true if it was successful.

SetValueString(in Value : BSTR) : HRESULT

Parameters *Value:* The string respective to value that should be set for the parameter. The host application needs to take care that the value is valid. The server dll will not check for it.

Return Returns true if it was successful.

IBUCSTrigger

This interface can trigger an action.

OperateTrigger() : HRESULT

Parameters -

Return Returns true if the action started successful.

IBUCSStatusContainer

A interface to retrieve status information from the server. This could be a single value of different types or an array of one or more dimensions.

Update() : HRESULT

Updates the status of the container.

Parameters -

Return Returns true if it was successful.

GetSingleValueAsString(out StringValue : BSTR) : bool

Parameters *StringValue:* Pointer to a string which is filled with the string of the current status of the container. The status is not allowed to be an array.

Return Returns true if it was successful.

GetSingleValueAsInt(out IntValue : int) : bool

Parameters *IntValue:* Pointer to a Int value which is filled with the value of the current status of the container. The status is not allowed to be an array.

Return Returns true if it was successful.

GetSingleValueAsDouble(out DoubleValue : double) : bool

Parameters *DoubleValue:* Pointer to a double value which is filled with the value of the current status of the container. The status is not allowed to be an array.

Return Returns true if it was successful.

GetStatusArray(out Dimensions : int, out DimensionExtents : SAFEARRAY, out StatusArray : SAFEARRAY) : bool

Parameters *Dimensions:* Pointer to an Int value which contains the number of dimensions of the status array after the call.

DimensionsExtents: Pointer to an array which contains a one-dimensional array with the extent for each dimension after the call.

Status Array: Pointer to an array which contains the complete status array after the call. Take care for consistent indexing.

Return Returns true if the status array is valid.

`C/C++/C#`: After using this function it is necessary to call `SafeArrayDestroy()` for DimensionExtents and StatusArray!

GetStatusProperties(out Caption : BSTR, out Unit : BSTR) : bool

Parameters *Caption:* Pointer to a string which is filled with the description of the current status value.

Unit: Pointer to a string which is filled with the unit of the current status value.

Return Returns true if the status is valid.

IBUCSFillStatusContainer

An interface to transfer a one-dimensional data array to the server.

FillDataArray(in DataArray : SAFEARRAY) : HRESULT

Parameters *DataArray:* The array with data (variant SAFEARRAY with data type VT_ARRAY | VT_R4 or VT_ARRAY | VT_BSTR)

Return Returns true if the array was transfered successful.

`C/C++/C#`: The server calls `VariantClear()` for the passed DataArray.

Table of interface GUIDs

With the method `GetSubSystemNameAndIId()` of IBUCSSubSystemsList it is possible to determine the GUID of the default interface of an object. The following table gives an overview about the available interfaces and their respective GUID (IId).

Interface Name	Interface GUID (IId)
IBUCSSubSystemsList	512E8C62-F2C9-4840-8C5C-746E7FCE3B5B
IBUCSCore	78D9E3C8-7E0A-4788-B4D8-EF22365D3648
IBUCSAccess	594ACBE6-938A-411F-A62E-E06FE6DBD35C
IBUCSSubSystemInfo	A548CB11-959F-46C0-BACD-C813837CB9C4
IBUCSSingleValue	A75ED3DF-D774-412C-8DF3-B7640EAE0551
IBUCSSingleValueHasLimits	5C4AF664-B397-4D35-8D98-8E2AE222A8EB
IBUCSInt	EFAE9411-A8E0-461D-A5F4-4887595AA830
IBUCSIntMinMaxValues	67B7266A-9EC3-4417-A900-7FCE26FD3AD6
IBUCSFloat	3EBB7227-74F9-4A0D-9AC9-7E3327AB5221
IBUCSFloatMinMaxValues	3D750FA2-17AB-4D75-AAFA-8D6DD5717289
IBUCSString	90C0EA65-0483-46BB-80FD-B1B536D73FC4
IBUCSBool	08862E7F-BE29-4DCF-B2FC-55A3DFAE33F7
IBUCSTrigger	923FC802-04D3-4BEE-AE63-A349051FE2E8
IBUCSEnum	CC1BD98A-2D74-4242-B5F9-0288FC58E339
IBUCSStatusContainer	5CAF623C-976F-46DC-9624-2F685B00D293
IBUCSFillDataArray	EC6F2072-5998-4318-8B49-6F4E995407E6

COM Subsystems

In WITec Control all available parameters, actions and status information are organized in a hierarchic structure of subsystems and their respective objects. Each subsystem may contain several subsystems, but not every subsystem is a discrete object. Some subsystems just group other subsystems below them without being an object (like a parameter, action or status) itself.

To create an object its complete path must be known. The path is comparable to a path in a file system. A pipe (|) is used as delimiter. A path can be i.e. A | B | C | X.

All objects belong to one of the four main groups that build the first part of the path: *UserParameters*, *MultiComm*, *Status* and *ApplicationControl*. They are further explained in the following.

All available parameters can be listed by executing the LabView vi "Get Available Subsystems.vi" or by using the method GetSubSystemsList() of IBUCSCore. The availability of single parameters depends on the microscope configuration.

Application Control	*ApplicationControl*	Actions of the main menu
User parameters	*UserParameters*	Parameters of the Control tree
Status container	*Status*	Retrieves status information or sends data
Additional parameters	*MultiComm*	Parameters of the video control window

Application Control

Selected actions found in the main menu of WITec Control are implemented as distinct subsystems. These subsystems have **ApplicationControl|** at the beginning of the path.

Name	Type	Description
LoadConfiguration	String	Retrieves or changes the current selected configuration.

		Retrieves/expects the name of the configuration (without file extension) and its path relative to the configurations folder i.e. "AFM\AFM AC".
UpdateHardware	Trigger	Apllies changes of the user parameters to the hardware and updates the status data afterwards.
ExitApplication	Trigger	Closes WITec Control.
FileNameToAppendToProject	String	Writing the filename of a WIP-file to this parameter will append the project to the current project.
NewProject	Trigger	Deletes the current project and creates a new project

For saving a project refer to the Auto save group in the default tree.

User parameters

Most of the parameters that can be found in the Control tree in WITec Control are organized in the UserParameters group. Select the left column of the regarding parameter in the Control tree and press Ctrl + C to copy its path to the clipboard. To create a complete path to the respective parameter the part **UserParameters|** has to be added at the beginning. The following types of parameters can be found:

- Integer parameters are objects of the type CBUCSIntSingleValueMinMax.
- Parameters using numbers with radix point are objects of the type CBUCSFloatSingleValueMinMax.
- String parameters are objects of the type CBUCSStringSingleValue.
- Parameters using a dropdown list are objects of the type CBUCSEnumSingleValue.
- Boolean parameter are objects of the type CBUCSBoolSingleValue. The difference between a list parameter with only two possibilities and a boolean parameter is not obvious.
- Buttons starting an action are objects of the type CBUCSTrigger.

If a client tries to create an object for a not implemented parameter the server raises an error and returns a null pointer.

Additional parameters

There are additional parameters in related to the Video Control window that have **MultiComm|MicroscopeControl|** at the beginning of the path. A list of available parameters can be found in the Menu of the Video Control window in the Advanced section.

Status Container

The status container is used to C to WITec Control. All status container have **Status|** at the beginning of the path and are implemented as CBUCSStatusContainer. If a non-implememted status is updated the application returns E_NOTIMPL. If not stated different the direction of data is from server to client.

Application status

Path Status|Software|Application|ProgramVersion

Type single value, string

Description	The name, version and ,if existing, build version of the application.
Path	Status | Software | Application | MemoryStatus | PhysicalMemory Status | Software | Application | MemoryStatus | PageFile Status | Software | Application | MemoryStatus | AddressSpace
Type	single value, integer
Description	The available space in % of the respective value.
Path	Status | Software | Application | CurrentFileName
Type	single value, string
Description	The name of the project file, if existing.

Sequencer status

Path	Status | Software | Sequencers | IsASequencerActive
Type	single value, bool
Description	Return true if a sequencer is active.
Path	Status | Software | Sequencers | ActiveSequencer | Name
Type	single value, string
Description	The name of the active sequencer. If no sequencer is active it returns E_ACCESSDENIED.
Path	Status | Software | Sequencers | ActiveSequencer | CurrentActivity
Type	single value, string
Description	The current activity of the sequencer (refer to the tables at the end of the page for more detailed information). If there is no specific action, it returns Sequence Busy. If no sequencer is active it returns E_ACCESSDENIED.
Path	Status | Software | Sequencers | SequencerTimeSeriesSlow | UserDataCaptions
Type	1-dimensional array, string
Description	The description of the external user data channels.
Direction	Client → Server
Path	Status | Software | Sequencers | SequencerTimeSeriesSlow | UserDataUnits
Type	1-dimensional array, string
Description	The units of the external user data channels.
Direction	Client → Server
Path	Status | Software | Sequencers | SequencerTimeSeriesSlow | UserDataValues
Type	1-dimensional array, float
Description	The values of the external user data channels.
Direction	Client → Server

All three parameters (UserDataCaptions, UserDataUnits and UserDataValues) must be filled with arrays of the same dimension before starting the Slow Time Series. The values are not visible in the GUI.

Data channel status

Path	Status | Hardware | Controller | DataChannels | [name of the data channel]
Type	single value, float
Description	The current value of this data channel (scale and offset are taken into account). The available data channels depend on the hardware configuration and can be readout by using the method GetSubSystemsList() of IBUCSCore.

COM subsystem status

Path	Status | Software | COMAutomation | AcceptsRemoteConnection
Type	single value, bool
Description	Returns true if the server allows write access.
Path	Software | COMAutomation | COMCallPerformance
Type	1-dimensional array with 2 values, long
Description	Index 0: Tic frequency of the host systems (low dword) Index 1: Tic count of the host systems (low dword) Used to measure the time between two COM calls.

Sequence activity strings for all sequencers

The status container Status | Software | Sequencers | ActiveSequencer | CurrentActivity retrieves the current action of a sequencer. If not stated different it can deliver one of the following strings for the current activity. (It is not possible to retrieve the numeric value.)

Sequence Activity String	Numeric value
Sequence Busy	0
Sequence not Active	-1
Cleaning up after Sequence End	-2
Unknown Sequence Activity (n)	n

The following sequencers retrieve specific activity strings.

SequencerAutoFocus

Auto Focus Activity String	Numeric value
Preparing Data-Channels and Microscope	1
Searching for Focus-Position Iteration #n	2
Moving Microscope to Focus-Position	3

SequencerScanImage

Image Scan Activity String	Numeric value
Preparing Data Objects and Scan Path	1

Scanning Image	2
Restarting	3
Collecting data	4
Moving to next Depth-Scan Line	5
Waiting for User to Continue Scan manually	6

SequencerTimeSeriesSlow

Slow Time Series Activity String	Numeric value
Preparing Data Objects	1
Waiting for next Measurement	2
Executing Sub-Sequencer	3
Acquiring Spectral Data	4
Acquiring Data Channels	5

Remote access

It is possible to access WITec Control (server) over network from a different PC (client) over DCOM (Distributed COM). It should be done only if really necessary. Reasons for doing so:

- Combined control of the WITec system and another device connected to the other computer
- no LabVIEW license available on the microscope PC

Instructions

- Both PCs (client and server) must be in the same network domain
- Install the COM Add-on on the client PC by executing "WITecControlCOMAddOn_6_X_Setup.msi".
- The user that wants to access from the client PC must be logged on and must have administrator rights on the server PC on which WITec Control is running. (WITec Control doesn't need to be executed with administrator rights)
- Configure the DCOM service using the Component services tool (dcomcnfg.exe) on the server PC (Fig. 1):
    - Open Properties of My Computer over the context menu (right-click) and go to the tab COM security click on both Edit Default buttons.
    - Make sure the local administrators have local and remote access, launch and activation rights.
- Search for BUCS Application in the DCOM Config folder and open the properties of it on the server
    - Security tab: Deny local and remote launch, allow local and remote activation, Access and configuration should be on Default.
    - General tab: Authentication level should be on Default
    - Identity tab: Interactive user should be used

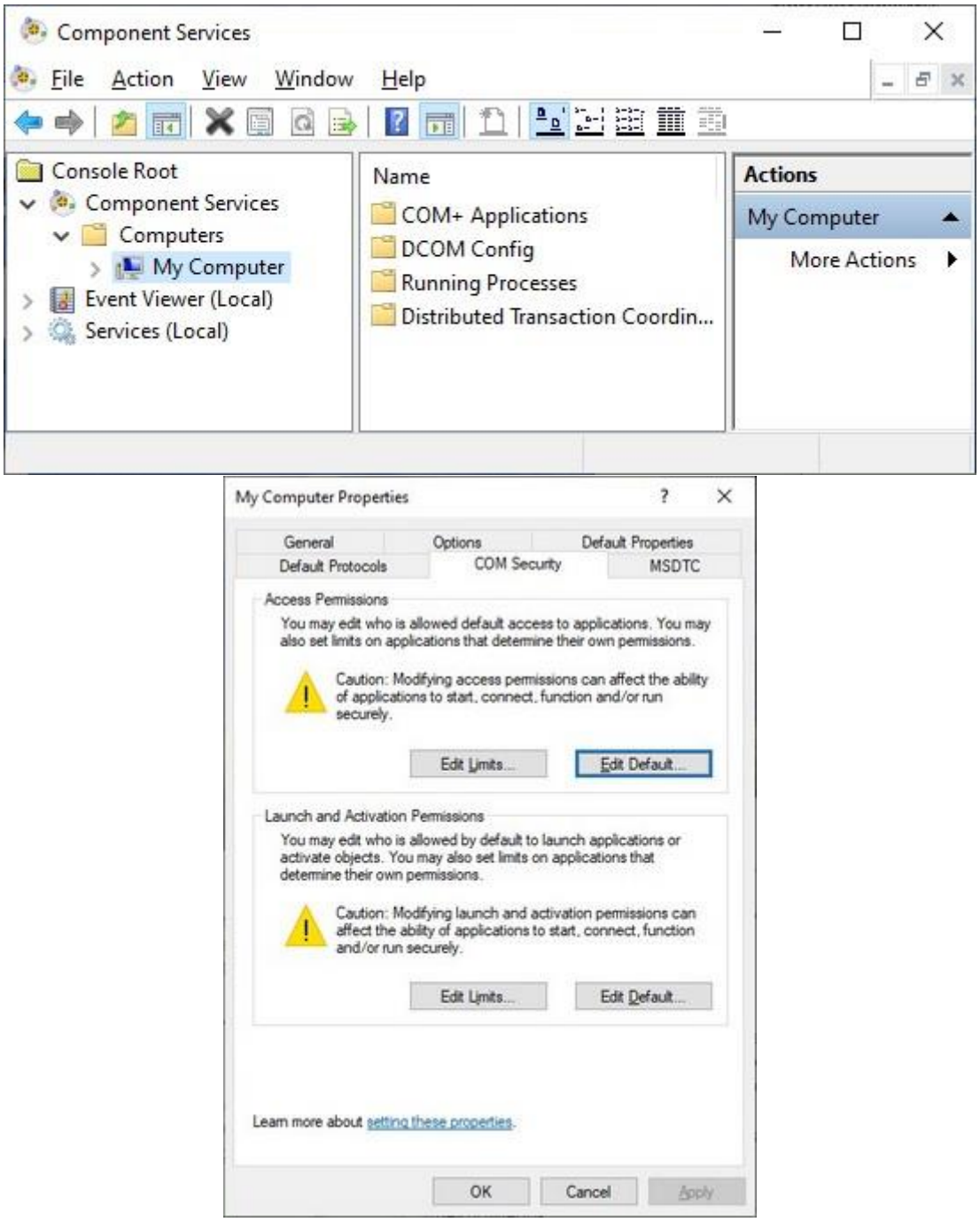


Fig. 1: Component services tool

Remarks

- The users that want to use the remote access need to be member of the same windows domain. Creating users with the same user name on both computers will not work.
- The Windows-DCOM-service must run on both computers.
- If NetBIOS is used, the computer name has to be not longer than 15 characters.
- Optional: Install the LabVIEW driver on the client PC, if LabVIEW should be used.
- Refer to the allow write access section for testing the connection. Use the "WITec Control COM Connection Tester" on the client PC.

LabVIEW Driver

Functional blocks in LabVIEW are called Virtual Instruments (VIs) and are implemented for most control functions offered by WITec Control.

This describes the installation of LabVIEW software components related to WITec products as well as verification of successful installation. A short description of the examples delivered with the interface is also included. These are additionally described in greater detail in separate documents.

For common questions and troubleshooting, the customer is referred to the FAQ (frequently asked questions) document which is also included.

> Assistance with writing LabVIEW code can be obtained and training purchased from National Instruments.

Preconditions

Before installing the WITec Control LabVIEW driver, LabVIEW must be installed:

- LabVIEW version 8.5 (or higher)

Installation

- Install the WITec Control LabVIEW driver by running the Program "WITec Control LabVIEW Driver Setup.msi".
- Select the correct LabVIEW instr.lib directory of your LabVIEW installation as the installation directory.

After the installation, the WITec Control LabVIEW driver is available in LabVIEW in the folder "Instrument IO/Instrument Drivers" as shown in Fig. 1.

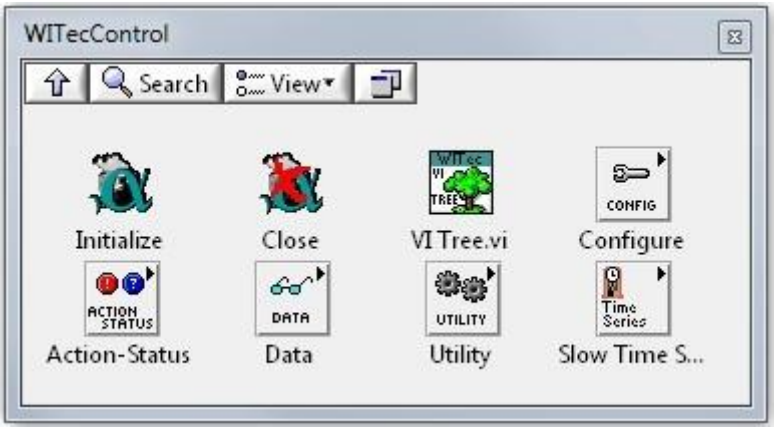


Fig. 1: The WITec Control LabVIEW drivers.

Examples

The WITec Control LabVIEW driver project contains a few additional examples to assist in

understanding this interface. These examples and the corresponding detailed descriptions are located in the LabVIEW driver directory and its subdirectory "Examples". The first example (Slow Time Series) is documented in the most detailed way including block diagrams to facilitate the understanding for the user.

- Slow Time series (EFM license and ADC + DAC boards needed)
- Fast Time series (EFM license and ADC + DAC boards needed)
- Image Stack (Piezo stage needed)

It is recommended that the users familiarize themselves with these examples prior to initiating their own project.

Test the connection

Run the example LabVIEW VI "QueryReadWrite" which is included in the WITec LabVIEW driver.

(If you use remotly access the microscope PC, enter its network name as server name in the corresponding field as shown in Fig. 2.)

After starting the VI, the "HasReadAccess" indicator should be green. After pressing "WantWriteAccess" the "HasWriteAccess" indicator should also be green as shown in Fig. 2 (if the write access is granted in WITec Control).

Fig. 2: Testing the Read and Write access of LabVIEW for WITec Control.

Python

For Python a ready to use package with classes for different measurement modes and actions is delivered with WITec Control. In the following the installation process is described. Further down it will be described how to use Python without this package.

WITec Python package

Preconditions

- Install the latest Python version (>= 3.10)

Package installation (online)

The WITec Python package can be installed using pip:

```
pip install WITecSDK
```

Package installation (offline)

If the computer is not connected to the internet, install the offline package and its dependencies:

- WITecSDK – download latest version or install delivered version: `pip install "C:\ProgramData\WITec\WITec Suite 6.X\Common Files\WITec.SDK\Python\WITecSDK-1.2.X.tar.gz"` (replace 6.X and 1.2.X with the current WITec Suite and package version)
- comtypes – a lightweight Python COM library – download and install

Examples

Example code using the WITec Python package:

```
from asyncio import run
from WITecSDK import WITecSDKClass

WITec = WITecSDKClass()
WITec.RequestWriteAccess()
videoImageAcquisition = WITec.CreateVideoControl()
bitmappath = run(videoImageAcquisition.AcquireVideoImageToFile())
```

Some more examples can be found here (replace 6.X with the current WITec Suite version):

C:\ProgramData\WITec\WITec Suite 6.X\Common Files\WITec.SDK\Python\Examples

- **LASExample.py:** Shows how to define and start different types of Large Area Scans
- **LAScanfromFile.py:** Can be used to define Large Area Scans in WITec Control and save the settings to a file with the method `WriteLAtoFile(filename)`. The file can be used to run the predefined scans automatically with `RunLAfromFile(filename)`. This example shows how to dynamically use the write access. While measuring the window allowing write access to WITec Control can be closed to use i.e. the filter viewer. The program will then wait with starting the next Large Area Scan until the COM Automation button in the Control tree is pressed again to allow write access for external software.
- **ManualTopography.py**: Shows a possibility to automate the manual topography correction by using the spectral autofocus.
- **HeatWITecTimed.py:** This shows the use of the WITec heating stage and how to feed additional data channels to a slow time series. Furthermore it implements a spectral Autofocus for the Slow Time series.
- **HeatWITecManual.py:** This is doing the same as the previous example, but uses the manual slow time series. This allows to trigger the next measurement individual.
- **AdditionalParameter.py:** Shows how to read all available parameters to a file and how to access a specific parameter.

Linkam stages

An additional package for controlling Linkam stages can be provided. Please contact us for further information.

Use without WITec Python package

Prerequisites:

- install comtypes (`pip install comtypes`)

or

- install pywin32 (`pip install pywin32`)

To use the interface IBUCSFillStatus:

- for comtypes: code how to define a BSTR array and a Float array can be found in COMClientComTyp.py of the WITec Python package.
- for pywin32: how to define a BSTR array and a Float array is shown at the end of the example code.

Example code using comtypes:

```
from socket import gethostname
from comtypes.client import GetModule, CreateObject

bucstlb_id = '{E84FEF4C-DD2F-4B8D-9C2F-D016FEBB01F4}'
GetModule((bucstlb_id, 1, 0))
from comtypes.gen.BasicUniversalCOMServerLib import *

# Connect to WITec Control
wcCoreInterface = CreateObject(CBUCSCore, interface = IBUCSCore, machine =
gethostname())

# Get a parameter modifier
parameterModifier =
wcCoreInterface.GetSubSystemDefaultInterface("UserParameters|SequencerAsTimeG
oesBy|IntegrationTime").QueryInterface(IBUCSFloat)

# Read the value of a float parameter
value = parameterModifier.GetValue()
print(value)

# Request Write Access
# Precondition: WITec Control must allow Remote Write Access (Control-Form,
Parameter: COM Automation -> Allow Remote Access)
wcCoreInterface.QueryInterface(IBUCSAccess).RequestWriteAccess(true)

# Write the value of a float parameter
parameterModifier.SetValue(0.42)
```

Example code using pywin32:

```
from pythoncom import CLSCTX_REMOTE_SERVER, VT_ARRAY, VT_R4, VT_BSTR
from win32com.client import DispatchEx, CastTo, VARIANT
from socket import gethostname

# Connect to WITec Control
CLSID = "{C45E77CE-3D66-489A-B5E2-159F443BD1AA}"
IBUCSAccess = DispatchEx(CLSID, machine = gethostname(), clsctx =
CLSCTX_REMOTE_SERVER)
IBUCSCore = CastTo(IBUCSAccess, 'IBUCSCore')
```

```
# Get a parameter modifier
parameterModifier =
IBUCSCore.GetSubSystemDefaultInterface("UserParameters|SequencerAsTimeGoesBy|
IntegrationTime")
parameterModifierFloat = CastTo(parameterModifier, 'IBUCSFloat')

# Read the value of a float parameter
value = parameterModifierFloat.GetValue()
print(value)

# Request Write Access
# Precondition: WITec Control must allow Remote Write Access (Control-Form,
Parameter: COM Automation -> Allow Remote Access)
IBUCSAccess.RequestWriteAccess(True)

# Write the value of a float parameter
parameterModifierFloat.SetValue(0.42)

# Define arrays for IBUCSFillStatus
stringarray = VARIANT(VT_ARRAY | VT_BSTR, ["str1","str2"])
floatarray = VARIANT(VT_ARRAY | VT_R4, [5.2,8.4])
```

C#

An example Visual Studio Solution can be found in

C:\Program Data\WITec\WITec Suite 6.X\Common Files\WITec.SDK

Example code using the SDK:

```
using (WITecSDK witecSDK = new WITecSDK())
{
   witecSDK.ConnectWithWriteAccess();

   using (VideoImageAcquisition videoImageAcquisition =
witecSDK.CreateVideoImageAcquisition())
   {
      Bitmap videoImage =
videoImageAcquisition.AcquireVideoImage().GetAwaiter().GetResult();
   }
}
```

Example code without SDK:

```
// PreCondition: Add a reference to "BasicUniversalCOMServerLib" (Referecnes
-> Add -> COM -> BasicUniversalCOMServerLib)
static void Main(string[] args)
{
    // Connect to WITec Control
    string serverName = Environment.MachineName;
    Guid bucsCoreGuid = new Guid("C45E77CE-3D66-489A-B5E2-159F443BD1AA");

    Type bucsCoreType = Type.GetTypeFromCLSID(bucsCoreGuid, serverName,
true);
    IBUCSCore wcCoreInterface = Activator.CreateInstance(bucsCoreType) as
IBUCSCore;
```

```
    // Get a parameter modifier
    IBUCSFloat parameterModifier =
wcCoreInterface.GetSubSystemDefaultInterface("UserParameters|SequencerAsTimeG
oesBy|IntegrationTime");

    // Read the value of a float parameter
    float value = parameterModifier.GetValue();
    Console.WriteLine("Float Value of parameter: " + value);

    // Request Write Access
    // Precondition: WITec Control must allow Remote Write Access (Control-
Form, Parameter: COM Automation -> Allow Remote Access)
    ((IBUCSAccess)wcCoreInterface).RequestWriteAccess(true);

    // Write the value of a float parameter
    parameterModifier.SetValue(0.42f);


    // Get a list of all available SubSystems
    CBUCSSubSystemsList subSystemNameList =
wcCoreInterface.GetSubSystemsList(null, 0);
    for (int i = 0; i < subSystemNameList.GetNumberOfSystems(); i++)
    {
        Console.WriteLine("Subsystem Name: " +
subSystemNameList.GetSubSystemName(i));
        var subSystemName = subSystemNameList.GetSubSystemName(i);

        var subSystemParameterList =
wcCoreInterface.GetSubSystemsList(subSystemName, 10);
        for (int j = 0; j < subSystemParameterList.GetNumberOfSystems(); j++)
        {
            Console.WriteLine("Parameter Name: " +
subSystemParameterList.GetSubSystemName(j));
        }
    }
}
```

MATLAB

It is possible to use MATLAB with the COM automation of WITec Control, but there are several issues.

1. MATLAB needs a ProgID to connect to the COM server. It is not set by default. Add the following entries in the registry:

```
[HKEY_LOCAL_MACHINE\SOFTWARE\Classes\Witec.COMAutomation]

[HKEY_LOCAL_MACHINE\SOFTWARE\Classes\Witec.COMAutomation\CLSID]
@="{C45E77CE-3D66-489A-B5E2-159F443BD1AA}"

[HKEY_LOCAL_MACHINE\SOFTWARE\Classes\WOW6432Node\CLSID\{C45E77CE-3D66-
489A-B5E2-159F443BD1AA}\ProgID]
@="Witec.COMAutomation"
```

2. The COM interface returns IUnknown interfaces and it is necessary to cast them to an appropriate interface. This is only possible in 32-bit versions of MATLAB. The last version available in 32-bit was R2015b. In a 64-bit MATLAB the last line in the example code will throw an error, so working with subsystems is not possible. (Refer to the help of actxserver: 64-bit MATLAB does not support custom interfaces.)
3. It is not possible to define the server type when connecting. By default MATLAB will connect as in-process server, but it needs to be connected as out-process server.

- For 32-bit MATLAB: Delete the following registry entry and restore it after MATLAB is connected.

```
HKEY_CLASSES_ROOT\WOW6432Node\CLSID\{C45E77CE-3D66-489A-B5E2-
159F443BD1AA}\InprocServer32
```

- For 64-bit MATLAB: This is not a problem, because it is not able to load the 32-bit DLL as in-process server and is using it as out-process server instead.

For these reasons it is not recommended to use MATLAB directly. One solution is to create i.e. a C wrapper for implementing the functions to MATLAB.

Example code:

```
witeccom = actxserver('Witec.COMAutomation')
witeccom.RequestWriteAccess(true)
witeccom.HasWriteAccess
witeccore = invoke(witeccom, 'IBUCSCore')
subsyslist = witeccore.GetSubSystemsList('',0)
inttime =
witeccore.GetSubSystemDefaultInterface('UserParameters|SequencerAsTimeGoesBy|
IntegrationTime')
inttimefloat = inttime.invoke('IBUCSFloat')
```

WITec Project

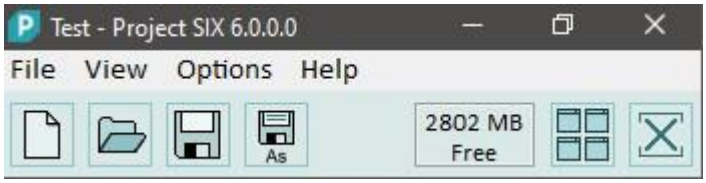
Welcome to WITec Project

Welcome to the WITec Project Data Evaluation Software Help.

Main Window	Load/Save Projects, Options, Help
Working with Projects	Managing Projects and Data, Import/Export, Project Manager
Data Objects	Different Kinds of Data Objects, Memory Requirements
Data Visualization	Handling of Image-, Graph- and Text Viewers
Data Analysis	Drop Action Dialogs, Analysis Software Concepts, Example Analysis
Math	Mathematical Definitions and Descriptions
Program Options	Viewer Defaults, Window Positioning, OpenGL Mode, Memory Strategy
Licensing	Licensing Information, Software Activation

Press the **F1 key** anywhere in the software to open the context help or browse the Help Menu to open the help contents.

Main Window



Test - Project FIVE
The title of the application shows the current file name and the application name.
If the project has been changed and not saved, an asterisk "*" character is displayed left from the file name.

Tool Buttons

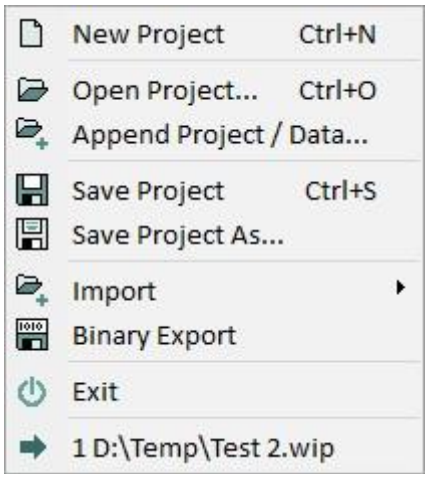
- New Project

- Open Project
- Save Project
- Save Project as

- Memory Information
- Reset Viewer Positions
- Close all Viewers

Memory Information
Hovering the memory information with the mouse shows the following memory information:
PM = Amount of free space in physical memory
PF = Amount of free space in page file
AS: Amount of free space in address space in percent
Free: Amount of free space in address space in Megabytes
If the text gets red, you should consider saving the file and creating a new project.

File Menu



New Project
Creates a new, empty project.

Open Project
Opens an existing project from file. See Loading or appending a Project.

Append Project/Data
Appends all data from a selected project file to the current project. See Loading or appending a Project.

Save Project
Saves the current project and overwrites the existing file. See Saving a Project.

Save Project As
Saves the current project and asks the user for a filename. See Saving a Project.

Import (Sub Menu)
See Import Overview.

View Menu

Drop Actions Window
Shows or hides the drop action window.

Cursor Manager Window
Shows or hides the cursor manager window. Here you can see all different cursor positions, e.g. spatial, spectral, CCD counts, ...

Graphic Export/Editor
Opens the graphic export window which can be used to export a bitmap to a desired image file format.

Close All (Sub Menu)
Here you can close all windows of a certain kind with one click. E.g. close all image viewers.

Reset Viewer Positions
This will realign all your viewer windows.

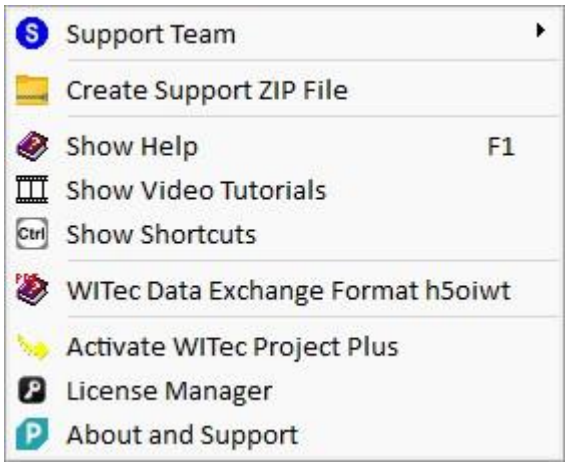
Point Viewer Window
Only visible in WITec Control.
Shows the point viewer for Raster Sample measurements.

Options Menu

Program Options
Shows the program options window.

Calibrate Space Transformations
For special systems: lets you define a spatial transformation for non-calibrated scan table measurements.

Help Menu



Support Team (Sub Menu)
Contains some special functions that should be used only by a WITec employee.
To send log files and further information to WITec, the sub menu-item "Create Support ZIP File" can be used.

Create Support ZIP File
Collects information about your system and saves it into a compressed ZIP file.
Used for WITec Support Team to help fixing software issues.

Show Help
Shows this help manual. You can press F1 anywhere in the software to open the context help:
The help for the window which has the focus will be shown.
In the Video Window: the help for the component which is under the mouse pointer will be shown.

Show Video Tutorials
Opens the Web page for WITec Video Tutorials.

Show Shortcuts
Shows the shortcut viewer

WITec Data Exchange Format h5oiwt
Opens the help PDF for the h5oiwt file format.

Activate WITec Project Plus
Shows the WITec Project Plus Activation licensing information.

License Manager
Shows the License Manager. Here you can add new or remove expired licenses.

About and Support
Shows the About Dialog. This will show the following information:

- Software Version Number
- Presence of Plus License
- System ID and Service ID for support
- OpenGL Hardware Acceleration
- Operating System Version

- Memory Information
- Buttons for sending emails to the support.

Working with Projects

Working with Projects Overview

A "**Project**" contains measurement data, with all additional information necessary for interpreting the data and further processing; also viewers and their current settings are stored in a Project.

A Project is stored in one .wip file.

Manage Projects

To learn how to manage projects, see Save and Load.

Manage Data

You can manage your Data Objects using the Project Manager.

Export and Import

Several export and import features are available, most of them via the Project Manager.

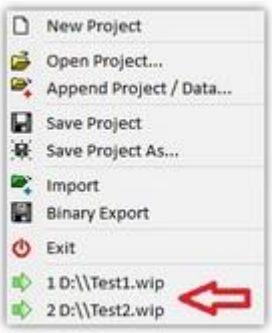
Save and Load

Loading or Appending a Project

You can load a Project by using the main menu "**File > Load Project**".
Note that the currently opened Project will be closed and all data will be lost if you don't save the Project before (a dialog may ask you before).

If you want to add all data from another Project to your currently opened Project you can append it using the main menu "**File > Append Project**".

Loading Recent Projects



You can load recently opened Project files by using the main menu "File" and selecting a file at the bottom of the menu.
Note that files which no longer exist on the hard drive will not be shown here.

Saving a Project

Save the entire Project

You can save your Project by using the main menu "**File > Save Project**" (or **"Save Project As..."**) to save all your data on a hard drive or flash drive. All data will be saved in one .wip file (WITec Project File).

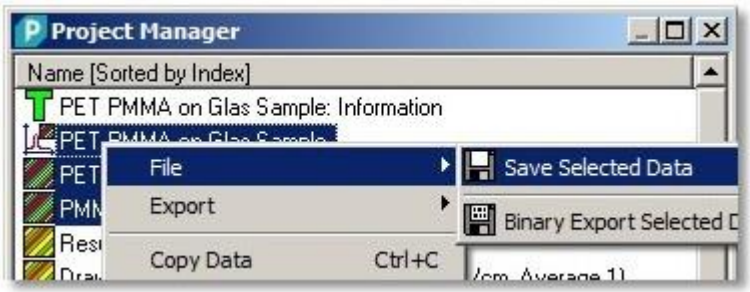
What will be saved when saving a Project?

- All data listed in the Project Manager are saved (including Data Objects that are not currently shown in the Project Manager, e.g. Transformation or Interpretation Objects).
- Viewer windows such as Image-, Graph-, Text- Viewers and their settings.
  The position and size of the Viewer windows are also saved but the windows will be automatically repositioned upon loading. You can deactivate this feature in the Viewer Positioning Options.
- Filter Viewers with their current filters. Preview windows of the filter Viewers are not saved. Only the drop action/analysis window is saved with the project.

What will NOT be saved:

- Currently opened **Drop Action Dialogs** and their temporary preview results and Viewers will **NOT** be saved.

Save or append several Data Objects (not the entire Project)

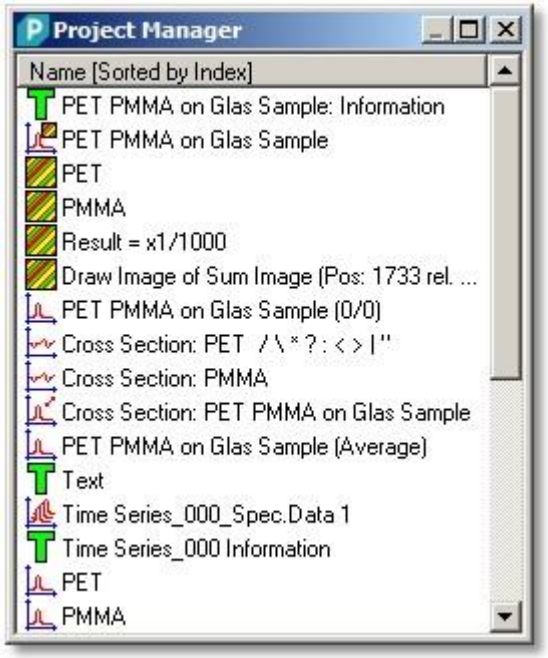


It is possible to save one Data Object only or even a selection of several Data Objects into a .wid file (WITec Data File).
This action can be performed by selecting several Data Objects in the Project Manager and opening the context menu **"File > Save Selected Data"**.

It is possible to append a previously saved data file to the current Project using the main menu "**File > Append Data**".

Project Manager

Project Manager Overview



Description

The Project Manager shows all measurement Data Objects as well as the results from the performed analysis. It is also the tool best suited to organize, visualize or analyze measured data.

The Project Manager is automatically created upon starting the program. Another Project Manager window can be added using the main menu **"Add > Project Manager"**.

Features

- Copy and Paste Data Objects
- Delete Objects
- Rename Caption

You can also show or hide data categories, change the sort mode or start data analysis via the
- Project Manager Circle Menu

To change the default behavior, also have a look at the
- Program options for the Project Manager.

You can also have a look at the context menu:
- Project Manager Context Menu

Data Visualization

To visualize a Data Object, double-click on it or select one or multiple Objects and press the enter

key.

For more information see Data Visualization.

Data Analysis

A Data Analysis is performed via the so-called Drop Action dialogs: You can drag and drop one or multiple selected Data Objects from the Project Manager onto one of the drop action buttons. Alternatively start the analysis via the Circle Menu "Actions".

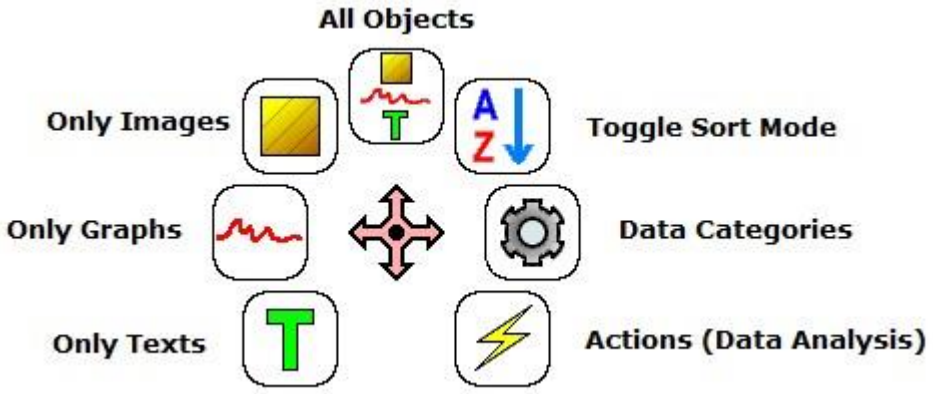
For more information see Data Analysis Overview.

Data Objects

To learn more about the different kind of Data Objects, see Data Objects Overview.

Project Manager Circle Menu

Use the Circle Menu (keep right mouse button pressed) for fast access to certain features:



All Objects
Shows Images, Graphs and Text Objects.

Toggle Sort Mode
Toggles between sort modes:

- Sort by Index (the newest Data Object is at the bottom of the list)
- Sort by Name
- Sort by Category

(You can also click on the "Name" heading of the Project Manager's list box in order to change the sort type.)

Data Categories
Opens a dialog for changing the visibility of data categories and the sort mode.

Actions
If Data Objects are selected, certain actions can be performed with them (see Drop Actions Window).

Only Texts
Shows only Text Objects.

Only Graphs
Shows only Graph Objects.

Only Images
Shows only Image Objects.

Copy and Paste Data Objects

It is possible to copy a selection of Data Objects into the Windows clipboard. You can do this by opening the Project Manager's context menu item "Copy Data" (shortcut Ctrl-C).

Note that the **clipboard will be cleared** upon starting any WITec Project or WITec Control instance. If you intend to copy data from one instance of WITec Project into another, you must open the second instance before you copy your data.

Simply use the Project Manager's context menu item "Paste Data" (shortcut Ctrl-V) to append the copied data to the Project.

If you would like to **copy and paste multiple Objects** make sure you paste them in one step; otherwise some analysis features will not work due a missing relation between between the single Objects.

Pasting Bitmaps
It is also possible to copy bitmap data such as screenshots into the Project using the "Paste Bitmap" function. A new bitmap object without transformation will be created and appended to the Project.

Delete Objects

You can delete one or multiple selected Data Objects using the context menu item "Delete Data" (shortcut Delete).

Be careful:

- Deleting Data Objects can **NOT** be reversed!
- Deleting Transformation or Interpretation Objects that are still used by Image- or Graph-Objects will lead to data loss and therefore inconsistent data; i.e. the data possibly can no longer be used for visualization or analysis. Please only delete Data Objects if you are certain they will no longer be used.

Rename Caption

You can rename a single Data Object by using the context menu item "Rename" (shortcut F2).

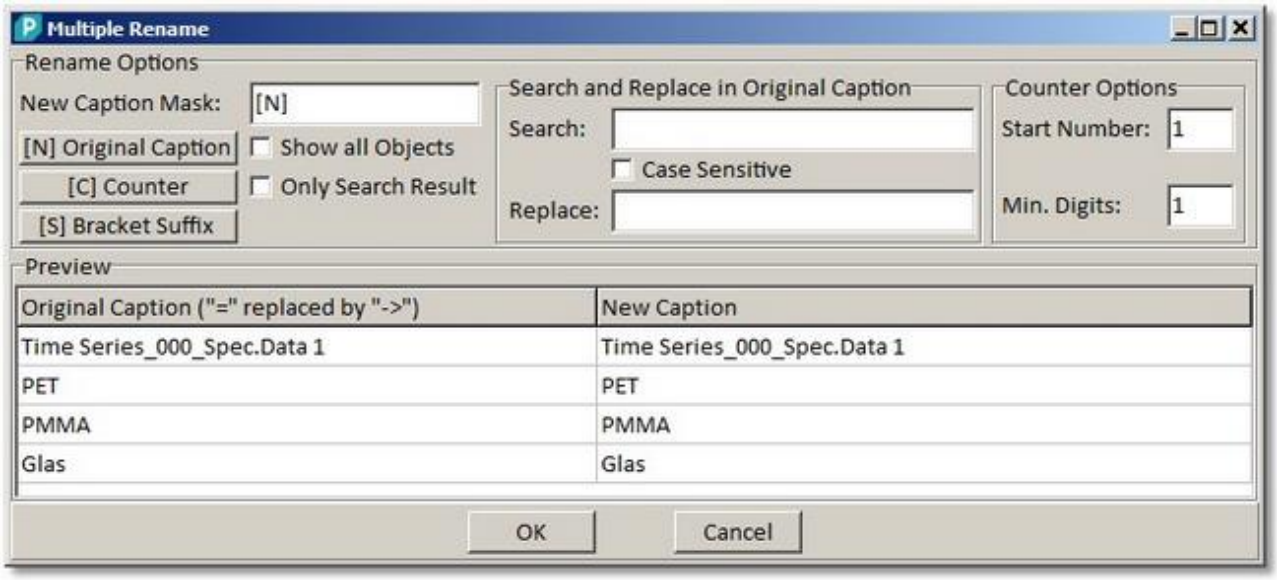
It is also possible to rename multiple Data Objects using the context menu item "Multiple Rename" which will open the Multiple Rename Tool.

Multiple Rename

Description

The Multiple Rename tool allows the changing of the caption/name of multiple Data Objects using a mask or by using search and replace. It's also possible to modify each caption separately after the caption-generation process.

User Interface



Rename Options

New Caption Mask
Enter a mask for the new caption; you can use the following variables for the mask:

[N] Original Caption
Inserts the original caption of the Data Object.

[C] Counter
Inserts a counter value.

[S] Bracket Suffix
Inserts all strings written in brackets in order to preserve information regarding data analysis results.

Show all Objects
If checked, all Data Objects in the complete Project are shown and renamed, otherwise only the selected objects are shown and renamed. Please only use this feature if you use search and replace. Save your project before renaming all data.

Only Search Result
If checked, the new caption mask is only applied on names that contain the current search string.

Search and Replace

Enter a search and replace string in order to replace all occurrences of the search pattern by the replace string.

Counter Options

Start Number
Define the start number for the counter value variable [C].

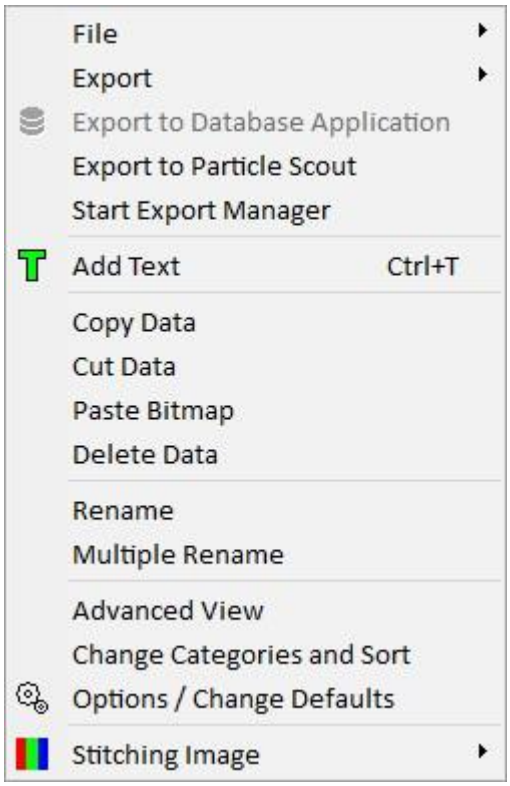
Min # of Digits
Define the minimum number of digits for the counter value (for preceding zeros).

Preview

The preview shows you the original caption and also the new caption which is created by changing the above mentioned Rename Options. You can also **change the new caption** in the second column to define a completely custom string, but be careful: this string is **overwritten upon changing** any Rename Options.

Project Manager Context Menu

The Context Menu of the Project Manager contains several management features. It can be opened by clicking and releasing the right mouse button anywhere in the Project Manager.



File
The File Menu enables a selection of Data Objects to be saved in a WITec Project file or exported through the WITec Binary Export file format.

Export
You can export one or multiple Data Objects using one of the export features.

Export to Database Application
Depending on the Database Export Options, the selected single spectrum data object(s) will be opened in an external database application such as the WITec TrueMatch Database Software or ACDLabs. Also see Database Search in WITec Project.

Export to Particle Scout
Exports a Video Image or a Stitching Image to the WITec ParticleScout.

Start Export Manager
Starts the WITec Export Manager.
If data objects are selected, they will be exported to the Export Manager.

Add Text
Adds a new text data object for adding custom notes.

Copy/Cut/Paste Data
Copies/Cuts/Pastes the selected Data Objects into/from the windows clipboard.

> The clipboard is cleared when starting a new WITec Project instance; data is temporarily stored on the hard drive in a WITec Temp Directory. When copying Data Objects the corresponding internal helper Data Objects (e.g. transformations) are also copied automatically.

Delete Data
Deletes the selected Data Objects.

Rename
Renames the currently selected Data Object.

Multiple Rename
Opens the multiple rename dialog which enables to rename multiple Data Object at once.

Advanced View
This will show all internal Data Objects (transformations, interpretations, ...). Please, use this feature only if you really know what you are doing. Deleting internal data objects can lead to data loss or crashes.

Change Categories and Sort
Opens the quick menu for changing Data Categories and the sort mode. You can also click on the Name column header of the project manager to change the category or open that quick menu via the circle menu.

Options / Change Defaults
This will open the program options for the Project Manager.

Data Object Sub Menu ("Stitching Image")
If **one** Data Object is selected, at the bottom of the context menu there is a special sub menu for this Data Object. The contents of this sub menu depends on the kind of data object which is selected.
See Graph Data, Image Data, Bitmap Data

Import/Export

Export

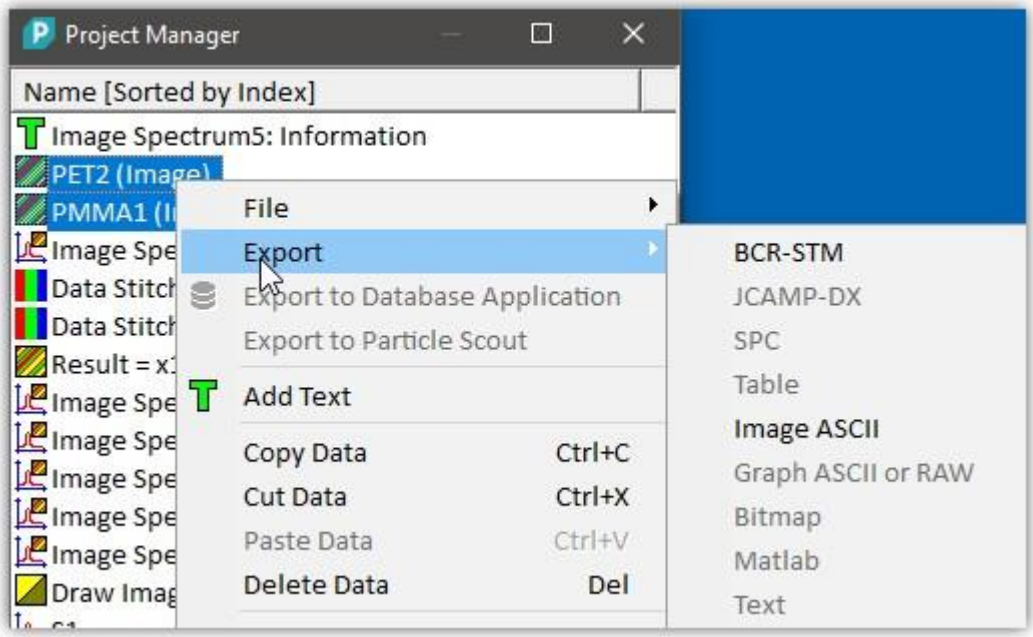
Export Overview

You can export your data in several ways:

- Export Data Objects into one or multiple files or the clipboard.
- Export the current **drawing/bitmap** or even the ASCII data from a **Viewer.**
  See Graph Viewer Export or Image Viewer Export.
- Export Data Objects using the WITec Export Manager.
- Binary export into the custom and public WITec Binary Export Format.
  To perform a binary export of the whole project, select from the main menu "**File > Binary Export**".
  To perform a binary export of selected items in the Project Manager, open the Project Manager's Context Menu and select "**File > Binary Export Selected Data**".

Export Data Objects

You can export one or several Images, Graphs or Text Data Objects into one or several files using the context menu of the Project Manager:



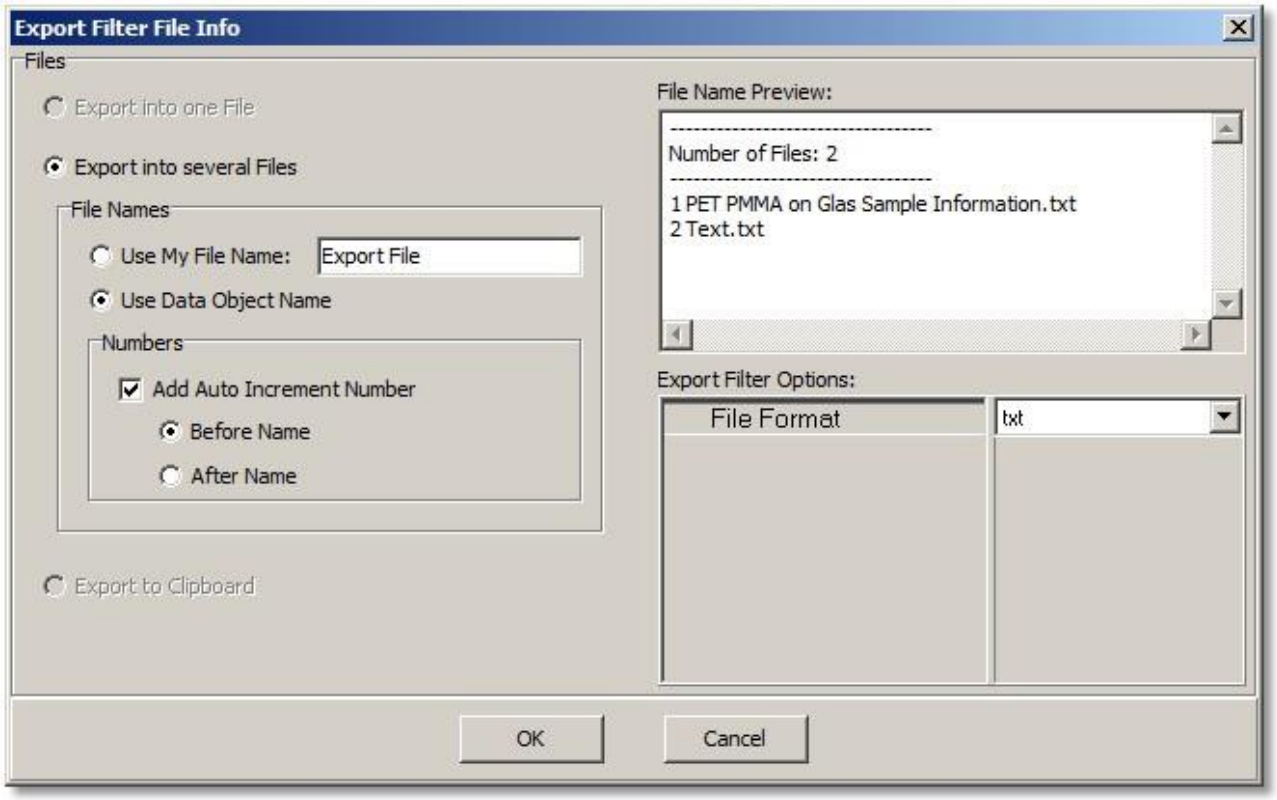
Available Formats

The following export formats are available:

- BCR-STM
- JCAMP-DX
- SPC
- Table (ASCII)
- Image ASCII
- Graph ASCII or RAW
- Bitmap
- Matlab
- Text

Export Dialog

The following dialog will open upon selecting one of the export features:



"Export Into One File":
Exports one or several Data Objects into the same file.
Available for certain export filters.

"Export Into Several Files":
Exports one or several Data Objects into several files.
Available for certain export filters.

"Export To Clipboard":
Exports one or several Data Objects into the clipboard.
Available for certain export filters.

File Names

"Use My File Name":
Define your own file name. All output files will use this file name together with an auto increment number.

"Use Data Object File Name":
Uses the caption of the exported Data Object as file name.

"Add Auto Increment Number":
If checked, an auto increment number is added to the file name. You can choose between adding the auto increment number before or after the file name. This feature is automatically used when using a single file name.

"File Name Preview":
Shows a preview of all file names that will be exported.

"Export Filter Options":
Here you can change some export options; each export filter uses different export options, i.e. the Text Export function for example has file format options allowing files to be saved in ASCII or Rich Text Format.

Export BCR-STM

Description

The BCR-STM format is a very old format for storing STM images. It is also used to store AFM images.

Input and Output

Input:
One or multiple (floating point) image objects.

Output:
One or multiple BCR-STM files, each containing one image.

Format

The format contains an ASCII header with some properties of the image. The header size is exactly 2048 characters. The image data is stored as binary data (floating point 32 bits per value).
In general one image is stored in one file, but you can export multiple images in one step into multiple files.

Export JCAMP-DX

Description

The JCAMP-DX format is a pure ASCII file format. It is used to export different kinds of spectra. There is no clear specification for this format because the header attributes are defined differently by each organization.

Input and Output

Input:
One or several spectral data objects (of any dimension).

Output:
- One or several files, each containing all spectra of each selected data object
- One file containing all spectra of all selected data objects
- All data can be exported to Windows Clipboard

Format

The file contains different header information followed by the spectral values, separated by spaces.
The x-axis values are equidistant.

Export Filter Options

X Unit:
The unit of the exported x-axis data.
Can be: rel. cm 1/cm, 1/cm, nm and µm.

Interpolation Type:
When the x-axis values are converted into equidistant values, the y-axis values have to be interpolated.
This parameter defines how the y-axis values are interpolated.
It can be a cubic spline or a linear interpolation.

Number of Points Type:
The number of exported supporting points / x-values can be defined in 3 different kinds.
For each type there is one parameter that you can change, see the following parameter descriptions.

Oversampling Factor:
The smallest distance between two x-values is calculated. This distance has the number of supporting points defined in this parameter.

Fixed Number of Points:
Defines exactly the number of x-values.

Wanted Data Spacing:
Defines exactly the data spacing (defined in the selected unit) of the x-values.
The actual spacing can vary slightly because the first and the last point of the spectrum are exactly on a supporting point.

Export SPC

Description

The SPC format is a binary file format for different kind of spectra.
See https://en.wikipedia.org/wiki/SPC_file_format

Input and Output

Input:
One or multiple spectral data objects (with any dimension).

Output:
One or multiple files, each containing all spectra of each selected data object

Export Filter Options

X Unit
The unit of the exported x-axis data.
Can be rel. 1/cm, 1/cm, nm and µm.

Export Table

Description

The export table format exports any kind of graph data objects into ASCII Tables.

Input and Output

Input:
One or multiple graph data objects (with any dimension).

Output:
- One or multiple files, each containing all spectra of each selected data object
- One file containing all spectra of all selected data objects (if all objects share the same x-axis values)
- All data can be exported to Windows Clipboard

Format

The first column of the table always contains the x-axis values (in the selected unit).
All other columns are y-values of the graph objects.

Export Filter Options

Column Delimiter:
The separation character used to separate the columns.
Can be Tabulator, Semicolon, Comma and Space.

Text Qualifier:
Column Labels and Column Units are surrounded by the text qualifier.
Can be nothing, quote sign or inverted comma.

Decimal Separator:
Can be point or comma.

Decimal Precision:
Defines the number of decimal places. The exponential format is used, e.g. 1234567 with a

precision of 3 is exported as 1.23E+06.

Export Column Label:
Exports the name of the data object as a table header.
If a graph object contains multiple spectra, the spectrum number is added as a suffix.

Export Column Unit:
Exports the unit strings of the x- and y-axis values as a table header.

X Unit:
The unit of the exported x-axis data. Can be all x-units that WITec Project can handle (see Interpretation Data).

Export Image ASCII

Description

The Image ASCII format exports floating point image data objects into an ASCII file.
Can be used as a compatible export from the ASCII export options in older software versions.

Input and Output

Input:
One or multiple (floating point) image data objects.

Output:
- One or multiple files, each containing one image
- Single images can be exported to Windows Clipboard

Format

The ASCII header gives some information about the image like the number of pixels and the scan size and position.
The image values are stored line by line as exponential floating point strings with a selected separator.

Export Filter Options

Export Header
Defines if the header should be exported, or only the image values.

Column Delimiter:
The separation character used to separate the columns.
Can be Tabulator, Semicolon, Comma and Space.
Is not used for column labels and column units, if no text qualifier is used.

Text Qualifier:
Column Labels and Column Units are surrounded by the text qualifier.
Can be nothing, quote sign or inverted comma.

Decimal Separator:
Can be point or comma.

Decimal Precision:
Defines the number of decimal places. The exponential format is used, e.g. 1234567 with a precision of 3 is exported as 1.23E+06.

File Suffix:
Only defines the suffix of the file name.

Export Graph ASCII or RAW

Description

The Graph ASCII or RAW export exports graph data objects into several ASCII or binary files. Can be used as a compatible export from the ASCII export options in older software versions.

Input and Output

Input:
One or multiple graph data objects.

Output:
Up to 3 files per data object (Header, X-Axis, Y-Axis)

Format

The ASCII header gives some information about the image like the number of pixels and the scan size and position. It is stored in an extra file.
The x-axis and y-axis values are also stored in extra files. The y-axis data first contains the spectral dimension, then the other dimensions (e.g. space line by line). Values are exported as exponential floating point strings with a selected separator or as binary 4-byte floating point values.

Export Filter Options

Export Header:
Defines if the header should be exported into an extra file.

Export X-Axis:
Defines if the x-axis values should be exported into an extra file.

Export Mode:
Graph ASCII - Exports all numbers as ASCII text
Raw as Float - Exports all numbers as 4-byte floating point numbers (binary export)

Text Qualifier:
Column Labels and Column Units are surrounded by the text qualifier.
Can be nothing, quote sign or inverted comma.

Decimal Separator:
Can be point or comma.

Decimal Precision:
Defines the number of decimal places. The exponential format is used, e.g. 1234567 with a

precision of 3 is exported as 1.23E+06.

X Unit:
The unit of the exported x-axis data. Can be all x-units that WITec Project can handle (see Interpretation Data).

File Suffix:
Only defines the suffix of the file name.

Export Bitmap

Description

The Bitmap export allows to export color bitmap data objects into the graphic file formats PNG, BMP, JPG.
The maximum bitmap size is 4096 x 4096 pixels.

Input and Output

Input:
One or multiple color bitmap data objects.

Output:
One or multiple graphic files.

Export Filter Options

Keep Aspect Ratio:
If not set, the pixel size in the result image are quadratic.
If set, the color bitmap transformation is taken into account. This makes sense if the scan size ratio is different to the pixel size ratio.

File Format:
Can be .bmp, jpeg, png.

Export Matlab

Description

The MATLAB export format exports any kind of graph data objects into a MATLAB structure or DSO format.
The export is limited to 2 GB of data. More data can be exported using the Graph ASCII or RAW Export.

Input and Output

Input:
One or multiple graph data objects (with any dimension).

Output:
One or multiple files, each containing all spectra of each selected data object

Format

Depending on the MATLAB export type "DSO" or "Matlab Structure", the format is one of the following.

DataSetObject (DSO 6.0)

This format is specified by "Eigenvector". A MATLAB package can be downloaded there.
See http://www.eigenvector.com/software/dataset.htm
It contains MATLAB functions, that can be used on the DSO data format. It is for free (BSD-License).
It only supports simple functions for the display of data.
The DSO is created as a MATLAB object mat-file, see
http://www.mathworks.com/help/pdf_doc/matlab/matfile_format.pdf
A description of each DSO field is available under
http://wiki.eigenvector.com/index.php?title=DataSet_Object
or in the downloaded package.

DSO Fields:

Name:
Contains the graph object name in the WITec Project project manager.

Type:
The type of the graph object. "image" if its an 2D graph object, otherwise "data".

Date:
Current date as yyyy.mm.dd hh.mm.ss

ModDate:
Same as Date. Will be changed if MATLAB changes the file.

ImageSize:
Null for 0d and 1D graph objects, SizeY and SizeX for 2D graph objects

ImageMode:
1 if its a 2D graph objects, otherwise 0

Data:
Contains the graph data. For single graph objects the graph data is saved as a row vector.
For 0D and 1D graph objects (e.g. / time series / line scan) the graphs are saved as sequential row vectors.
The data is stored in a matrix with <NumberOfSpectra> rows and <SpectrumSize> columns.
For 2D graph objects the matrix has SizeY*SizeX rows and <SpectrumSize> columns.
The image columns are stored sequentially, then the rows.

Label:
Is always null.

AxisScale:
The graph scale is stored in a cell with 2x2 elements. For this the X Unit setting in the export filter options is used.
For 1d graphs (e.g. time series), the cell element (1,1) contains the series scale (e.g. the time scale).
The cell element (1,2) is the string of the unit of the series (e.g. "seconds").
For single spectra the cell elements (1,1) and (1,2) are empty.

The cell element (2,1) is always the graph x-axis scale, the cell element (2,2) is the unit string of the x-axis scale (e.g. "rel. 1/cm").

ImageAxisScale:
The image axis scale is stored in a cell with 2x2 elements. A standard unit is used.
For 0D and 1D graph objects the elements are empty. For 2D graph objects element (1,1) contains the scale of the image columns, element (2, 1) contains the scale of the image rows, element (1, 2) and (2, 2) contain the unit string of the x/y scale (e.g. µm).

Title:
Is always null.

Class:
Used for MATLAB to recognize the file as a DSO format. Contains "dataset".

Include:
Used to set an image- or graph mask to tell MATLAB which pixels should be used. WITec Project always sets all pixels in the mask.
The field is a cell with 2 elements. Element 1 has the image mask as row vector with <SizeY*SizeX> elements.
Element 2 has the graph mask as row vector with <SpectrumSize> elements.

ClassLookUp:
Is always null.

AxisType:
Can be used to characterize the spectral axis scale (data will not be changed by this). Can be "none", "continuous" and "discrete". WITec Project uses "none".

ImageAxisType:
See AxisType, but for the image axis scale.

Description:
Is always null.

UserData:
Is always null.

DataVersion:
WITec Project uses DSO 6.0, so "6.0" is written.

History:
Contains "Created by WITec Project (Version XXX)".

UniqueID:
For each graph object a unique ID is generated from the caption + date.

Usage of DSO in MATLAB

The download package contains a folder @dataset. To open an exported DSO file in MATLAB, the file must be in the same folder than the folder @dataset (or the file must be a sibling of this folder).
In the MATLAB window "Current Folder" the file can be double clicked or via command "load('filename_dso-file').
MATLAB shows it then in the MATLAB window "Workspace". You can list the DSO fields in the command window of MATLAB by entering the DSO file name.

You can use the fields by using "dso-filename.fieldname". Fields with multiple values like AxisScale can be addressed via e.g. "dso-filename.axisscale{2,1}". For the DSO the functions in the folder @dataset can be used.
There is a documentation on
http://wiki.eigenvector.com/index.php?title=DataSet_Object_Methods

MATLAB Structure

This is a custom structure format defined by WITec.
The fields Name, Date, Data, AxisScale, ImageSize, ImageAxisScale are the same like the DSO format.

Usage in MATLAB

You can double-click the exported file in the "Current Folder" window of MATLAB or via the load command.
The structure is shown in the MATLAB window "Workspace". You can double-click on the structure to show the fields, they can also be double-clicked.
The fields "date", "data" and "imagesize" are matrices, which can be addressed using brackets, e.g. structurename.data(1,:) returns the first line of data that means the first spectrum of the dataset.
The fields "axisscale" and "imageaxisscale" are cells and are addressed via curly brackets, e.g. structurename.axisscale{2,1} returns the line vector of the spectral axis scale.
You can also press the tabulator-key to show the possible fields after typing "structurename.".

Here are some examples to plot graph objects in MATLAB. Just copy one of the functions in the gray boxes into a MATLAB file (filename.m) and load it in MATLAB:

```
function PlotGraph(aGraph, aImagePosition, aYLim)
% Plots a single graph from the whole dataset in a 2d coordinate system.
% aGraph: struct from WITec Project Export
% aImagePosition: x- and y- image coordinates of the graph, e.g. [30,50] means
x=30, y=50
% aYLim: lower and upper limit of the spectral y-axis, e.g. [-5,250] plots the
graph in a range of -5 to 250
% Note that the Rayleigh-Peak may make your important raman peaks invisible due
to automatic scaling.

aSpectrum = aGraph.data(aImagePosition(1) * aGraph.imagesize(1) +
aImagePosition(2),:);
aXAxisscale = aGraph.axisscale{2, 1};
plot(aXAxisscale, aSpectrum);
aXLabel = sprintf('[%s]',aGraph.axisscale{2, 2});
xlabel(aXLabel)
ylabel('CCD cts.')
ylim(aYLim)
aTitle = sprintf('%s (y = %i, x = %i)',aGraph.name, aImagePosition(2),
aImagePosition(1));
title(aTitle)
end
```

```
function PlotAverageGraph(aGraph, aYLim)
% Plots the average spectrum
% aGraph: struct from WITec Project Export
% aYLim: lower and upper limit of the y-axis

aAverageSpectrum = mean(aGraph.data);
```

```
aXAxisscale = aGraph.axisscale{2, 1};
plot(aXAxisscale,aAverageSpectrum)
aXLabel = sprintf('[%s]',aGraph.axisscale{2, 2});
xlabel(aXLabel)
ylabel('CCD cts.')
ylim(aYLim)
aTitle = sprintf('%s (Average Spectrum)',aGraph.name);
title(aTitle)
end
```

```
function PlotImage(aGraph)
% Plots the intensity image
% aGraph: struct from WITec Project Export

aSizeY = aGraph.imagesize(1);
aSizeX = aGraph.imagesize(2);
aSumImage = sum(aGraph.data,2);
aImage = zeros(aSizeY,aSizeX);
for x=1:aSizeX
    for y=1:aSizeY
        aImage(y, x) = aSumImage((x - 1) * aSizeY + y);
    end
end
figure
aXAxisScale = aGraph.imageaxisscale{2, 1};
aYAxisScale = aGraph.imageaxisscale{1, 1};
imagesc(aXAxisScale, aYAxisScale, aImage)
%colormap(gray)
colormap(hot(256))
aTitle = sprintf('%s (Intensity image)',aGraph.name);
title(aTitle)
aXLabel = sprintf('[%s]',aGraph.imageaxisscale{2, 2});
xlabel(aXLabel)
aYLabel = sprintf('[%s]',aGraph.imageaxisscale{1, 2});
ylabel(aYLabel)
end
```

```
function SpectrumToImage(aGraph, aSpectralPosition)
% Plots the intensity image of the specified spectral position
% aGraph: struct from WITec Project Export
% aSpectralPosition: spectral position

aSizeY = aGraph.imagesize(1);
aSizeX = aGraph.imagesize(2);
aSumImage = sum(aGraph.data(:,aSpectralPosition),2);
aImage = zeros(aSizeY,aSizeX);
for x=1:aSizeX
    for y=1:aSizeY
        aImage(y, x) = aSumImage((x - 1) * aSizeY + y);
    end
end
figure
aXAxisScale = aGraph.imageaxisscale{2, 1};
aYAxisScale = aGraph.imageaxisscale{1, 1};
imagesc(aXAxisScale, aYAxisScale, aImage)
%colormap(gray)
colormap(hot(256))
aTitle = sprintf('%s (Intensity image)',aGraph.name);
title(aTitle)
```

```
aXLabel = sprintf('[%s]',aGraph.imageaxisscale{2, 2});
xlabel(aXLabel)
aYLabel = sprintf('[%s]',aGraph.imageaxisscale{1, 2});
ylabel(aYLabel)
end
```

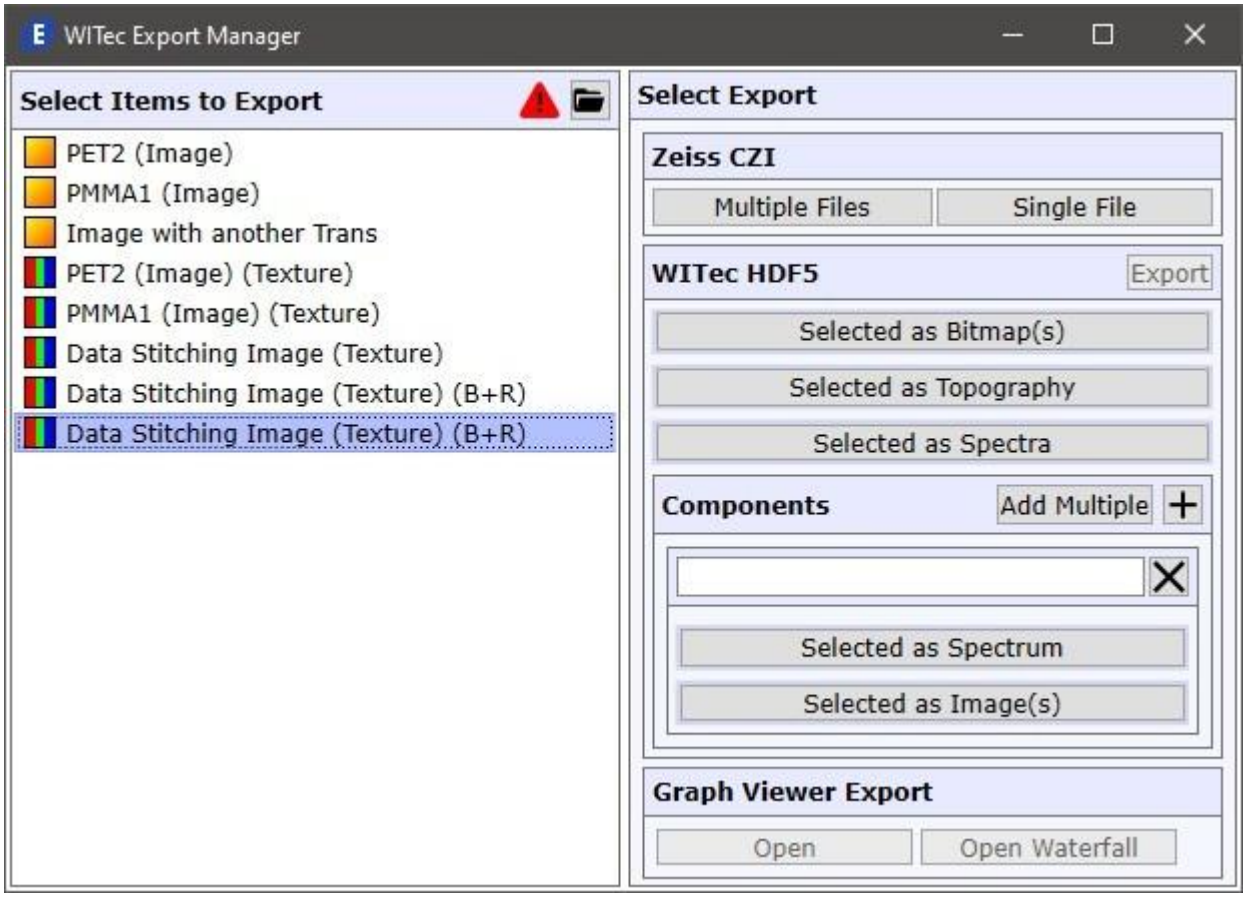
Export Filter Options

Matlab Export Type:
Can be DataSetObject (DSO 6.0) or Matlab Structure.

X Unit:
The unit of the exported x-axis data. Can be all x-units that WITec Project can handle (see Interpretation Data).

WITec Export Manager

The WITec Export Manager can load a WIP file and export data into several formats.
You can open the Export Manager from the Windows Start Menu or in WITec Project (Project Manager Context Menu).



Select Items to Export
Here you can select items that should be exported.

Open WIP File
Opens a WIP file. You can also drag and drop a WIP file from the file explorer onto this window.

 The red warning is displayed, if there are data objects in the loaded WIP file, that can not be used in this program.
Move the mouse over the warning icon in order to see more details.
The following data objects can be loaded:

- Images with spatial X/Y information
- Bitmaps with spatial X/Y information
- Single Spectra with spectral axis information (old WIP files might contain a wrong format for spectral axis information)

Zeiss CZI

Multiple Files / Single File
Exports the current selection to the Zeiss CZI format.
Can be opened e.g. in Zeiss ZenBlue Software.

WITec HDF5 / .h5oiwt

For detailed information about the WITec HDF5 format, please open the documentation:

C:\ProgramData\WITec\WITec Suite X.X\Common Files\Help\WITec Data Exchange Format h5oiwt.pdf

Export
Exports the objects that were assigned previously to the WITec HDF5 File Format.

Selected as Bitmap(s)
Assigns one or multiple selected data objects as bitmaps for the export.

Selected as Topography
Assigns one selected data object as topography image for the export.

Selected as Spectra
Assigns one or multiple selected data objects as spectra for the export.

Components

Add Multiple
Automatically assigns multiple selected images/graphs to different components.

 **Add Component**
Adds a new empty component.
The Images and Spectra of all components must have the same size and spatial/spectral positions.

Component Group Box

Name Edit
Enter a name for the component. The name is automatically set to the first assigned data object.

Selected as Spectrum
Assigns one selected single spectrum to this component.

Selected as Image(s)
Assigns one or multiple selected images to this component.

Remove Component
Removes the component and all its assigned objects from the component list.

Graph Viewer Export

Open
Opens a graph viewer export preview.
Can be used with one or multiple single spectra only.
See Spectrum Viewer Export and Spectrum Viewer.

Open Waterfall
Opens a graph viewer in waterfall spectra mode.
Can be used with multiple single spectra or with spectral line data objects (e.g. time series).
Also see Spectrum Viewer Export.

Spectrum Viewer Export

The Spectrum Viewer enables to export the viewer using several settings.

To File ...
Exports the current view to an image file.

To Clipboard
Exports the current view to the windows clipboard.

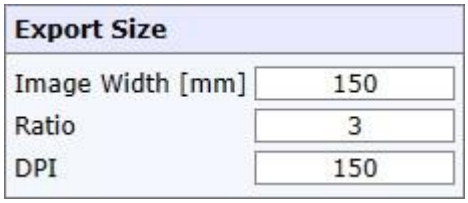


Image Width
Defines the image width in [mm].
This will automatically calculate the correct pixel size in combination with the DPI.

Ratio
Defines the ratio of the viewer.

DPI
Defines the DPI (Dots per inch).
This will automatically calculate the correct pixel size in combination with the Image Width [mm].

Line Width
Defines the line width in [mm].

Use AntiAliasing
If checked, the line will be smoothed by using anti aliasing.

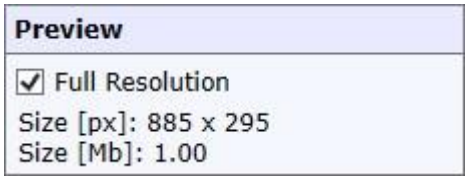
Font Size
Defines the font size in [pt].

Font Family
Defines the font family used in the axis ticks and caption labels.

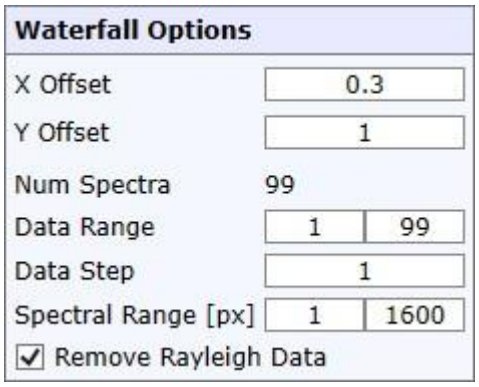
Line Width
Defines the line width of the axes and ticks.

Show Legend
If checked, a legend is shown on the viewer.
You can drag and move the legend box using the left mouse button.

Font Size
Defines the font size of the legend captions.



Full Resolution
If checked, the preview will be shown in the full pixel resolution of the resulting bitmap.



Only visible in Graph Export Waterfall mode.

X / Y Offset
Changes the offset between each spectrum in order to adjust the waterfall effect.

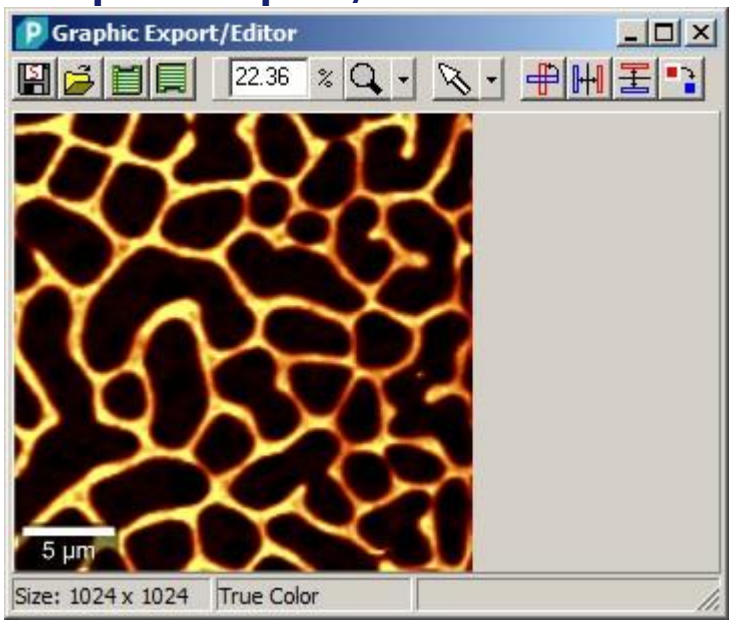
Data Range
Defines which spectra should be shown in the waterfall graph.

Data Step
Enables to skip every <n>th spectrum. This is necessary if there is a high number of spectra in the dataset.

Spectral Range
Defines which spectral pixels should be shown in the waterfall graph.

Remove Rayleigh Data
If checked, the Rayleigh area is automatically removed from the data.

Graphic Export/Editor



Description of Tool Buttons (from left to right):

Save Graphic:
Saves the current graphic into a graphic file.

Load Graphic:
Loads graphic from a graphic file.

Copy Graphic To Clipboard:
Copies the current graphic as bitmap into the clipboard.

Copy Graphic From Clipboard:
Copies a bitmap from the clipboard into the graphic editor.

Zoom:
Changes the zoom of the graphic.

Mouse Mode:
- Drag
- Select
- Move Text

Rotate Image:
Rotates the graphic.

Flip Vertical:
Flips the image vertically.

Flip Horizontal:
Flips the image horizontally.

Auto Crop White Border:
Removes the white border around the image.

WITec Binary Export Format

This chapter will describe the WITec Binary Export file format in detail. With this format it is possible to export WITec Project objects (graph, image, bitmap and text objects) as binary data in a straightforward and flexible format. The Binary Export format allows storing multiple objects in one file: Not only the data itself is stored but also all information that is needed to interpret the data, such as dimensions, transformations, additional meta information etc.

In the first section some basic information about the component technique of the file format can be found. In the second part, the file header is described. Finally, the storage of data in general and in particular how WITec Project objects are stored in the Binary format is explained.

Please ask the WITec Support Team for the C header files and example pseudocode!

Structures, Pointers and Data

The WITec Binary Export format consists of several components:

- **Structures** (C structs), that simply can be loaded into memory using the structure size. These structures hold information necessary to read the data from the file, e.g. what data is stored in the file, what is the file position of the data, what kind of data is stored and more.
- **File pointers**, which are part of all structures and which are 64 bit pointers that point to a file address at which another structure or data value can be found. All file pointers are absolute pointers inside the file.

- **Data values**, which can have different data types - multiple values are stored sequentially.

It is possible to create instances of structures inside the memory and fill them by reading <structure size> bytes from the file into this structure.

> The structures use structure packing, i.e. that small memory gaps are used between the variables to optimize performance and therefore the structure size is not necessarily the sum of all variable sizes (see „Data Structure Alignment“ on the web). A pack size of 8 bytes (?) is used in all structures. Most C/C++ compilers use 8 byte structure packing or at least have a possibility to change the pack size (e.g. by a #pragma pack(n) statement).

Header

The exported binary file starts at file position 0x00 with a header - the **TWITecBinExportHeader** structure - which is the only static positioned structure in this format. Other structures can be stored anywhere in the file and to find the position of the structures their file positions must be read from file pointers first.

```
struct TWITecBinExportHeader
{
    char Identifier[8];
    unsigned int Version;
    TWITecBinExportDataInfo GlobalMetaStringDataInfo;
    FILE_POINTER FirstObjectInfoFilePointer;
};
```

- **Identifier:**
  Describes a file format identifier (ASCII). When starting an import the identifier should be compared with the identifier in the WITecBinaryExport.h header file (8 bytes without null termination).
- **Version:**
  Represents an unsigned integer which means the version of this file format. If the version number changes it can be expected that some structures changed and therefore are no longer compatible to older export formats. The read version number should just be compared with the version in the WITecBinaryExport.h header file.
- **GlobalMetaStringDataInfo:**
  Is a TWITecBinExportDataInfo structure which holds information about a meta information string. The TWITecBinExportDataInfo structure is used for all kind of data (also for strings) and is described later on in this documentation. This meta information string contains global information like:
  - the original file name of the WITec Project file that was opened before exporting the data objects
  - the number of exported data objects
  - the export date.
- **FirstObjectInfoFilePointer**
  Is a 64 bit unsigned integer which stores the address of the first object information Structure (TWITecBinExportObjectInfo). In order to load the first object info structure a TWITecBinExportObjectInfo structure must be created and filled with the bytes from the file at this file pointer position.

Data

The **TWITecBinExportDataInfo** structure defines information about data with any number of values and any type of data. So this struct is able to store data of a WITec Project data object as well as strings that are used in other export structures in this file format, e.g. the GlobalMetaStringDataInfo in the header structure.

```
struct TWITecBinExportDataInfo
{
    unsigned __int64 NumberOfValues;
    TWITecDataType DataType;
    FILE_POINTER DataFilePointer;
};
```

- **NumberOfValues**
  Defines the number of data values.
- **DataType**
  Defines the data type and therefore the size of one data value. DataType can for example be integer or floating point values, RGB color values or even ASCII characters.
  TWITecDataType is an enumeration (treated as a 4 byte integer, like all enumerations) which is defined in the **WITecDataType.h** header file.
- **DataFilePointer**
  This is a 64-bit file pointer which points to the file position of the first data value. From this file position the data can be loaded by reading <NumberOfValues * sizeof(DataType)> bytes.

String Data

In case of TWITecBinExportDataInfo representing string data (DataType = ASCII) the **NumberOfValues** variable defines the number of characters (without null termination).
Note: If null-terminated strings are used <NumberOfValues+1> bytes of memory must be allocated and the last byte must be set to '\0'.

Meta Information

The TWITecBinExportHeader structure as well as TWITecBinExportWITecData both have variables for meta information which are struct variables of the type TWITecBinExportDataInfo. These meta information strings contain keys and values - each value string belongs to a key string.
A meta information string is one big string that can store any number of key-value string pairs. The strings are separated from each other by the two control characters CR and LF (Carriage Return 0x0D and Line Feed 0x0A). Therefore two lines represent one key-value pair. The following example shows two keys with their corresponding values:
"`ExcitationWavelength[CR+LF]532.0[CR+LF]Caption[CR+LF]My Spectrum Caption[CR+LF]`"

Data Objects

To allow storing more than one Data Object into one file this file format uses linked lists that can be jumped through to get all of the exported data objects.

The TWITecBinExportHeader structure exhibits a file pointer to the first TWITecBinExportObjectInfo structure of the linked list. This first object info in turn points to the first WITec Data Object structure as well as to the next object info (which is the info for the second data object).

The **TWITecBinExportObjectInfo** structure is defined as follows:

```
struct TWITecBinExportObjectInfo
{
    TWITecBinExportObjectType ObjectType;
    FILE_POINTER ObjectFilePointer;
    FILE_POINTER NextObjectInfoFilePointer;
};
```

- **ObjectType**
  The object type defines which structure is stored in this object. In case of a data object linked list (that starts with the first file pointer in the header structure) this should normally be a TWITecBinExportObjectWITecData, where information about a WITec Project data object like Graph, Image, Bitmap or Text objects is stored. Other object types are described later on in this documentation.
- **ObjectFilePointer**
  Points to the structure which holds all necessary information about the object.
- **NextObjectInfoFilePointer**
  Points to the next object info structure, which is also of type TWITecBinExportObjectInfo. If this pointer is null, there is no further data object.

The **TWITecBinExportObjectWITecData** structure is used to store information about one WITec Project data object:

```
struct TWITecBinExportObjectWITecData
{
    FILE_POINTER FirstTransformationObjectInfoFilePointer;
    TWITecBinExportUnitKind DataUnitKind;
    TWITecBinExportDataInfo DataUnitStringDataInfo;
    TWITecBinExportDataInfo DataInfo;
    TWITecBinExportDataInfo MetaStringDataInfo;
};
```

- **FirstTransformationObjectInfoFilePointer**
  Points to the first transformation object info which is of type TWITecBinExportObjectInfo. The **TWITecBinExportObjectWITecData** structure uses a linked list for storing multiple transformation objects. Transformations are important describing the dimensionality as well as the dimension sizes of the data and how all the data values should be interpreted.
- **DataUnitKind**
  This enumeration describes the data value's unit kind, like e.g. spatial, spectral, temporal etc. Each unit kind has a standard unit, e.g. the spatial standard unit is [µm], the temporal standard unit is [s] e.c. All unit kinds and the standard units for each unit kind can be found in the **WITecBinaryExport.h** header file.
- **DataUnitStringDataInfo**
  Normally the data unit string is the standard unit of the respective data unit kind, e.g. "µm". In the case of the unit kind ZValue (that is a free unit), it can be anything, e.g. "CCD cts", "Intensity", "RGB Value" or just "Arbitrary Unit" / "a.u." (can also be a user defined unit via the calculator tool in WITec Project).
- **DataInfo**
  This struct of the type TWITecBinExportDataInfo stores the information about the main data array of this data object: the number of data values, the data type and a pointer to the file position of the data.

  The number of dimensions and the number of data values in each dimension can either be obtained from the meta information string (see below) or by walking through all transformation informations.
  The first transformation describes the first dimension(s), the second transformation describes

the next dimension(s) e.c. A transformation can have one or more dimensions.
For example, if there is a image graph with 200 x 150 image pixels and 1024 spectral pixels, the first transformation will describe the 1024 spectral pixels, the second transformation will be a spatial transformation that has 3 dimensions with the sizes 200 for X, 150 for Y (and 1 for Z, because there is only one image layer at the moment). So the dimension sizes are 1024, 200, 150, 1.
Therefore the data is stored in the file as follows:

```
(x=1, y=1) Value1, Value2, …, Value1024 (end of first spectrum)
(x=2, y=1) Value1, Value2, …, Value1024
…
(x=200, y=1) Value1, Value2, …, Value1024
- End of first line -
(x=1, y=2) Value1, Value2, …, Value1024
(x=2, y=2) Value1, Value2, …, Value1024
…
(x=200, y=2) Value1, Value2, …, Value1024
```

- **MetaStringDataInfo**
  This string describes a meta info list that contains several information about the data object.

  The meta string contains some important keys:
  - "**WITecProjectObjectType**" (required)
    Declares the object type like "**Image**", "**Graph**", "**Bitmap**" or "**Text**".
    If an object type is known, some things can be assumed, e.g. Bitmap or Image data is (up to now) always two dimensional and Graph data can be four dimensional (e.g. when using an image graph with a spectrum at each image pixel - the fourth dimension is 1 because there is only 1 layer). It can also be assumed that a **Image** or **Bitmap** always has one transformation, which is a three dimensional linear spatial transformation. A **Graph** may have one or two transformations, the first one is for the values in one graph, the second one is a 3D spatial transformation if the graph consists of more than one "Spectrum".
  - "**Caption**" (optional)
    Stores the caption of the data object, which is also the caption of the data object in the WITec Project "Project Manager".
  - "**NumDataDimensions**" (required)
    Stores the number of dimensions of the data
  - "**DataDimension0**", "**DataDimension1**" […] (required)
    Stores the number of data values in each dimension.
  - "**ExcitationWavelength**" (optional)
    Stores the excitation wavelenth, if the object is a spectrum (Type "Graph").

Transformations

All WITec Project data objects (except **Text** objects) need information about the dimensionality and the sizes of each dimension as well as the interpretation of the dimension axes. This information is stored in so called "Transformations" (if the interpretation and transformation is not needed, it may be sufficient only to read the dimensions from the meta string, see above).

The **TWITecBinExportObjectWITecData** has got a pointer to a linked list of **Transformation** objects. More precisely, the pointer points to a **TWITecBinExportObjectInfo**, which in turn points to an object of the type **TWITecBinExportTransformationInfo**. The object info also has got a pointer to the next object which will be the next object info containing a pointer to the next transformation info.

```
struct TWITecBinExportTransformationInfo
{
    TWITecBinExportUnitKind TransformationUnitKind;
```

```
    TWITecBinExportDataInfo TransformationUnitStringDataInfo;
    TWITecBinExportTransformationType TransformationType;
    FILE_POINTER TransformationFilePointer;
};
```

- **TransformationUnitKind**
  Defines the unit of the axis, which is described by the transformation. See DataUnitKind of TWITecBinExportObjectWITecData.
- **TransformationUnitStringDataInfo**
  Stores the string of the transformation unit, e.g. "nm". See DataUnitStringDataInfo of TWITecBiNExportObjectWITecData.
- **TransformationType**
  Defines the type of the transformation (see below).
- **TransformationFilePointer**
  A file pointer to a structure of a type which is selected by the TransformationType enumeration.

Transformation Types

At the moment, there are two different types of transformations:

- **Lookup Table**
  A lookup table transformation is a structure of the type TWITecBinExportDataInfo. It stores a value for each data value to define the x position of this data value (so the number of values of the Lookup Table is the number of data values for this dimension).
  E.g. the table stores 1024 wavelength numbers (in [nm]) for each pixel in a spectrum, while the 1024 data values itself represent the intensity in CCD cts for each pixel.
- **Linear Transformation**

```
struct TWITecBinExportTransformationLinear
{
  unsigned int NumberOfDimensions;
  FILE_POINTER DimensionArrayFilePointer;
  FILE_POINTER BinOriginFilePointer;
  FILE_POINTER OriginFilePointer;
  FILE_POINTER ScaleMatrixFilePointer;
  FILE_POINTER RotationMatrixFilePointer;
};
```

  - **NumberOfDimensions**
    The linear transformation can be one dimensional, e.g. when describing a graph which x axis represents the time or a phase. There is also a three dimensional linear transformation, for example representing 3 spatial dimensions X/Y/Z to define the position, scale and rotation of an image layer. With such a 3D transformation it is possible to calculate the real world position by a given pixel number in each dimension (see below)
  - **DimensionArrayFilePointer**
    Points to an unsigned integer array with <NumberOfDimensions> unsigned integers that describe how many data values are stored for each dimension.
  - **BinOriginFilePointer**
    Points to a double array with <NumberOfDimensions> double values that describe the pixel numbers that correspond to the real world origin, which is stored in OriginFilePointer
  - **OriginFilePointer**
    Points to a double array with <NumberOfDimensions> double values that describe the real world origin for the corresponding BinOrigin

- **ScaleMatrixFilePointer**
  Points to a double array with <NumberOfDimensions*NumberOfDimensions> double values that represent the scale matrix which defines the size of a pixel in each dimension in the real world (normally only the main diagonal is used)
- **RotationMatrixFilePointer**
  Points to a double array with <NumberOfDimensions*NumberOfDimensions> double values that represent the rotation matrix which defines the rotation of the data in all dimensions in the real world.

  For example, if there is an single graph 1024 pixels, the origin is 500.0 nm and the bin origin is 512, then the graph values can be interpreted from 0 µm to 1000 µm (assuming the scale is 1.0).

  The following formula can be used to get the position of a pixel in the real world:

$$\vec{x} = R \cdot S \cdot (\vec{b} - \vec{b}_0) + \vec{x}_0$$

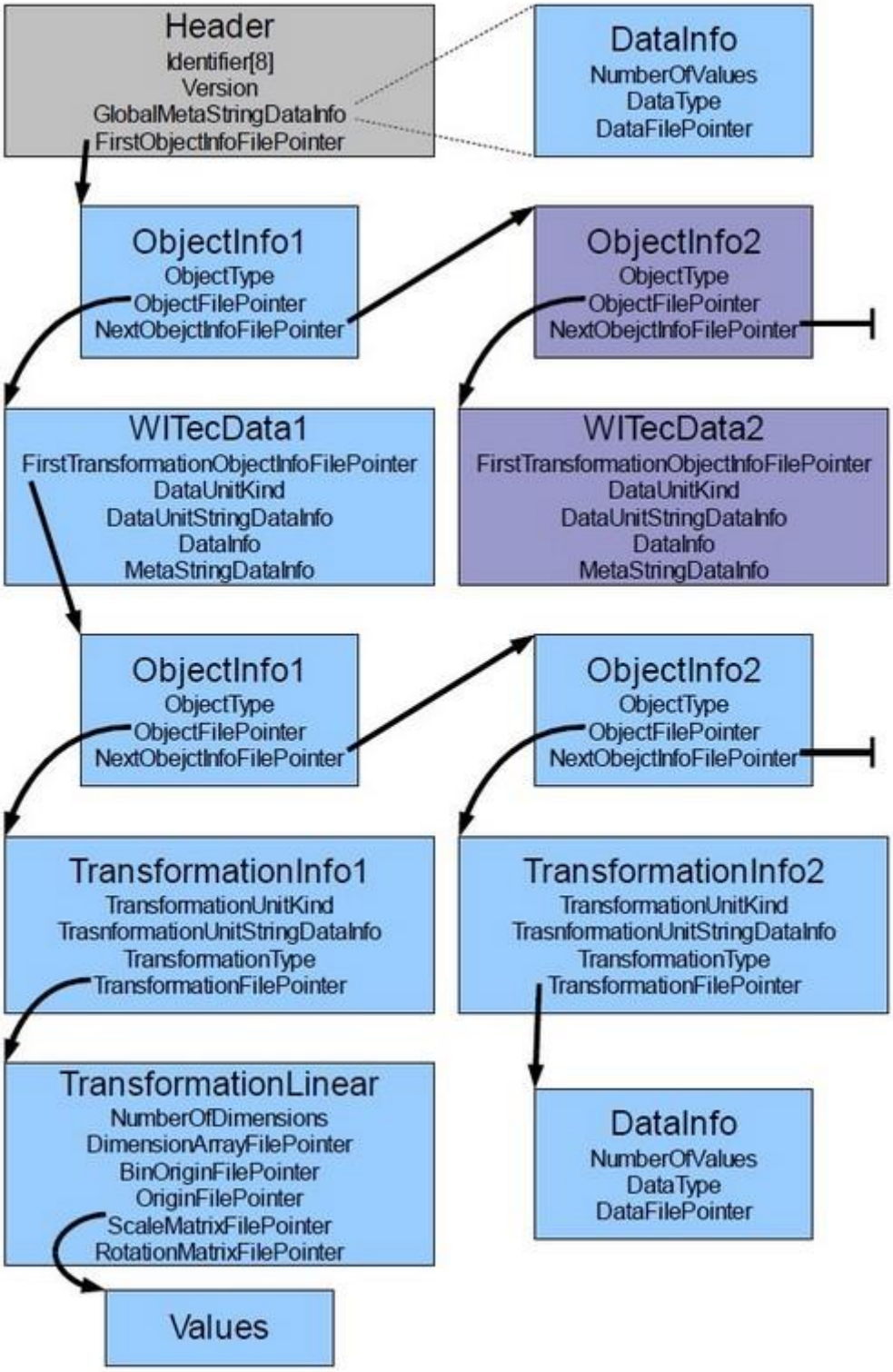
$$\begin{aligned} \vec{x} &= Real\ World\ Position \\ \vec{x}_0 &= (Real\ World)\ Origin \\ \vec{b} &= Pixel\ Number \\ \vec{b}_0 &= (Pixel)\ BinOrigin \\ R &= RotationMatrix \\ S &= ScaleMatrix \end{aligned}$$

When using a three dimensional (spatial) linear transformation, the memory alignment of matrices is defined as follows:

$$R = RotationMatrix = \begin{pmatrix} Value[0] & Value[3] & Value[6] \\ Value[1] & Value[4] & Value[7] \\ Value[2] & Value[5] & Value[8] \end{pmatrix}$$

$$S = ScaleMatrix = \begin{pmatrix} Value[0] & Value[3] & Value[6] \\ Value[1] & Value[4] & Value[7] \\ Value[2] & Value[5] & Value[8] \end{pmatrix}$$

The following diagram might help understanding the object structure of this file format.


Header
Identifier[8]
Version
GlobalMetaStringDataInfo
FirstObjectInfoFilePointer
DataInfo
NumberOfValues
DataType
DataFilePointer
ObjectInfo1
ObjectType
ObjectFilePointer
NextObejctInfoFilePointer
ObjectInfo2
ObjectType
ObjectFilePointer
NextObejctInfoFilePointer
WITecData1
FirstTransformationObjectInfoFilePointer
DataUnitKind
DataUnitStringDataInfo
DataInfo
MetaStringDataInfo
WITecData2
FirstTransformationObjectInfoFilePointer
DataUnitKind
DataUnitStringDataInfo
DataInfo
MetaStringDataInfo
ObjectInfo1
ObjectType
ObjectFilePointer
NextObejctInfoFilePointer
ObjectInfo2
ObjectType
ObjectFilePointer
NextObejctInfoFilePointer
TransformationInfo1
TransformationUnitKind
TrasnformationUnitStringDataInfo
TransformationType
TransformationFilePointer
TransformationInfo2
TransformationUnitKind
TrasnformationUnitStringDataInfo
TransformationType
TransformationFilePointer
TransformationLinear
NumberOfDimensions
DimensionArrayFilePointer
BinOriginFilePointer
OriginFilePointer
ScaleMatrixFilePointer
RotationMatrixFilePointer
DataInfo
NumberOfValues
DataType
DataFilePointer
Values

Import

Import Overview

ScanCtrl or DPFM Images
Imports Data from the old WITec software "ScanCtrl".

WinSpec
Imports Data from the WinSpec software.

ScanCtrl and WinSpec
Imports Data combined from ScanCtrl and WinSpec.

PFMControl Data (DPFM Images and DPFM Curves)
Imports PFM Data.

DPFM Data
Imports DPFM Data. The dialog allows you to select in detail the DPFM curves that you would like to import.

Bitmap
Imports one or multiple bitmap files from hard drive as new bitmap data objects to the project.

SEM Images (only available if license present)
Imports TIF or PNG image files that were created using a scanning electron microscope.
TIF must have the format 8/16-bit gray scale or 8-bit color.

H5 OINA Files
Imports EDS-, Electron- and Layered Images from .h5oina files (from AZtec Software).
It's also possible to drag and drop one or multiple .h5oina files from the Windows Explorer onto the main window of WITec Project.

Data Objects

Data Objects Overview

Data Categories

WITec Project and WITec Control feature several Data Object categories. In general, only Image, Graph, Bitmap and Text objects are required.

- **Image Data**
- **Bitmap Data**
- **Graph Data**
- **Text Data**

Internally, the following Data Object categories are used (and can be made visible for advanced users):

- Color Profile Data
- Transformation Data
- Interpretation Data
- Cursor Data
- Filter Data (deprecated)

Common Data Properties

All different kind of Data Objects contain of:

- **A data caption / name**
  Can be changed by the user; has no effect on the operation of the software

- **A unique Index** (used for "Sort by index" in Project Manager)

Some Data Objects are linked to other Data Objects in order to use shared information, e.g. two images from different channels that were created by the same measurement may link to the same spatial transformation

Data Objects are created by WITec Control during measurements, via a Drop Action Dialog or the Filter Viewer/Filter Manager in WITec Project/Control.

Memory Consumption

Please also have a look at the Data Object Memory Consumption in order to be able to estimate the size of a measurement.

Measurement Data

Graph Data

Graph Data Objects contain:

- the graph data: a 3D array of floating point numbers; the three dimensions represent the following values:
    a. SizeGraph ("spectral" dimension, typically 1024 or 1600 pixels for spectra)
    b. SizeX (= 1 for single spectra, > 1 for image graph objects or line graph objects)
    c. SizeY (= 1 for single spectra or line graph objects, > 1 for image graph objects)
- a link to a spatial transformation (defines the graph object's position and size in physical space)
- a link to a z-value interpretation (defines the unit of the graph values. This can be virtually anything, e.g. "CCD cts")
- a link to an x transformation (defines the graph object's x-axis scale, e.g. pixel to wave numbers)
- a link to an x interpretation (defines the unit of the x-axis, e.g. rel. 1/cm - can be switched to other units, see Interpretation)

Different kinds of Graph Data Objects:

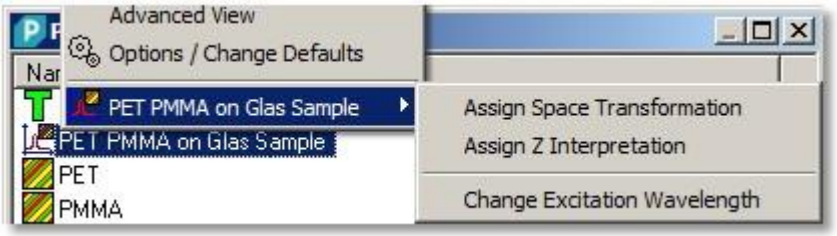
- **Single Spectrum:**
  Contains 1 spectrum, SizeX = 1, SizeY = 1;
  e.g. created by single spectrum measurement or average graph drop action.

- **Line Graph Object:**
  Contains n spectra, SizeX = n, SizeY = 1;
  e.g. created by the line scan, time series or the cross-section drop action.

- **Image Graph Object:**
  Contains n*m spectra, SizeX = n, SizeY = m;
  e.g. created by the image or large area scan.

Graph Objects are visualized in the Graph Viewer, which can be opened by double clicking the object in the Project Manager.

Data Object Context Menu:



Assign Space Transformation:
This feature allows you to assign a space transformation to the selected Graph Data Object. Please only use this feature if you exactly know what you are doing.

Assign Z Interpretation:
This feature allows you to assign a z-interpretation (defining the value unit) to the selected Graph Data Object.
Please only use this feature if you exactly know what you are doing.

Change Excitation Wavelength
If the Graph Data Object contains spectral data, it's possible to change the excitation wavelength, see Interpretation Data (Spectral Interpretation).

Image Data

Image Data Objects contain:

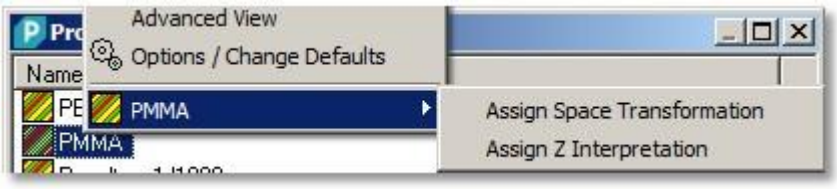
- the image data: a two-dimensional array of floating point numbers
- a link to a spatial transformation (defines the image's position and size in physical space)
- a link to a z-value interpretation (defines the unit of the image's values. This can be virtually anything, e.g. "CCD cts")

Examples for Images:

- Sum Image (one image pixel value is the CCD count sum of a number of CCD pixels of the corresponding spectrum)
- T-B Image (one image pixel value is the voltage of the T-B A/D Converter)
- Mask Image (one image pixel value is 0.0 or 1.0, the image is used as a mask)

Images are visualized by the Image Viewer using a false color mapping (Color Profile). The viewer can be opened by just double clicking the object in the Project Manager.

Data Object Context Menu:



Assign Space Transformation:
This feature allows you to assign a space transformation to the selected image;
please only use this feature if you exactly know what you are doing.

Assign Z Interpretation:
This feature allows you to assign a z-interpretation (defining the value unit) to the selected image;
please only use this feature if you exactly know what you are doing.

Bitmap Data

Bitmap Data Objects contain of:

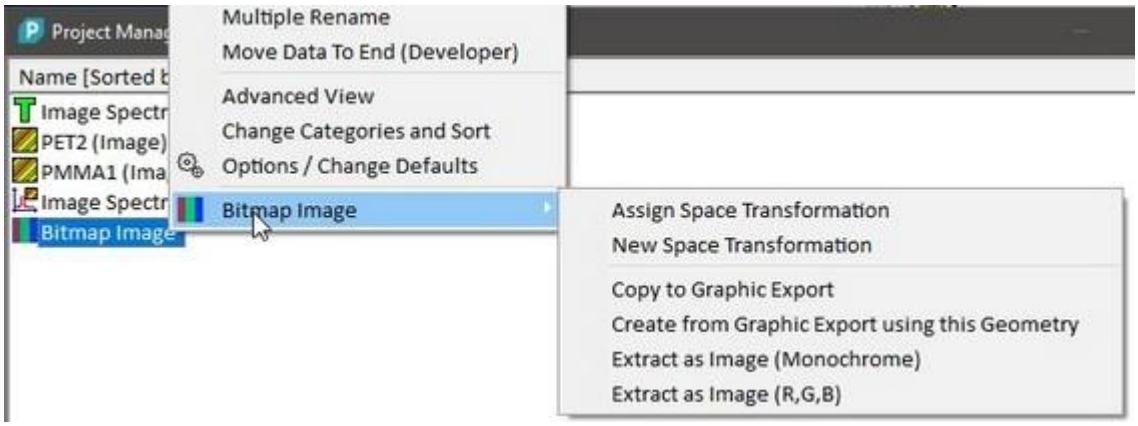
- the bitmap data: a two-dimensional array of RGB color values (24 bit)
- a link to a spatial transformation (defines the bitmap's position and size in physical space)

Examples for Bitmaps:

- Video Image
- Imported Windows Bitmap
- Texture Bitmap created by the Image Viewer

Bitmaps are visualized in the Image Viewer, which can be opened by just double clicking the object in the Project Manager.

Data Object Context Menu:



Assign Space Transformation
This feature allows you to assign a space transformation to the selected bitmap; please only use this feature if you exactly know what you are doing.

New Space Transformation
Creates a new custom space transformation and assigns it to this bitmap.

Copy to Graphic Export
Copies the bitmap to the Graphic Editor / Export window.

Create from Graphic Export using this Geometry
Creates a new bitmap object from the Graphic Export window and uses the transformation / geometry from this bitmap.

Extract as Image (Monochrome)
Create a monochrome floating point image (Intensity = 0.299 * R + 0.587 * G + 0.114 * B).

Extract as Image (R, G, B)
Create 3 Floating Point Images (R, G and B) using the bitmap's color channels.

Text Data

Text Data Objects contain text in rich text format (RTF).

Measurements in WITec Control create text objects containing measurement information.

Text objects can be visualized and edited with the Text Viewer, which can be opened by just

double clicking the object in the project manager.

You can add a new empty text object via main menu "**Add > Text**"

Data Object Memory Consumption

The required memory size for a Data Object depends on the dimensionality/size of the scan:

Size in Bytes = Pixels per Row * Rows in Image * Number of Channels * 4 [Bytes]
Size in MB = Size / 1024 / 1024

Examples:

Raman Image Scan with 128 x 128 Spectra (CCD Camera with 1024 Channels):
Size = 128 * 128 * 1024 * 4 = 64 MB

AFM Image Scan with 512 x 512 Pixels 4 Channels (T-B, R-L, Aux1, Aux2):
Size = 512 * 512 * 4 * 4 = 4 MB

Internal Data

Color Profile Data

Color Profiles are used by the Image Viewer to convert the image floating point numbers to colors.

The color scale can be defined in the Image Viewer:

- **Top Color Scale Value:**
  Defines the image value that will get the "furthest right" color of the color table (in most color tables this will be the brightest color).

- **Bottom Color Scale Value:**
  Defines the image value that will get the "furthest left" color of the color table (in most color tables this will be the darkest color).

Color Profile objects have the following properties:

- **A Color Table:**
  Defines the RGB color values. You can choose the desired table via the Color Profile Editor.

- **Color Cycle:**
  With this option it's possible that color tables are repeated multiple times, e.g. a cycle of 2 leads to:
  

- **Top Color:**
  If top color is turned on, the defined top color will be used if the image value is higher than the defined top color scale.

- **Bottom Color:**
  If bottom color is turned on, the defined bottom color will be used if the image value is lower than the defined bottom color scale.

Use the Color Scale Circle Menu in the Image Viewer in order to change the Color Profile:

Transformation Data

Transformation Data Objects are used to convert from a pixel position to a real physical position.

There are different kind of transformation objects:

- **Spatial Transformation:**
  Converts image/bitmap/graph-image pixel positions (x,y) into real spatial coordinates; uses the space interpretation (see Spatial Transformation (Math)).

- **Spectral Transformation:**
  Converts x-axis pixel positions of spectral Graph Objects into a spectral unit (e.g. relative wave numbers);
  uses the spectral interpretation.

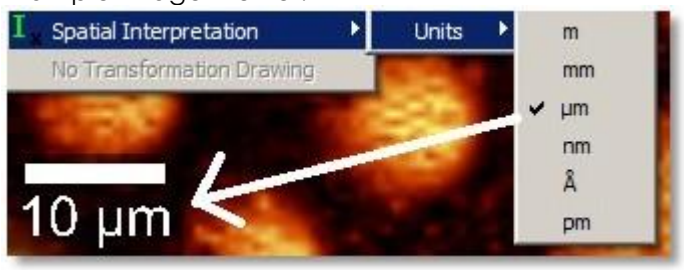
- **Linear Transformation**
  Converts x-axis or line graph pixel position of Graph Objects into a real unit;
  uses e.g. time, frequency, phase interpretation.

Interpretation Data

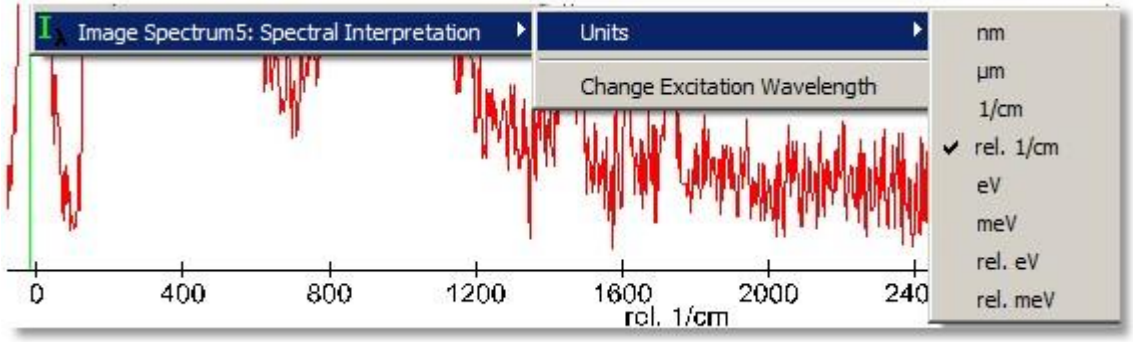
Interpretations can convert between physical units.

To change the unit, use the **context menu** of a viewer that uses this interpretation, open the submenu of the interpretation object and again open the "Units" submenu, then select a unit. This will affect all viewers that use this interpretation object.

Example Image Viewer:



Example Graph Viewer:



There are different kind of interpretation objects:

- **Z Interpretation:**
  Used to define the unit of image or graph floating point values, e.g:
  CCD cts, Volts, microns (Topography), e.c.
  Unit is static here, i.e. you can not convert between units.

- **Space Interpretation:**
  Standard unit is µm;
  can convert between: m, mm, µm, nm, Å, pm.

- **Inverse Space Interpretation:**
  Standard unit is 1/µm;
  can convert between: 1/m, 1/mm, 1/µm, 1/nm, 1/Å, 1/pm.

- **Spectral Interpretation:**
  Standard unit is rel. 1/cm;
  can convert between: nm, µm, 1/cm, rel. 1/cm, eV, meV, rel. eV, rel. meV.

  For relative units ("rel."), a reference wavelength is used. It is stored in the spectral interpretation.
  Thus it is possible to compare spectra with different wavelengths.
  To change the excitation wavelength after a measurement the Spectral Interpretation context menu "Change Excitation Wavelength" or the Graph Data Object Context menu (see Graph Data) can be used.
  Please avoid using this feature by doing a correct configuration of the spectrometer before doing the measurement!

- **Time Interpretation:**
  Can convert between: h, min, s, ms, µs, ns, ps, fs

- **Frequency Interpretation:**
  Can convert between: µHz, mHz, Hz, kHz, MHz, GHz, THz.

- **Phase Interpretation:**
  Can convert between: rad, mrad, °, grad, mgrad.

Cursor Data

Cursors are used to send mouse positions (in physical units) from one software component or window to other components or windows. This allows e.g. to show the corresponding spectrum from a spectral image scan in a graph viewer window when clicking on a pixel in the image viewer.

The cursor object is also used in the Listen Cursor Mechanism.

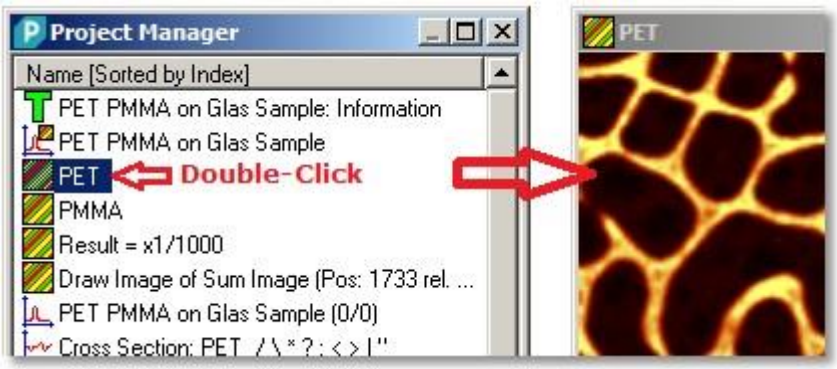
Cursor Positions are shown in the status bar of image- and graph-viewers.
You can also show more detailed cursor position information using the cursor manager window (press 'P' in Viewers).

Data Visualization

Data Visualization Overview

How to visualize

You can visualize Data Objects by simply doing **double click** on a Data Object in the Project Manager.
It is also possible to select one or multiple Data Objects first and to press the **Enter key**.

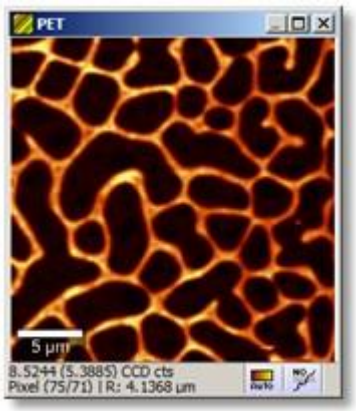


Viewers

There are different kind of viewers for showing images, bitmaps, graph and text objects:

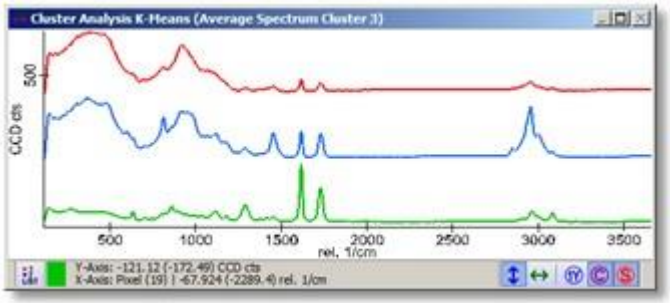
- **Image Viewer**

  shows Floating-Point-Images as False-Color-Image and Bitmaps.



- **Graph Viewer**

  shows one or multiple Graph/Spectral Data objects.

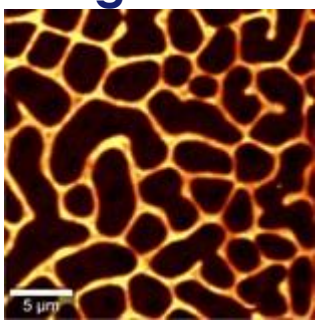


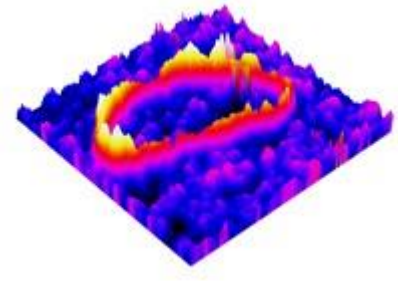
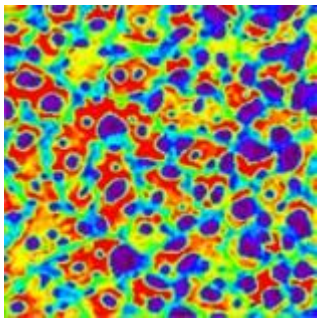
- **Text Viewer**

  shows Text Objects (using Rich Text Format).

Image Viewer

Image Viewer Overview



Description

The Image Viewer is able to visualize

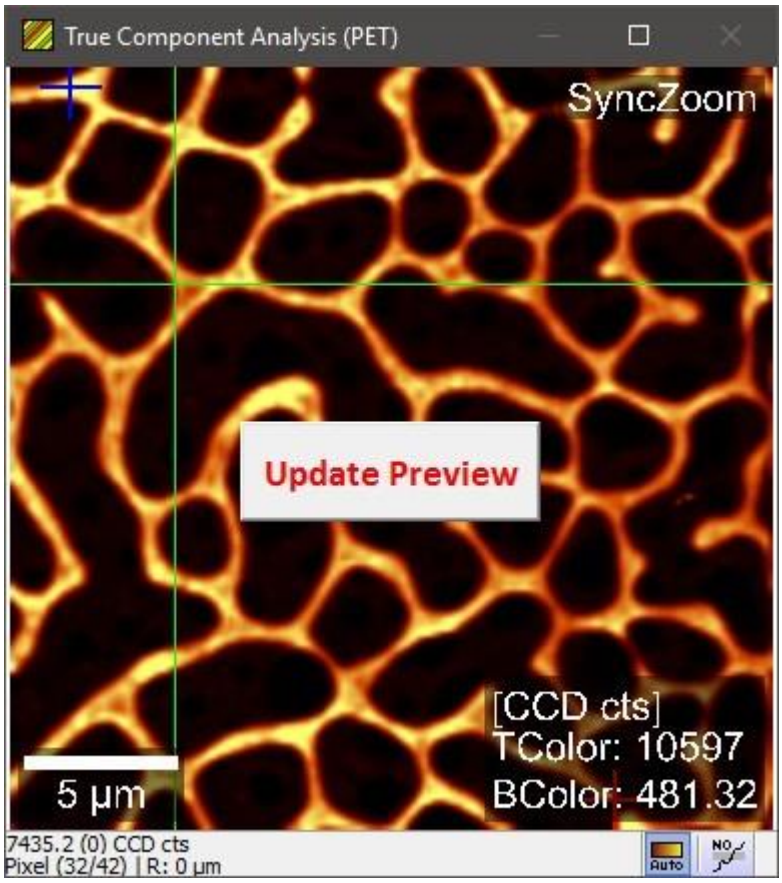
- Image Data Objects using a Color Profile Data Object to convert float values into color information.
- Bitmap Data Objects using the color data information in the bitmap.

A new Image Viewer can be created via the the Project Manager by simply double-clicking an image or bitmap object or by selecting one or multiple images and pressing enter.

Features

- Look at the general Image Viewer Appearance for a description on the image viewer window itself.
- Check out the Image Viewer Circle Menu to learn all the different Image Viewer Features:
    - Misc Visuals (Show/Hide Elements, Contour Plot, ...)
    - Draw Tools (Draw custom mask)
    - Actions (Data Analysis)
    - Color Scale (Usage of Color Table Scale, 3D-Z-Scale, Brightness Scale)
    - Camera (3D Mode, Camera Rotation/Tilt, Pixel-Zoom)
    - Export (Bitmap Export, Create Image Object, ..).
- Image Viewer Drag and Drop will show you some additional features which can be started by dropping other objects onto the viewer.

Image Viewer Appearance



SyncZoom:
This label is shown if synchronized Zoom mode is turned on (can be switched on at circle menu section Camera).

Update Preview (Button):
This Button can show up if the image is a preview of a calculation dialog and is no longer valid because of some parameter change. You can simply click on this button to calculate / update the preview image.

TColor / BColor:
This label is shown if additional information is turned on. It will show the minimum and maximum color scale values (can be switched on at circle menu Misc Visuals; only available if the color information is not a bitmap).

5 µm:
This is the color scale with label. Double-click on the scale to change its color or right-click on the scale to change more options. Alternatively the Misc Visuals section from the circle menu can be used.
To change the unit from µm to another unit, open the context menu and change the spatial interpretation object.

Status Bar:
The first line of the status bar shows the floating point value of the image at the current cursor position.
In brackets you can see the difference value between the value at the current cursor position and the value of the previous left-mouse-clicked cursor position.
The second line shows the Pixel Position of the cursor and the spatial distance of the current cursor position to the previous left-mouse-clicked cursor position.

Automatic Color Scale (First Tool Button):
Click to execute an automatic color scale.
Click with the right mouse button to activate automatic color scale on image change.
A second Tool button for automatic color scale will show up during measurements which allows to automatically scale using only the values of the last changed line.

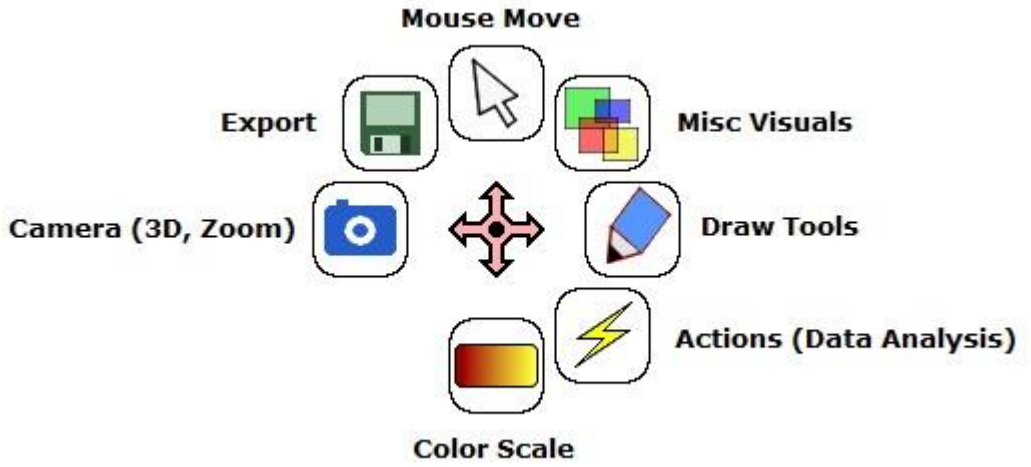
Line Subtraction (Second Tool Button):
Choose between one of the line subtraction modes:

- No Line Correction
- Line Correction by Average Subtraction
- Line Correction by Average Division
- Line Correction by Slope Subtraction.

Mathematical description see: Line Correction (Math)

Image Viewer Circle Menu

Use the circle menu by keeping the right mouse button pressed and moving the mouse for fast access of almost all features:



- Mouse Move (Change to Mouse Move Mode)
- Misc Visuals (Show/Hide Elements, Contour Plot, ...)
- Draw Tools (Draw custom mask)
- Actions (Data Analysis)
- Color Scale (Usage of Color Table Scale, 3D-Z-Scale, Brightness Scale)
- Camera (3D Mode, Camera Rotation/Tilt, Pixel-Zoom)
- Export (Bitmap Export, Create Image Object, ..).

Some features which are not available in the circle menu can be found in the context menu opening if clicking the right mouse button once.

Image Viewer Mouse Modes

You can change the image viewer mouse mode using the Image Viewer Circle Menu or the context menu (using a right mouse button click).

Mouse Move:
Change to the Mouse Move mode if have selected another mouse mode before and you do not want to use special mouse mode anymore.
This is the case for example after you have finished drawing with the draw tools. If you do not change the mouse mode, the selected draw tool is still active and you might accidentally change the mask in the image viewer.

Draw Tools:
see Image Viewer Draw Tools.

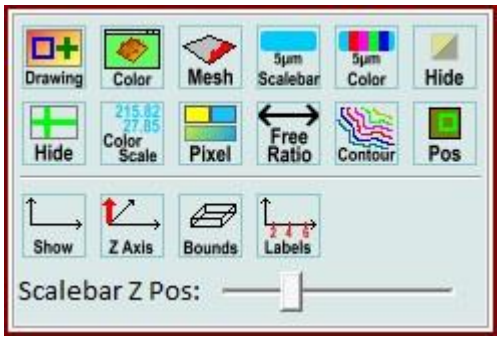
Marker Modes (Mark Region / Mark Line):
The Image Viewer automatically changes to the mark region or mark line mouse mode if some other software part listens to a spatial region or line (e.g. the cross section dialog).
It will automatically change back to mouse move mode if the other software part stops listening (or e.g. the listening dialog is closed).
Thus you don't have to change this mouse mode on your own (it's available via the right mouse button context menu).

Zoom:
see Image Viewer Camera.

Color Scale:
see Image Viewer Color Scale

Image Viewer Misc Visuals

You can open these features using the Image Viewer Circle Menu.



Drawing:
Opens the transformation drawing options. Here you can change the line drawing of transformations from other images / data objects.

Color:
Changes the background color

Mesh:
Shows nice mesh borders in 3D mode. The marginal pixels are turned down to close the mesh at the borders.

Scale Bar:
Show or hides the scale bar.

Color:
Changes the scale bar color.

Hide (Mask):

Hides the mask temporarily (e.g. masks that are drawn by the user with the draw tools or are calculated from a dialog).

-

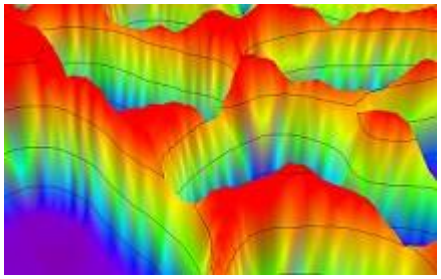
Hide (Crosshair):
Shows or hide the green cursor crosshair (useful if exporting an image).

Color Scale:
Shows the current color scale top and bottom values as overlay text on all image viewers.

Pixel:
Turns the pixel color interpolation on or off (e.g. if you would like to see pixel borders).

Free Ratio:
Turns the free ratio mode on or off. If turned on the image fits to the Viewer Window size without preserving the original ratio.

Contour:
Turns contour line drawing on or off. A dialog is shown to change the number and values of the contour lines.
In 2D-Mode the contour lines are calculated using the color image floating point values;
In 3D-Mode the contour lines are calculated using the 3D-Z image floating point values.



Pos:
Sends the position of the currently displayed image to all other viewers in order to see the region of this image in other viewers.

-

Show:
Shows or hides the coordinate system axes.

Z Axis:
Shows or hides the Z Axis, if coordinate system axes are shown.

Bounds:
Shows or hides the bounding box if the coordinate system axes are shown.

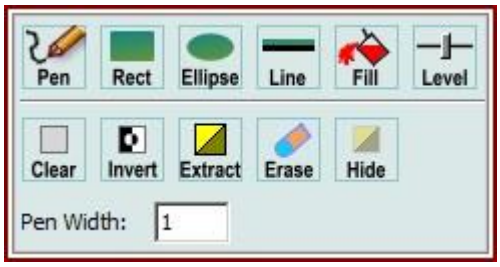
Labels:
Shows or hides tick labels and axes titles if the coordinate system axes are shown.

-

Scale Bar Z Pos:
Changes the Z-Position of the Scale Bar and other labels in 3D Display Mode.

Image Viewer Draw Tools

You can open these features using the Image Viewer Circle Menu.



Mouse Draw Modes

Pen:
Draws a custom line or marks single pixels.

Rectangle:
Draws a filled rectangle. Keep the Control-Key pressed while drawing to draw a square.

Ellipse:
Draws a filled ellipse. Keep the Control-Key pressed while drawing to draw a circle.

Line:
Click to draw a horizontal line. Keep the Control-Key pressed while drawing to draw a vertical line.

Fill:
Flood fills an area.

Level:
Opens a slider window where you can define threshold for mask drawing:

Changing the threshold will set the whole image mask on pixels that are higher or equal than the threshold and clear the mask on pixels that are lower or equal than the threshold.
You can also invert the mask logic in order to set mask pixels that are lower than the threshold.

> You can also use all tools to remove certain parts of the previously drawn area or line by keeping the shift key pressed and dragging the mouse.

Mask Tools

Clear:
Clears the current mask.

Invert:

inverts the current mask.

Extract:
extracts the current mask as a new image object to the current project.

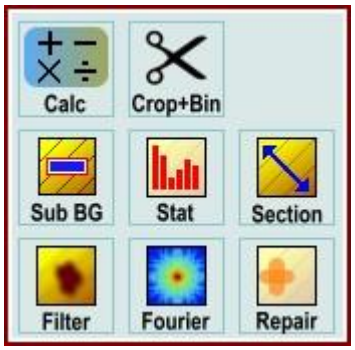
Erase:
turns the erase mode on or off. When turned on, all draw tools will erase the mask while drawing.

Hide:
hides the drawn mask (e.g. to have a better, quick look at the original image features).

Pen Width:
changes the pen with for the pen draw tool.

Image Viewer Actions

You can open these features using the Image Viewer Circle Menu.

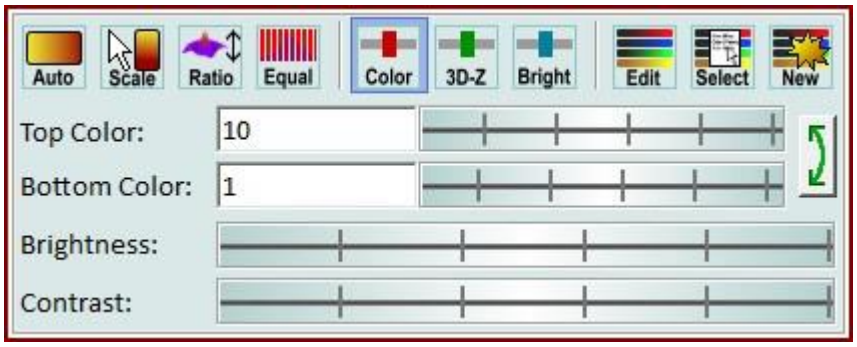


Select any of the available actions to use the current color image as an input Data Object for a data analysis tool.

See Data Analysis Overview.

Image Viewer Color Scale

You can open these features using the Image Viewer Circle Menu.



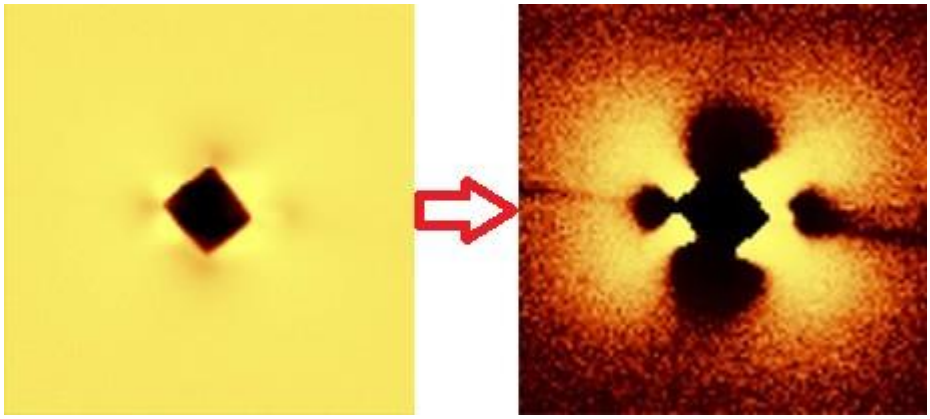
Automatic Color Scale:
Executes the automatic color scaling.

Mouse Color Scale:
Changes the mouse mode to the "scale color mouse mode".
Click somewhere in the image to set the image value at the current cursor position as the new color scale top value.
Hold down the shift-key while clicking to set the bottom color scale value instead.

Ratio:
Adjusts the 3D-Z scale to a correct XY/Z ratio. Works only with topography images.

Equal:
Equalize the color histogram. Used to show a high contrast for all dynamics of a floating point image. This might be useful if your image has two "plateaus" with each containing small value changes that could be interesting.

If checked, the color table colors are no longer assigned in a linear way using the top and bottom color scale values. Instead they are assigned in a non linear way by equalizing the histogram in order to get the same amount of "color changes" for the same amount of "floating point changes":



Color Scale:
Changes to Color Scale Mode. If selected, the edits and sliders will change the color scale.
This button is disabled if only one kind of scale can be changed.

3D-Z Scale:
Changes to 3D-Z Scale Mode. If selected, the edits and sliders will change the 3D-Z scale.
Only works if a floating point image is used as 3D-Z information image.

Brightness Scale:
Changes to Brightness Scale Mode. If selected, the edits and sliders will change the brightness scale.
Only works if a floating point image is used as brightness information image.

Edit Color Profile:
Opens the editor for currently used color profile object (see Color Profile Data).
Here you can choose another color table or change the color cycle, define top and bottom color to mark e.g. "extrema pixels".
All Image Viewers that use the same color profile object will also change their color drawing.

Select another Color Profile Object:
Opens a selector window to select another color profile object that you have created before using the following option.

Create a new Color Profile Object:
creates a new color profile object and assigns it to the current Image Viewer.
You can use the new color profile object in another Image Viewer by using the two features above.
If no additional Color Profile Object is created, all viewers always will use the same automatically created default color profile.

Top / Bottom edits + wheels:
here you can change the top and bottom color/3D-Z/brightness values.
The wheels can be moved infinitely; the mouse position is always moved back to the center when leaving the wheel area.

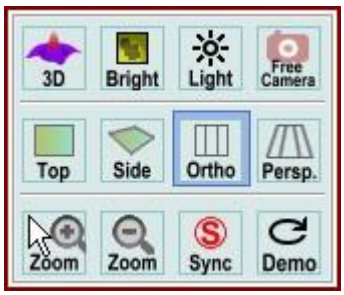
 **Swap Top and bottom Scale:**
swaps the top and bottom scale values.

Brightness (Wheel):
Changes the top and bottom values synchronously, this changes the brightness.

Contrast (Wheel):
Changes the top and bottom values in the opposite direction, this changes the contrast.

Image Viewer Camera

You can open these features using the Image Viewer Circle Menu.



3D Mode:
Turns on or off the 3D Display Mode.
Only works if a image data (not bitmap data) is used as 3D-Z information image.
You can assign another image to an Image Viewer to be used as the 3D-Z information image by drag & drop from the project manager and choosing the right option in the Image Viewer Drag and Drop menu. Otherwise the current image is used as color as well as 3D-Z information.
This mode also automatically enables the free camera mode, the camera side view and the perspective projection.

Use the left mouse button to tilt and rotate the image. Turn the wheel to zoom the camera. Hold down the Shift-Key and drag the image to pan.

Brightness Mode:
Turns on or off the Brightness Display Mode. Only works if a floating point image is used as a brightness information image.
You can assign another image to an Image Viewer to be used as the brightness information image by drag & drop from the Project Manager and choosing the right option in the Image Viewer Drag and Drop menu.
Brightness mode means that a brightness information image will define the brightness of a color, whereas the color image (+ color table) or a bitmap defines the color of a pixel.

Lighting:
Shows the Image Viewer Lighting dialog for rendering a nice 3D surface with light and reflections.

Free Camera:
Turns on or off the free camera mode.

If turned on, you can
- Tilt and rotate the image using the middle mouse button (press down the mouse wheel) and dragging the mouse cursor; or use the arrow keys to rotate and tilt
- Use the mouse wheel to do a free camera zoom.

If turned off, you can
- move the cursor with the arrow keys to exactly move through the pixels
- use the mouse wheel for a pixel zoom (camera distance is fixed)
- click the mouse wheel / middle button and drag the cursor to move the image around when zoomed in (pixel zoom).

Top View:
Changes to camera top view (automatically executed when turning off the 3D Mode).

Side View:
Changes to camera side view (automatically executed when turning on the 3D Mode).

Orthographic Projection:
Changes to orthographic camera projection (automatically executed when turning off the 3D Mode).

Perspective Projection
Changes to perspective camera projection (automatically executed when turning on the 3D Mode).

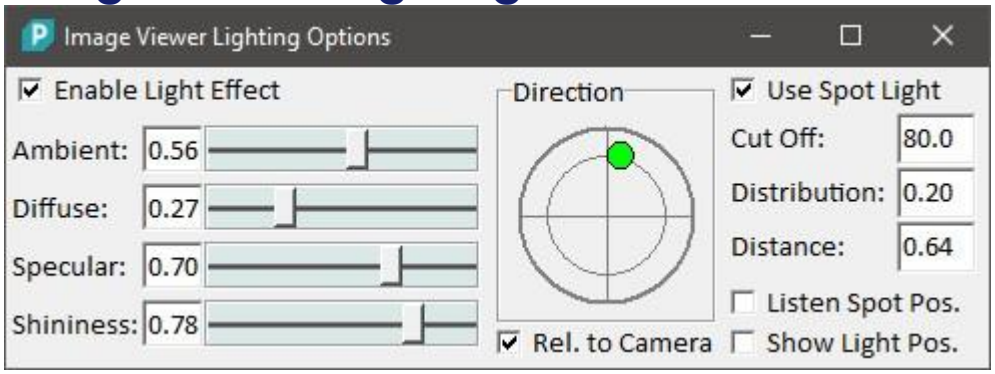
Mouse Zoom:
Turns on the Mouse Zoom Mode. If the mouse zoom mode is selected, you can click and drag a rectangle in the image and thus define an exact pixel zoom. Only the pixels of the selected range are displayed.
Don't forget to turn off this mouse mode by activating the mouse **move mode**.

Zoom Out:
Zooms out to see all image pixels (or in free camera mode will zoom to fit to the window).
If the **synchronized zoom** mode is turned on, all image viewers will zoom out to see all image pixels.

Synchronized Zoom:
Turns on or off the synchronized zoom mode; this mode is identical for all image viewers. When turned on, the pixel zoom will send the current spatial area to all other viewers that will in turn use the same pixel zoom.

Demonstration:
Lets the image rotate for demonstration purposes.

Image Viewer Lighting



Enable Light Effect
Turns on or off the light effect.

Ambient / Diffuse / Specular / Shininess
With those parameters you can affect the kind of "material", e.g. how much the surface reflects light.

Direction
Here you can click somewhere in the circle to define the light source position and direction of the light.

Rel. to Camera
If checked, the light position is relative to the camera/viewing position. If not checked, the light is relative to the sample/image.

Use Spot Light
If checked, a spot light source is used instead of an "all-over" plain light.

Cut Off
Lower values will do a hard cut at the light cone.

Distribution
Changes the angle of cone.

Distance
Changes the distance of the light source to the sample/image.

Listen Spot Pos.
If checked, you can click somewhere in the image to define the light spot position.

Show Light Pos.
If checked, shows the light position in the image viewer.

Example

Left: Without Lighting
Right: With Lighting

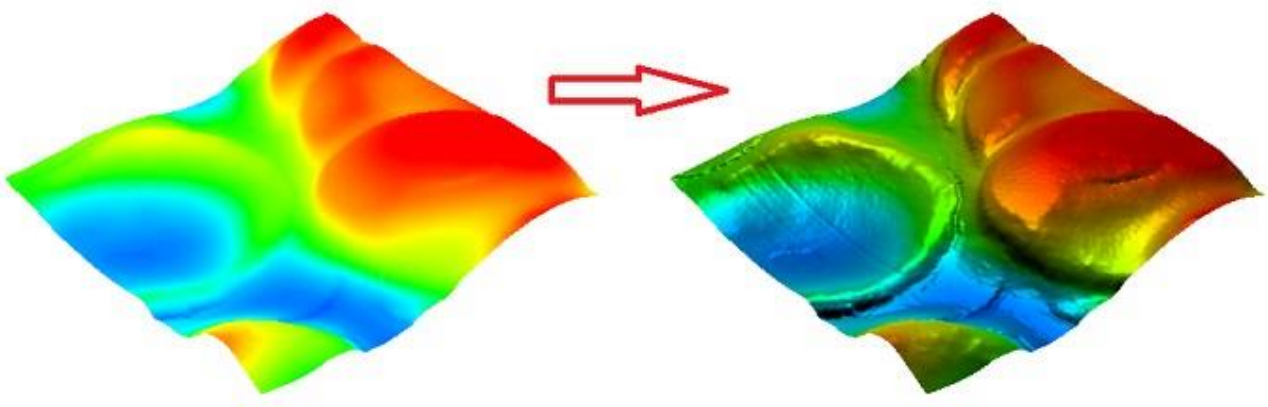
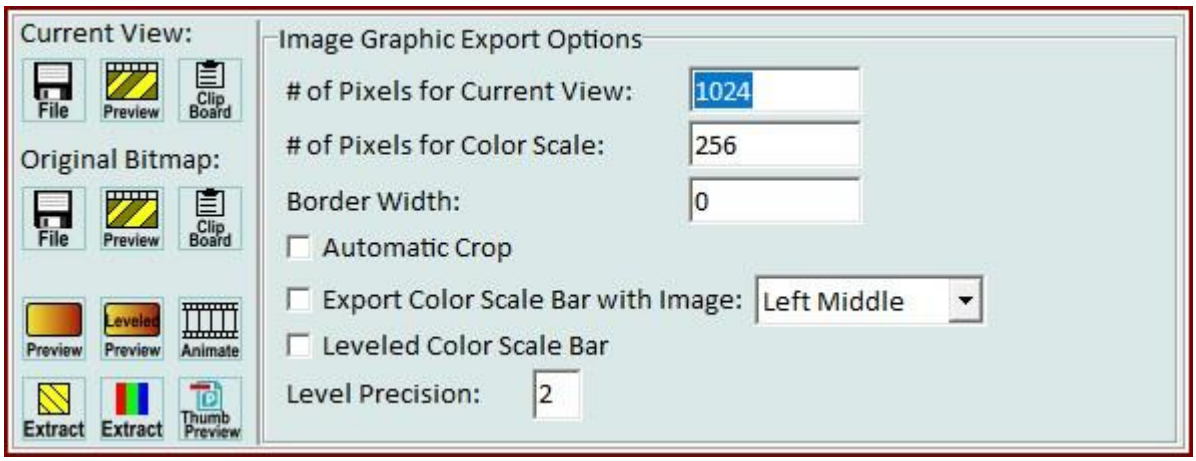


Image Viewer Export

You can open these features using the Image Viewer Circle Menu.



Export Bitmap to File:
Exports the current view to a file. You can choose between several image formats.

Export Bitmap to Preview:
Exports the current view to the built-in graphic editor. Here you can flip or rotate the bitmap and save it to a file or into the windows clipboard.

Export Bitmap to Clipboard:
Exports the current view as a bitmap into the clipboard. This allows you to paste the image into another graphic software (Shortcut: Ctrl+C)

Original Bitmap:
Exports the original bitmap in its original size without any scale bar or other overlay drawings instead of the current view of the image viewer.

Export Color Scale Bar to Preview:
Exports a the color scale bar to the graphic editor.

Export Leveled Color Scale Bar to Preview:
Exports a leveled color scale bar to the graphic editor (i.e. bottom value is set to 0).

Create and Export Animation:
Shows the Animation Editor for exporting a user defined animation.
The following parameters are animated:

- Rotation / Tilt / Camera Distance
- X / Y / Z Shift of the Image
- Background Color
- Color Scale and Z-Scale (if checked in the options)
- Pixel Zoom (if checked in the options)
- All light options
- Color Table index

Extract Image to Project:
Extracts the current floating point image as a new image object to the current project.

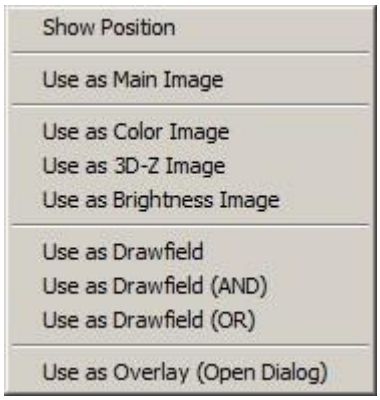
Extract View as Bitmap to Project:
exports the current view as a new bitmap object to the current project.

Set as WIP-File Thumbnail Preview in Windows Explorer:
sets the current view as the thumbnail preview for the WIP-File. You have to save the project afterwards to take effect.
The windows file explorer may cache thumbnails so you might not immediately see the new thumbnail.

Image Graphic Export Options
see Image Viewer Options.

Image Viewer Drag and Drop

You can drag and drop other Data Objects (image/graph/bitmap objects) onto an image viewer in order to execute the following actions:



Show Position:
shows the position of the dropped object. You can drop any number of images, bitmaps and graph objects to see their position:
Images, Bitmaps and Image-Graph Objects are shown as a rectangle.
Line-Scan Graph Objects or Cross Section Graph Objects are shown as a line.
Single Spectrum Graph Objects are shown as a cross.
You can change the line width of drawings, see the Drawing Button in Image Viewer Misc Visuals circle menu.

Use as Main Image:
to use the dropped image as a new main image. If the dropped image has the same size than the current color image, the draw field mask is preserved, otherwise it is cleared.

Use as Color Image:
the dropped image is used as color information. This is useful if you opened a topography image displayed as a 3D image and then you would like to use a chemical image as a color texture / color information.
Only works if the dropped image has the same size and space transformation as the current color image.

Use as 3D-Z Image:
the dropped image is used as 3D-Z information. This is useful if you opened a chemical image and you would like to use a topography image as 3D-Z information for the 3D display mode.
Only works if the dropped image has the same size and space transformation as the current color

image.

Use as Brightness Image:
the dropped image is used as brightness information. This is useful if you opened a chemical image and then you would like to use a mask or an error-mask image as brightness information to highlight masked pixels.
If the color image is a floating point image object, a rainbow color scale (containing colors only with maximum brightness) makes the most sense for the original image.
Only works if the dropped image has the same size and space transformation as the current color image.

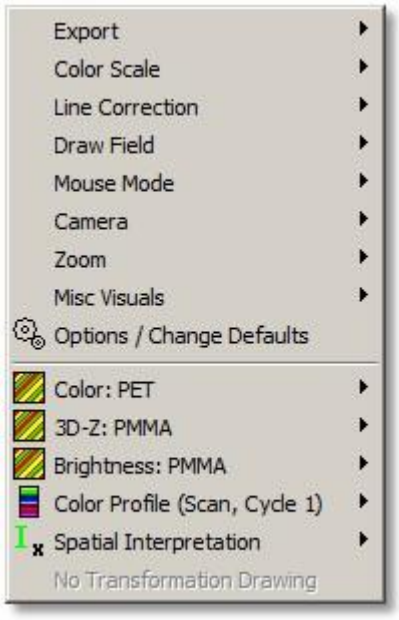
Shortcut: press the shift key on the keyboard while dropping.

Use as Draw field (and, or):
the dropped image is used as the draw field mask. You have to drop a mask image, otherwise you will most likely get a complete cleared or set mask.
Use and/or feature for a boolean and/or operation on the existing draw field mask.
Only works if the dropped image has the same size and space transformation as the current color image.

Shortcut: Press the control key on the keyboard while dropping.

Use as Overlay:
this action will open the Image Transform and Overlay dialog which can be used to draw an overlay and to change the spatial transformation of the dropped image/measurement.

Image Viewer Context Menu



The context menu is an alternative way for accessing the features that you also can find in the circle menu. Just click and release the right mouse button anywhere in the viewer.

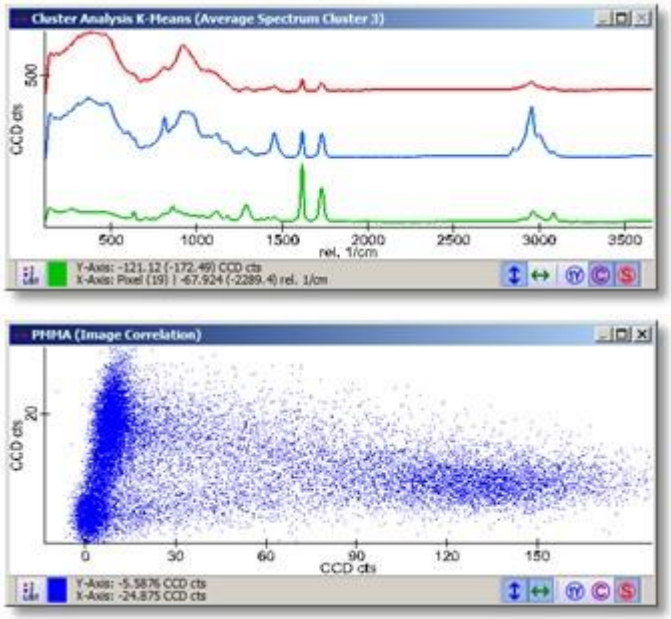
Options / Change Defaults:
This will open the program options for the image viewer.

At the **bottom of the context menu** you can find a list of data objects that are used by the viewer:

- the color image object that is displayed (also optionally the brightness and the 3D-Z information images)
- the color profile object in case of showing a floating point image as a color image
- the spatial interpretation, which you can use to switch between different spatial units
- Transformation Drawing, if you dropped other data objects to see their positions.

Graph Viewer

Graph Viewer Overview



Description

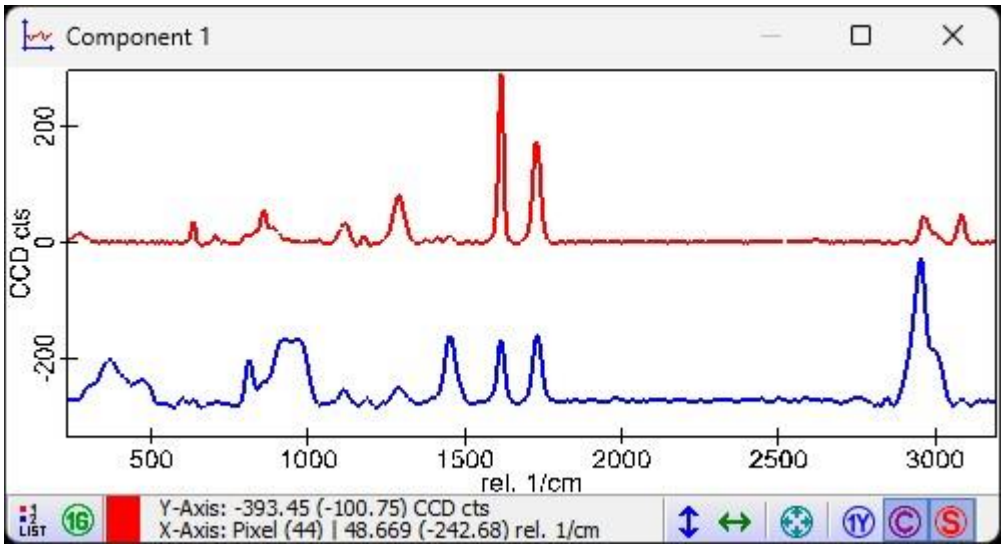
The Graph Viewer is able to visualize Graph Data Objects.

A new Graph Viewer can be created via the the Project Manager by simply double-clicking a Graph object, or by selecting one or multiple Graph objects and pressing enter.

Features

- Look at the general Graph Viewer Appearance for a description on the Graph Viewer window itself.
- Check out the Graph Viewer Circle Menu to learn all the different Graph Viewer Features.
- Graph Viewer Drag and Drop will show you some additional features that can be started by dropping other objects onto the viewer.

Graph Viewer Appearance



Title Bar

The text in the title bar shows the caption of the selected graph data object.

Status Bar

Graph List Button and Selection Information:
Shows the color of the currently selected graph object. Click on the color to select the next graph object (in order to change its scale or drawing or to export it).

Click on the "List" Button of the icon to show the Graph Viewer List at the left side of the graph viewer.

Cursor Positions:
The first line of the status bar shows the Y-Axis Cursor Position and the Graph Object Value Unit. The value in brackets is the distance between the current cursor position and the cursor position of the last position where the left mouse button was pressed previously.

Zoom Modes

See Graph Viewer Zoom Modes

Coordinate Axis and Units

The X-Axis is always the same for all graph objects that are shown in the same viewer.
The Y-Axix shows the values for the currently selected graph object.
The unit can be changed by changing the interpretation data objects.

Graph Viewer Zoom Modes

Zoom Modes

For more Information, see Graph Viewer Scale and Zoom.

Zoom Out Y-Axis Scale:
Left-Click to zoom Out the Y-Axis Scale,
Right-Click to toggle the "Zoom Out Y-Axis On Change Mode".

Zoom Out X-Axis Scale:
Left-Click to zoom Out the X-Axis Scale,
Right-Click to toggle the "Zoom Out X-Axis On Change Mode".

Auto Zoom Out Y-Axis Only:
If checked, the automatic Y-Axis Zoom on change will only zoom out, not in.

Same Y-Axis Scale:
If checked, all graph data objects share the same Y-Axis Scale.

Cascade Graphs:
Cascades multiple graph objects using a cascade distance that can be changed in the Scale and Zoom Circle Menu.

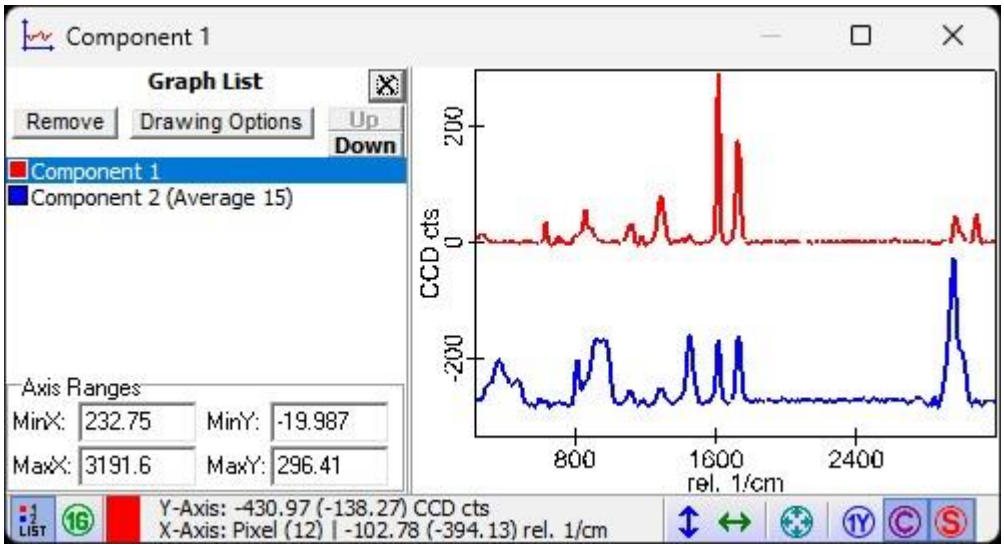
Synchronized Zoom
If checked, changing the Y-Axis via Mouse Wheel will zoom all graph objects in the viewer (only if graphs are not cascaded).

Fast Zoom Shortcuts

- Zoom fast into the current mouse/crosshair position by pressing the **space key.**
- You can zoom into a region fast by **holding down the control key and marking a region using the mouse.**
- You can use the **mouse wheel** to zoom the Y axis.
- Holding down the **control key while turning the mouse wheel** will zoom the X axis.
- Holding down the **shift key while zooming** will increase the zoom speed.
- **Press X** to zoom out the X axis (Y is automatically zoomed if the "Zoom Out Y-Axis on Change Mode" is turned on).
- **Press Y** to zoom out the Y axis.
- **Press R** to zoom out the Y axis with rayleigh peak.
- **Press Control-A** to toggle the "Auto Zoom Out Only" mode.

Graph Viewer List

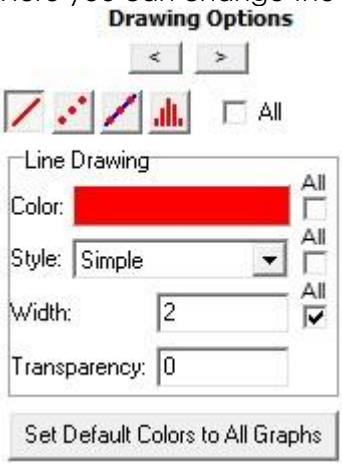
Click on the "List" Button in the status bar to show the graph list at the left side of the graph viewer:



The Graph List shows all graph objects that are displayed by the graph viewer.

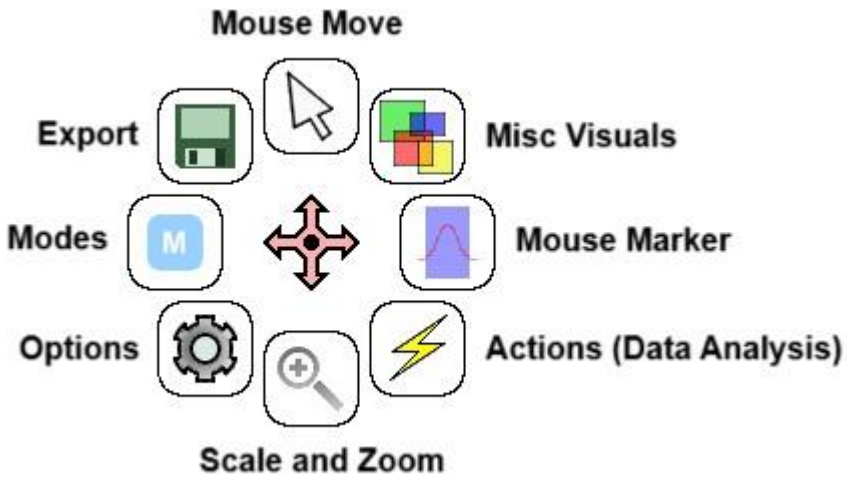
The following actions can be done here:

- You can just click on any graph to make it the selected graph (e.g. for scaling or changing the drawing options, or use the shortcut key Page-Up / Page-Down in the viewer without the list).
- You can delete a graph from the viewer (not from the project) by pressing the delete key in the Graph List or by pressing the "Remove" button above.
- You can change the displayed X axis range for all graphs or the Y axis range for the selected graph.
- A double-click will show the drawing options of the selected graph object.
  Here you can change the line style (e.g. line, dots, line and dots, histogram, line width etc.):



Graph Viewer Circle Menu

Use the circle menu by keeping the right mouse button pressed and moving the mouse for fast access of almost all features:



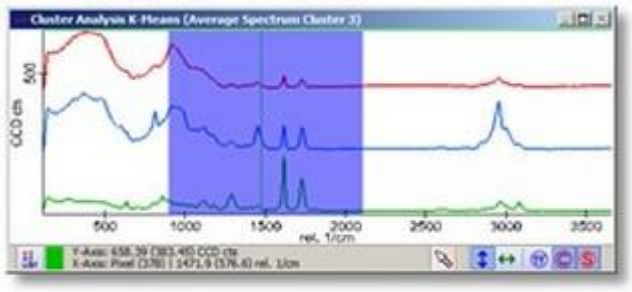
- Mouse Move (Change to Mouse Move Mode)
- Misc Visuals (Show/Hide Elements, Contour Plot, ...)
- Mouse Marker (Change to Mouse Marker Mode)
- Actions (Data Analysis)
- Scale and Zoom (Change the X- and Y-Axis scale, Cascade, Logarithmic Scale)
- Modes (Parametric Display, Listen Mode)
- Options (Graph Viewer Default Options)
- Export (Bitmap Export, Create Graph Object, ..).

Graph Viewer Mouse Modes

You can change the Graph Viewer mouse mode using the Graph Viewer Circle Menu.

Mouse Move:
changes to the mouse move mode if you selected another mouse mode before and do not want to use the special mouse mode anymore.

Mouse Marker:
The mouse marker mode is used to manipulate masks or to send a region to other software parts. The currently selected region is highlighted in the viewer:



This mode is automatically selected if another software part listens to a range so you can draw a range in a graph viewer. It will be automatically changed back to mouse move mode if that other software part stops to listen.

If you do an ASCII Export to an external program, only the current marked region is exported. If no region is selected, the complete graph/spectrum is exported.

Click and release the mouse at the same position to clear the region that was set by the mouse

marker.

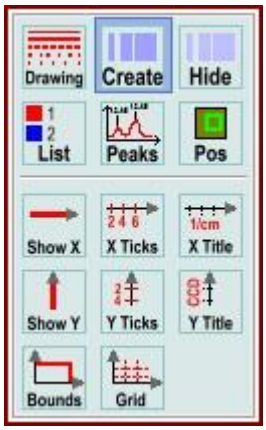
For further possibilities manipulating the mask see Graph Viewer Mask Manipulation.

Mouse Zoom:
See Graph Viewer Scale and Zoom.

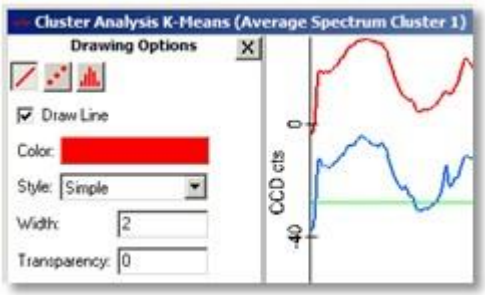
Mouse Follow Data:
See Graph Viewer Modes.

Graph Viewer Misc Visuals

You can open these features using the Graph Viewer Circle Menu.



Drawing:
Shows the graph drawing options at the left side of the viewer. Here you can change the color, line style etc. of each graph:



Create:
Switches to the mask creation mode. See Graph Viewer Mask Manipulation.
Changing the mouse mode to "Move" or clicking again on the Create Button will turn off the mask creation mode.

Hide:
Hides mask(s) temporarily. Only available on preview graph viewers that show a mask for manipulation or if the mask creation mode is turned on.

List:
Shows the Graph List of all graph objects that are displayed in the graph viewer.

Peaks:
Finds and labels peaks, see Graph Viewer Peak Labeling

Pos:
Sends the position of the currently selected spectrum to all other viewers in order to see the position of a single spectrum, line spectrum or image spectrum in an image viewer.

Show X:
Shows or hides the X Axis drawing.

X Ticks:
Shows or hides ticks and tick labels on the X Axis.

X Title:
Shows or hides the X Axis Title.

Show Y:
Shows or hides the Y Axis drawing.

Y Ticks:
Shows or hides ticks and tick labels on the Y Axis.

Y Title
Shows or hides the Y Axis Title.

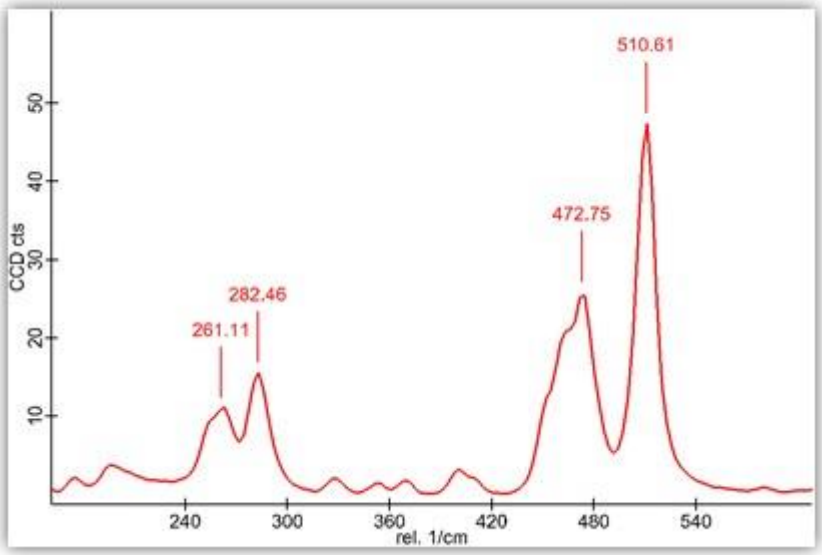
Bounds:
Shows or hides closing lines at the top and right bounds of the coordinate system.

Grid:
Shows or hides grid lines at the tick positions.

Graph Viewer Peak Labeling

The Graph Viewer allows to find and label peaks automatically; additionally the user can add or remove peaks manually. Peaks are saved in the project file.

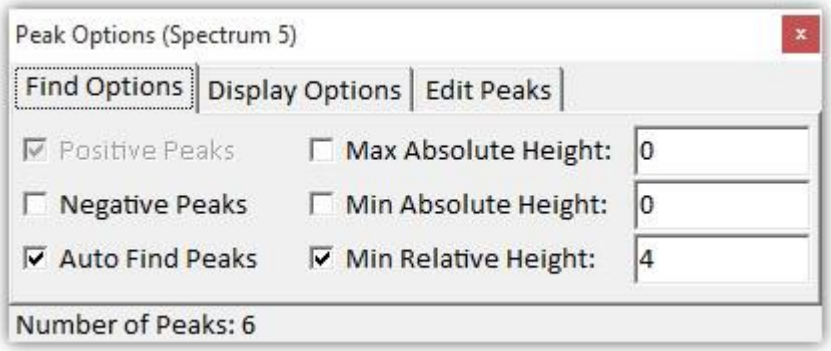
You can turn on or off the peak labeling using the Misc Visuals Circle Menu, the Context Menu or the Shortcut "F" ("Find peaks"):



User Interface

A peak find dialog will open if you enable the peak labeling feature; note that the labeling remains enabled if you close this dialog.
You can reopen the dialog by selecting the "Show Peaks" feature again in the circle menu / context menu / via shortcut.

Find Options Tab



These options can be used to change the automatic peak find algorithm.

Positive Peaks (Check Box):
Enables the peak find calculation for positive peaks.

Negative Peaks (Check Box):
Enables the peak find calculation for negative peaks.

Auto Find Peaks (Check Box):
Enables or disables the automatic peak find algorithm. If you add or remove peaks manually, the automatic peak find algorithm will be disabled.

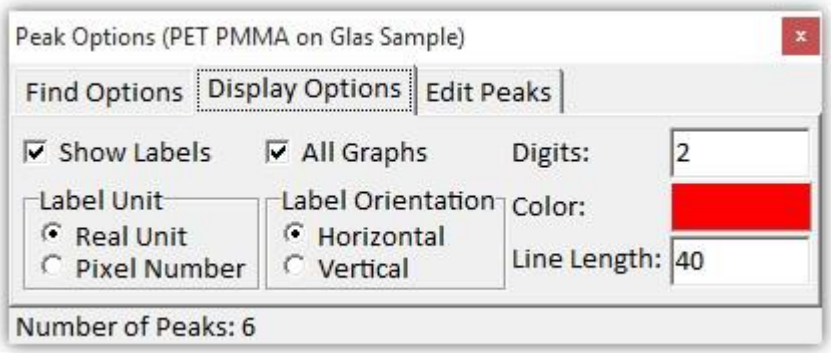
Max Absolute Height (Float Edit):
Peaks higher than this value will be ignored in the automatic peak find algorithm.

Min Absolute Height (Float Edit):
Peaks smaller than this value will be ignored in the automatic peak find algorithm.

Min Relative Height (Float Edit):
Peaks whose relative height is smaller than this value will be ignored in the automatic peak find algorithm.

Number of Peaks:
Shows the current number of peaks (including peaks added by the user).

Display Options Tab



Show Labels (Check Box):
Here you can switch between labels or vertical lines.

All Graphs (Check Box):
If checked, all graph objects in the current graph viewer are labeled.

Label Unit (Radio Group):
Defines, whether the real unit (e.g. "rel. 1/cm") or the pixel number should be used for labeling.

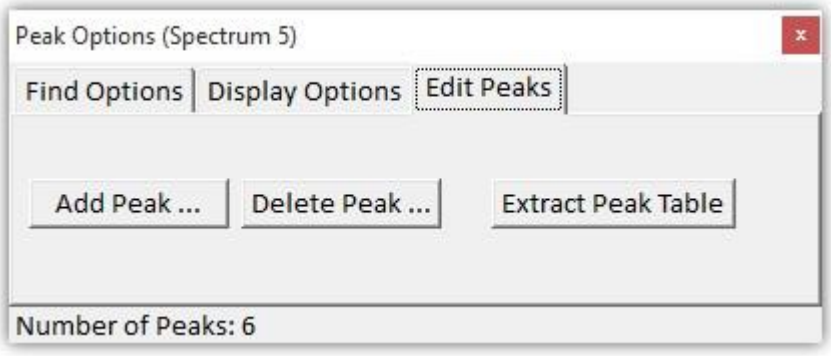
Label Orientation (Radio Group):
Defines the orientation of the labels.

Digits (Integer Edit):
Defines the precision of the peak label; note that the peak find has sub-pixel accuracy.

Color (Color Selector)
Defines the color of peak labels.

Line Length (Integer Edit)
Defines the length of the line that connects the peak to its label.

Edit Peaks Tab



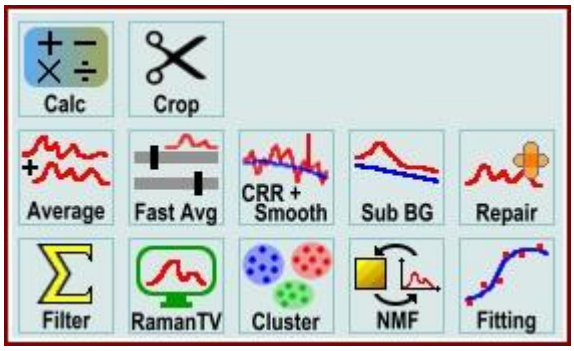
Add Peak (Button)
Opens a dialog where you can manually add a peak label at a desired X and Y Position.
Note that the automatic peak find algorithm will be disabled if you add or delete peaks.

Delete Peak (Button)
Opens a dialog where you can select one of the displayed peaks and remove it from the labeling list.
Note that the automatic peak find algorithm will be disabled if you add or delete peaks.

Extract Peak Table (Button)
This will extract all peaks with extended information as a table into a Text Data Object.

Graph Viewer Actions

You can open these features using the Graph Viewer Circle Menu.



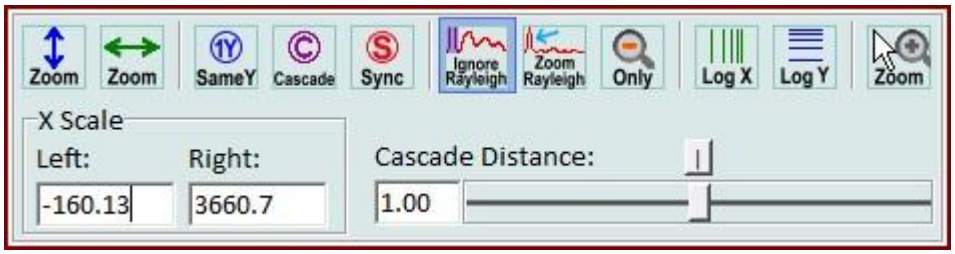
Press on any of the available actions to use the current selected graph as input data object for a data analysis tool.

See Data Analysis Overview.

Graph Viewer Scale and Zoom

You can open these features using the Graph Viewer Circle Menu.

In general the **X-Axis** is always identical for all displayed graph objects in the same viewer.
The **Y-Axis** can be different for all displayed graph objects in the same viewer. The values for the Y-scale of each single graph can be defined by using the Graph Viewer List. Additionally there are different actions that will change the Y-Axis Zoom behavior quickly (see below).



Zoom Out Y-Axis Scale:
Zooms out the Y-Axis scale to see the all Y-pixels of the currently selected graph object.
Click with the right mouse button toggle on or off the **Auto-Zoom-Y-Mode**. This will automatically execute the zoom out if the graph changes. Shortcut "Y".

Zoom Out X-Axis Scale:
Zooms out the X Axis scale to see the all X-pixels of all graph objects.
Click with the right mouse button to toggle on or off the **Auto-Zoom-X-Mode**. This will automatically execute the zoom out if the graph changes. Shortcut "X".

Same Y-Axis Scale:
If checked, all graph Data Objects share the same Y-Axis Scale. It is synchronized automatically upon changing the scale on any graph.

If the "**Auto-Zoom-Y-Mode**" is on: You can deactivate the Synchronized-Zoom and selected a desired graph (using the color area or by double-clicking the graph) in order to define which graph should be used for auto-zooming the Y-Scale. Shortcut "1".

Cascade Graphs:
Cascades multiple Graph Objects. Change the Cascade Distance to define the gap size between the graphs.
Note: if "Same Y-Axis Scale" is also active, you can zoom all graphs with the mouse wheel, but make sure that you position the mouse at the correct (selected) graph position. Shortcut "C".

Synchronized Zoom:
If checked, changing the Y-Axis via the mouse wheel will zoom all graph objects in the viewer. This only works if multiple graphs are not cascaded. Shortcut "S".

Ignore Rayleigh:
If checked, any Zoom Out Y-Axis Scale Action will ignore the Rayleigh Area. Only works with spectral Graph Objects.

Zoom out Rayleigh Peak:
Zooms out the Rayleigh peak. This might be useful when calibrating on the Rayleigh peak. Shortcut "R".

Auto Zoom Out Y-Axis Only:
If checked, the automatic Y-Axis Zoom on change will only zoom out, not in.

Logarithmic X-Axis:
Turns on or off the logarithmic X-Axis scale. Shortcut "Control-L".

Logarithmic Y-Axis:
Turns on or off the logarithmic Y-Axis scale. Values smaller than 0 will be drawn at the very bottom of the window. Shortcut "L".

Mouse Zoom:
Changes the mouse mode to the "mouse zoom mode". Click and drag a rectangle in the graph viewer to zoom the selected Graph Object exactly to this area.
Instead of turning to mouse zoom mode, you can **hold down the control key to zoom a rectangular region**.

X-Scale Left/Right (Float Edits):
Defines the X-Axis scale for all graphs. You can also hold down the Control-Key on the keyboard while turning the mouse wheel to zoom the X-Axis.

Cascade Distance (Slider)
Here you can define a gap size if you are cascading multiple graph objects.
A value of 0.0 means no cascade, a value of 1.0 means full cascade. The small button on top of the slider can be used to set the value to 1.0.

Graph Viewer Modes

You can open these features using the Graph Viewer Circle Menu.

Mouse Follow Data:
If checked, the cursor always follows the exact data points in X and Y direction.

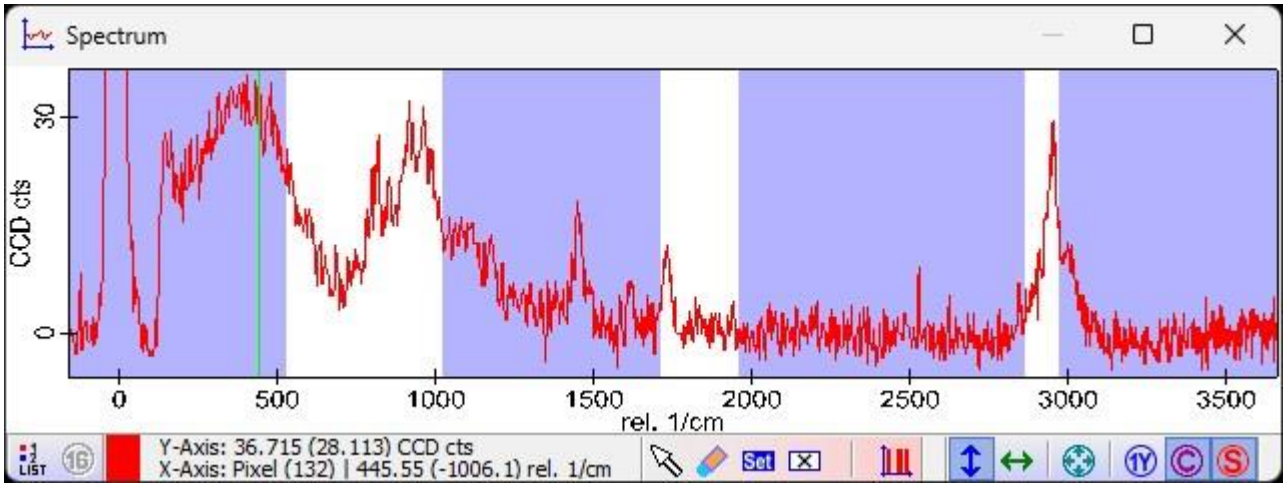
Listen to all Graphs
If checked, all multidimensional graph objects will listen; i.e. if two image graph objects are shown in the same viewer and you click on another pixel in an image viewer, the graph viewer will show the corresponding graph/spectrum of this position for all the graph data objects.
You can turn this feature off to compare particular single spectra of multidimensional graph data objects.

Parametric Display
Turns on the parametric plot. The X-Axis is defined by the graph that is selected at the moment you activate the parametric display. So just deactivate the parametric display, select another graph and activate it again to change the X-Axis-Graph.
In the Graph Viewer List the X-Axis-Graph used in the parametric display is marked by a small cross:

Graph Viewer Mask Manipulation

Some Drop Action Dialogs / Data Analysis Actions exhibit graph viewers that show a mask.With graph masks you can define, which pixels should be used for the calculation.

You can manipulate those masks directly in the preview graph viewer:



Usage

- If not already selected: select the mouse marker tool in the graph viewer.
  The mouse marker tool is automatically selected in graph viewers that show and allow manipulation of a mask.
- Just click down the left mouse button at a certain position and drag an area.
- If you release the left mouse button, the selected region of this mask is **set**.
- If the shift key is pressed while you release the left mouse button, the selected region is **cleared**.
-  You can also toggle the **Eraser mode** (visible in the status bar of the graph viewer) to clear the mask without having to press the shift button.

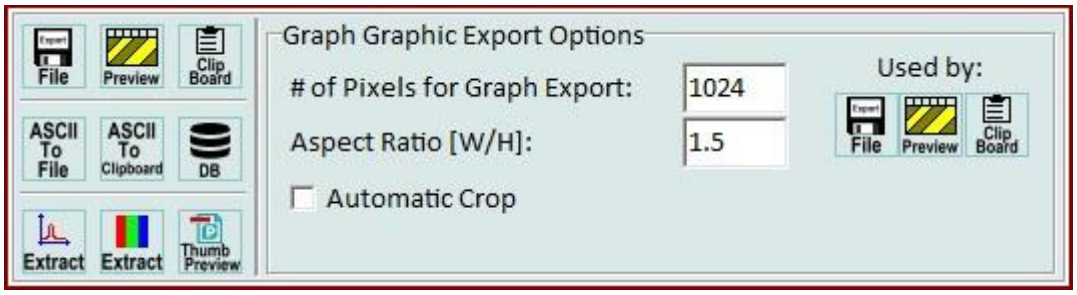
-  Press the extract button to extract the current mask as a new mask data object into the current project in order to reuse the mask.

- You can drag and drop an existing mask object from the project manager onto the current mask in order to overwrite the mask

Though this is the fastest way to change a mask, you can also use the Mask Manipulation Tools of a belonging drop action dialog in order to change the mask. There you can exactly define which pixels should be masked and its also possible to export a mask to the project for later usage.

Graph Viewer Export

You can open these features using the Graph Viewer Circle Menu.



Graph Graphic Export Options
Here you can change the size of exported bitmaps
See Graph Viewer Options

Export Bitmap to File:
Exports the current view to a file. You can choose between several image formats.

Export Bitmap to Preview:
Exports the current view to the built-in graphic editor. Here you can flip or rotate the bitmap and save it to a file or clipboard.

Export Bitmap to Clipboard:
Exports the current view as a bitmap into the clipboard. This allows you to paste the bitmap into another graphic software.

Export ASCII To File:
Exports the current graph in ASCII Format to a file.
For all ASCII exports, the format is: <Interpreted X-Axis Value> <Tab> <Y-Axis Value>

Export ASCII To Clipboard:
Exports the current graph in ASCII Format to the clipboard.

Export to Database Application
Exports all spectral data objects (using the selected region, if any set) to a database application which can be defined in the Database Export Options.
Also see Database Search in WITec Project.
Note 1: The export only works if the graph has a spectral X-Transformation and the interpretation unit of the X-Axis is "rel. 1/cm"
Note 2: If a region is marked in the graph viewer only the marked region is exported. Clear the marked region by switching to the mark region mouse mode and click somewhere in the window.

Extract Graph to Project:
Extracts the current displayed single graph as a new graph object to the current project.

Extract View as Bitmap to Project
Exports the current view as a new bitmap object to the current project.

Set as WIP-File Thumbnail Preview in Windows Explorer:
Sets the current view as the thumbnail preview for the WIP-File. You have to save the project afterwards to take effect. The windows file explorer may cache thumbnails so you might not immediately see the new thumbnail.

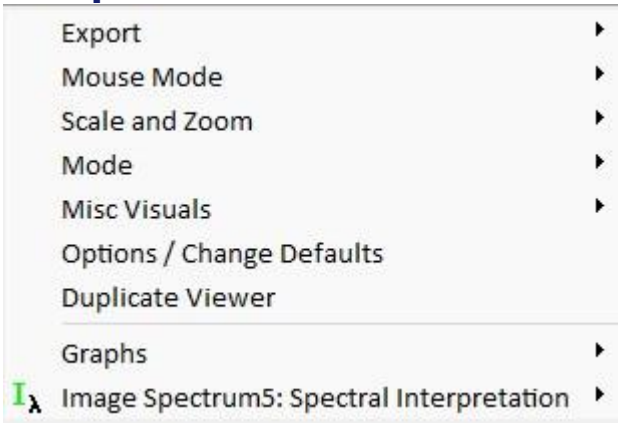
Graph Viewer Drag and Drop

You can drag and drop the following items on a graph viewer:

- Graph Objects (to display and compare multiple graph objects in the same viewer):
  Only works if the dropped object uses the same x axis unit kind

- Graph Mask Objects (to reuse a previously saved mask):
  Only works if the graph viewer is a preview containing a mask for manipulation (see Graph Viewer Mask Manipulation)
  or if the graph viewer is in the mask creation mode.
  If multiple masks are shown by the viewer, the mask below the mouse position is overwritten when dropping a mask.

Graph Viewer Context Menu

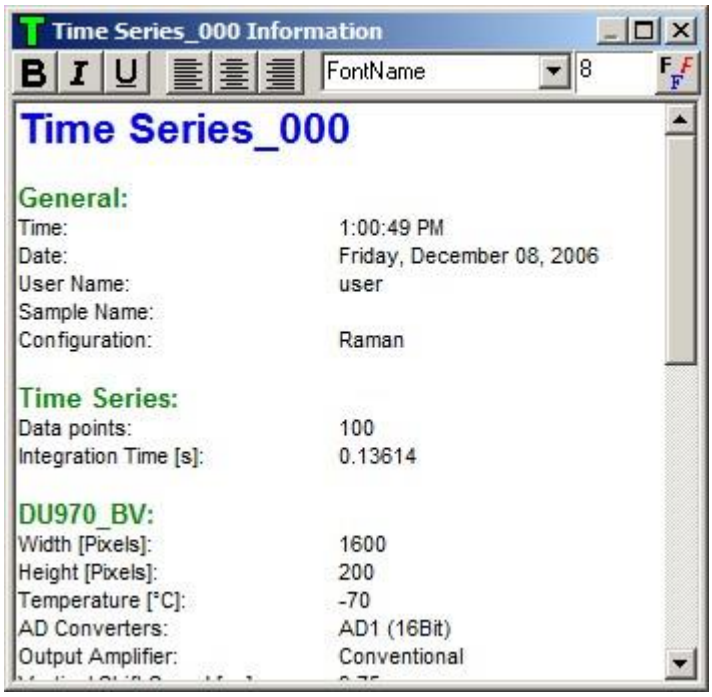


The context menu is an alternative way for accessing the features that you also can find in the circle menu. Just click and release the right mouse button anywhere in the viewer.

Options / Change Defaults
This will open the program options for the graph viewer.

At the bottom of the context menu you can find a list of data objects that are used by the viewer:
- graphs that are displayed (sub menu)
- the X-Axis Interpretation, which you can use to switch between different units (e.g. rel. 1/cm or eV).

Text Viewer



The Text Viewer can show text data objects that contain text in rich text format (RTF).

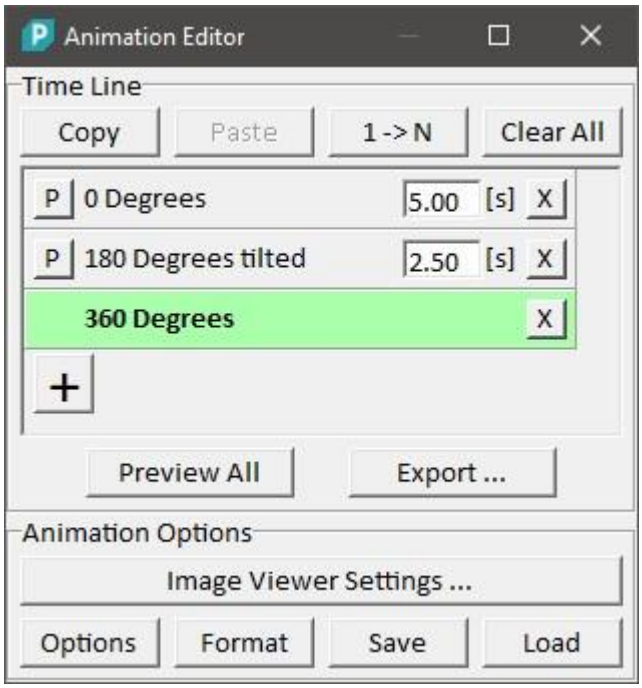
Just double-click a text object to create a Text Viewer. If the text object is already shown in a text viewer, no new viewer is created but the existing text viewer will come on top of all windows.

You can type, format, copy and paste text in the text viewer.

It's possible to **export** one or multiple text objects into a .txt (ASCII) or .rtf (Rich Text Format) file, see Export Overview.

Animation Editor

The animation editor is used to create user defined animations by defining any number of steps. The editor is used by the Image Viewer and the Image Transform and Overlay Dialog.



Copy:
Copies the selected step to the "local clipboard".

Paste:
Pastes the copied step from the clipboard after the selected step.

1 -> N:
Copies the first step to the end.

> Angles are measured continuously (e.g. -200° or +540°). This can result in a complete rotation or even more back to e.g. 45° when using this button, To prevent this, select the first step in the list and open the Image Viewer Settings. Enter e.g. 360 and click on Custom Rotate to add one full rotation to the first step as new step.

Clear All:
Clears all steps.

Preview Step ("P" Button):
Shows the preview for the step.

Step Name ("0 Degrees" Label / Edit):
This is the step name.
You can select a step or edit the step name by clicking on the text.

Interval Time ("5.00 [s]" Edit):
Changes the interval time for the step.

Delete ("X" Button):
Deletes the step.

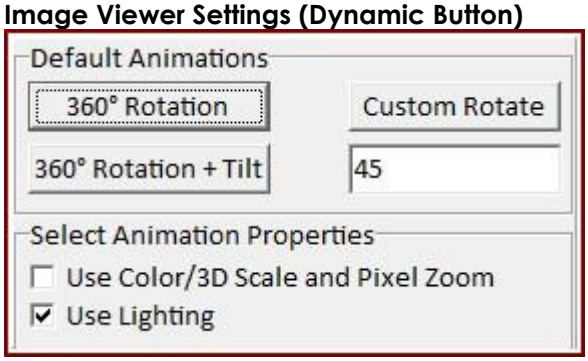
Add New Step ("+" Button):
Adds a new step.

Preview All (Button)

Shows a preview animation for all steps.

Export (Button)
Exports the animation using the current Image Export Options.
The aspect ratio and resolution of the exported data is defined by the window size.

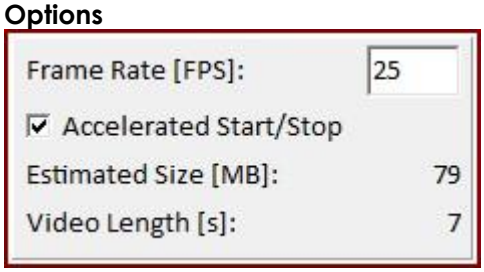
Image Viewer Settings (Dynamic Button)



Depending on which software component uses the animation editor, it shows options for the special component:

- Default Animations: one or more default animation, overwrites the current steps.
- Custom Rotate: Adds a new step with a rotation defined by the value below in °.
- Use Color/3D Scale and Pixel Zoom: Also changes of the Color or z scale are recorded.
- Use Lighting: Also in changes of the Lighting are recorded

Options



Shows animation options:

- Frame Rate: defines the frame rate for AVI videos
- Accelerated Start/Stop: if checked, the first and last step are accelerated and not linear.
- Estimated Size: Shows the estimated size of the file(s). Not valid for compressed AVI files.
- Video Length: Shows the video length / sum of all step intervals.

Format
Opens the Image Export Options to define the export format and open settings.

Save
Saves the current animation to the hard drive.

Load
Loads an animation from the hard drive.

Data Analysis

Data Analysis Overview

This chapter shows all important software features for analyzing your data.

- Drop Actions Window
  contains Technical Documentation of all Drop Action Dialogs.

- Raman Analysis
  shows a list of all important analysis features used for a Raman Image Scan analysis.

- Database Search in WITec Project
  shows an overview of using a database search in WITec Project.

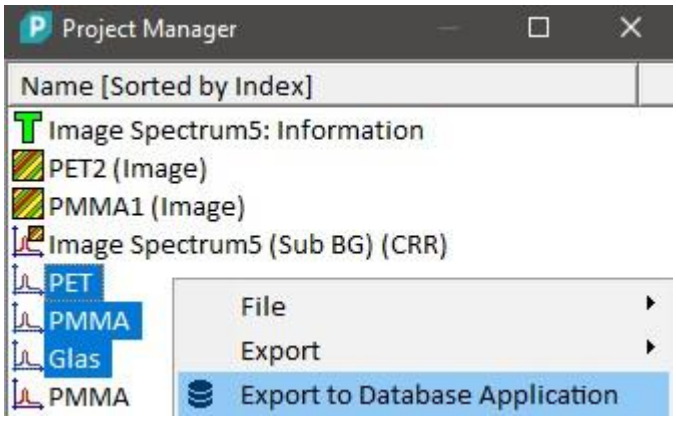
Database Search in WITec Project

It's possible to use a Spectrum Database to search for unknown components.
For this WITec developed the search software WITec TrueMatch. It's also possible to use the ACDLabs Software.
You can create your own databases or use the commercial database ST Japan.

There are several ways to get spectra into the WITec TrueMatch Database Software or ACDLabs. Before you use one of them, make sure you have configured the Database Export Options.

Project Manager

You can select multiple single spectrum data objects in the Project Manager and use the Context Menu "Export To Database Application":



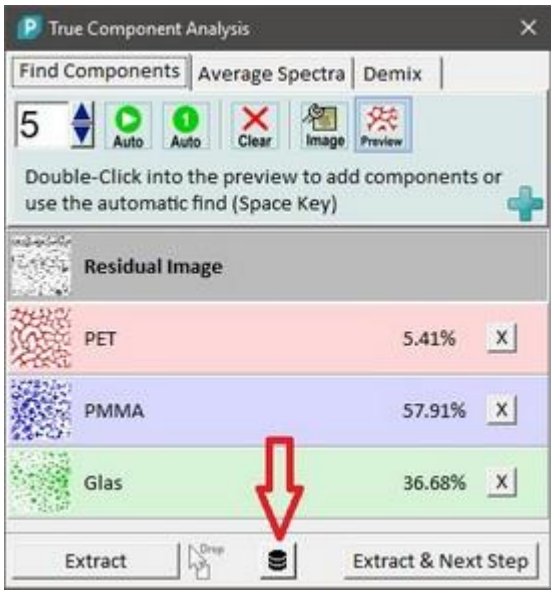
Graph Viewer

All visible spectra in the Graph Viewer can be sent to the TrueMatch Software using Circle Menu or Context Menu "Export -> Export to Database Application" (Shortcut E):

If a certain region is marked using the Graph Viewer Mouse Marker, only the selected region is sent to the TrueMatch Software.

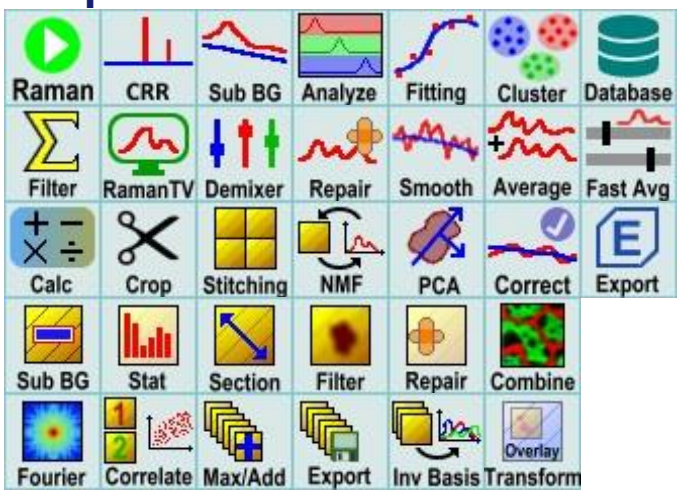
TrueComponent Analysis

You can directly put your analysis result spectra into the TrueMatch Software using the Database Button in the TrueComponent Analysis Dialog:



Drop Actions

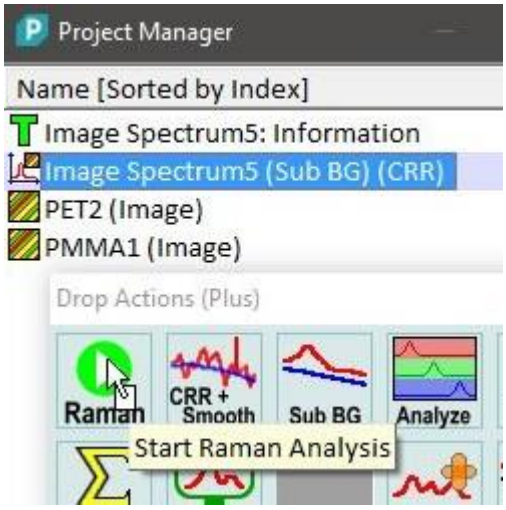
Drop Actions Window



Click on one of the Drop Action buttons to see the corresponding help page.

The Drop Actions window is the portal for all data analysis features of WITec Project.

To use the Drop Action features you have to drag one or several selected Data Objects from the Project Manager and drop them onto one of the Drop Action buttons:



Depending on the chosen Drop Action this will either start a calculation or open a dialog together with a preview window. The latter allows the effect of changing the parameters to be observed before starting the calculation on all data.

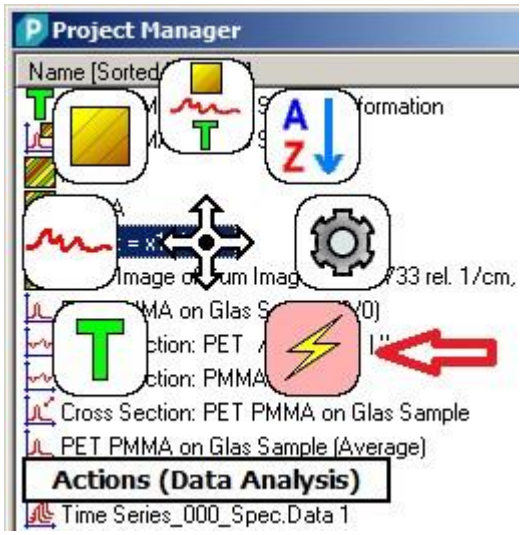
The "**Extract**" button(s) in each of the Drop Action dialog windows will calculate the result and create new Data Objects in the current Project.

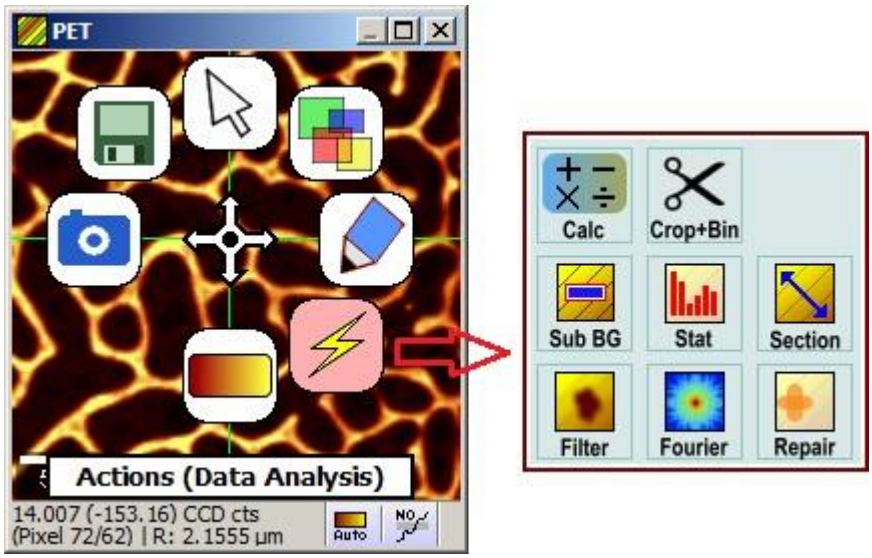
You can show or hide the Drop Actions window using the main menu "**View > Drop Actions Window**".
If the window is hidden, it will automatically pop up temporarily near the mouse position upon dragging items from the Project Manager.

A good way for starting a Raman analysis is the "Start Raman Analysis" button, which will start the first analysis step.

Another way of starting data analysis

Alternatively, you can directly open the action circle menu in the Project Manager or Image- or Graph-Viewer and click on one of the Action buttons:





Raman Analysis

If you have measured a Raman Image, you can just start your analysis by dropping your dataset on the Start Raman Analysis Drop Action. The "Extract & Next Step" Buttons in the dialogs will guide you through the different steps of the analysis.

Start Raman Analysis

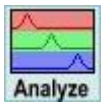
This will open the first dialog for the analysis, the Cosmic Ray Removal Drop Action:

Cosmic Ray Removal

After removing the cosmic rays, you can go on with the Background Subtraction:

Background Subtraction

Now the data is prepared for further analysis. The TrueComponent Analysis can be used in the next step. It helps finding all the components, creates nice images and spectra for each component:



TrueComponent Analysis

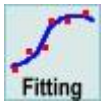
The result component images can be presented very nicely using a smoothing algorithm, if desired:

Image Smoothing

Multiple component images can be combined in a single, multicolored bitmap using the image combination dialog:

Image Combination

If your measurement has peak shifts due to certain effects, you can use the Advanced Fitting Tool to fit your peaks automatically:



Advanced Fitting Tool

The Cluster Analysis helps finding out differences in spectra and how many components are in your measurement:

Cluster Analysis

Graph Cosmic Ray Removal Dialog

Description

This dialog allows to remove cosmic rays from a spectral dataset.
The cosmic ray removal should be done before any other spectral data analysis (especially for spectral images).

Input and Results

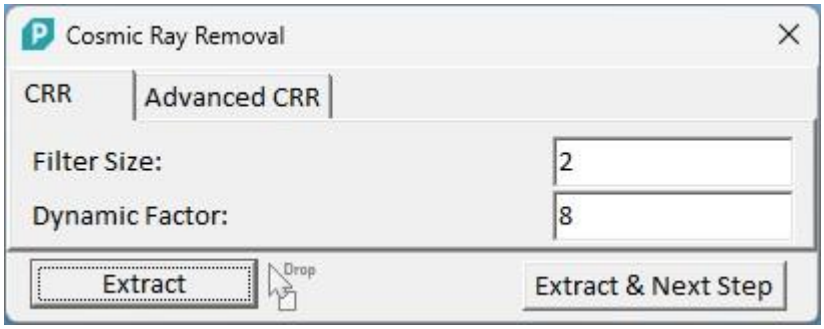
Input:
One graph object (single spectrum, line- or image-spectrum).
Advanced CRR works only with image-spectrum .

Result:
A cosmic ray removed graph data object.
The Advanced CRR additionally extracts a cosmic ray map.

User Interface

CRR (Simple Cosmic Ray Removal)

If the CRR tab is selected, cosmic rays will be removed from the graph fully automatically using a simple sorting threshold algorithm.



Filter Size:
Filter size of the cosmic ray detection algorithm. The total window size is 2*<Filter Size> + 1.

Dynamic Factor:
This factor changes the sensitivity of the cosmic ray detection algorithm (a smaller value means a higher sensitivity).

Advanced CRR

The Advanced CRR shows a list of detected cosmic rays and guides the user through multiple steps where the user can check and decide if a detected cosmic ray will be removed or not.
The dialog uses an advanced algorithm that uses neighbour spectra to detect cosmic ray, which leads to a better detection e.g. for broad cosmic rays hitting multiple CCD columns at once.

Processing all steps in the order of the tab controls will lead to the best result.

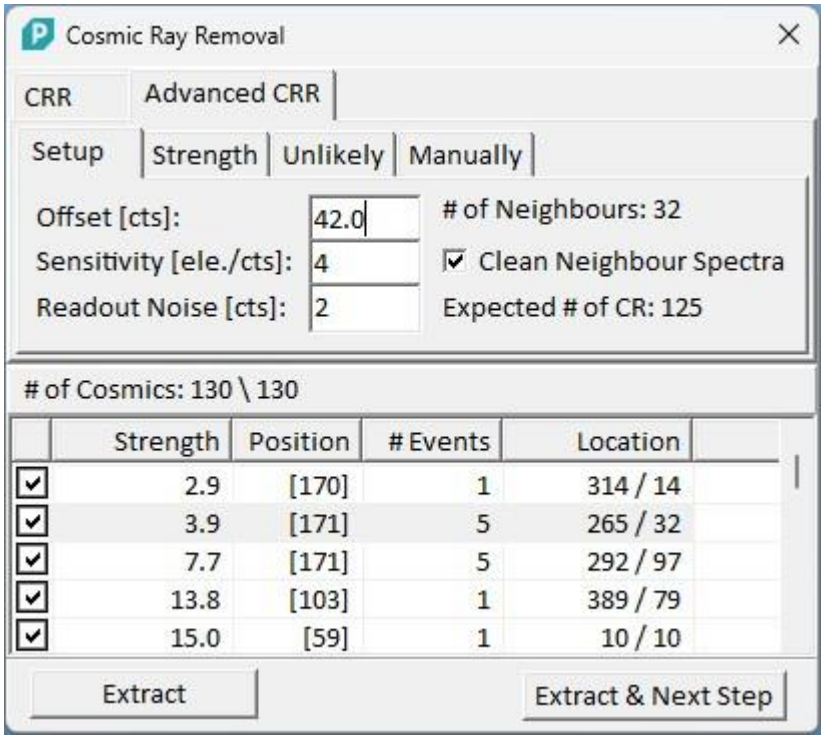
Only raw datasets should be used, no background subtracted data.

Only image spectrum data objects can be used

Spectra measured in EMCCD mode can not be used

Setup

Before doing the analysis, please check and set the correct offset, sensitivity and readout noise. In this step, it's possible to change the mask in the "Frequencies of Cosmics" graph viewer in order to filter out cosmics by their spectral position.



Offset (Float Edit)
Defines the offset of the data (+- 10 counts is fine). The value is auto-estimated when the dialog opens.

Sensitivity (Float Edit)
Defines the sensitivity of the CCD chip. Typical values for each CCD model see table below.

Readout Noise (Float Edit)
Defines the readout noise of the CCD chip. Typical values for each CCD model see table below.

Model	Mode	Sensitivity [ele./cts]	Readout Noise [cts]	Horizontal Shift Speed [MHz]	Pre amp
DR324 FI	Low Noise	2.3	2.5	0.035	4
	High Dynamic Range (Fast)	9	3	1.48	1
DR316 LDC-DD	Low Noise	1.5	3	0.13	4
	High Dynamic Range (Fast)	6	1.5	1.48	1
DV401A FI	Low Noise	2	1.5	0.033	1
	High Dynamic Range (Fast)	9	1	0.1	1.5 - 1.7
DV401A BVF	Low Noise	2	3.5	0.033	1
	High Dynamic Range	9	1.3	0.1	1.5 - 1.7

	(Fast)				
DU401A FI	Low Noise	2.5	1.2	0.033	1
	High Dynamic Range (Fast)	11	0.8	0.1	1.5 - 1.7
DU401A BVF	Low Noise	2.5	2.8	0.033	1
	High Dynamic Range (Fast)	11	1.1	0.1	1.5 - 1.7
DU401A BR-DD	Low Noise	2.5	2	0.033	1
	High Dynamic Range (Fast)	12	0.8	0.1	1.5 - 1.7
<XX>420A OE	Low Noise	2	2	0.033	1
	High Dynamic Range (Fast)	9	1	0.1	1.5 - 1.7
<XX>420A BU, BU2, BVF	Low Noise	2	3	0.033	1
	High Dynamic Range (Fast)	9	1.1	0.1	1.5 - 1.7
<XX>420A BEX2-DD, BR-DD	Low Noise	2.5	1.6	0.033	1
	High Dynamic Range (Fast)	11	0.9	0.1	1.5 - 1.7
DU970P BVF, FI, UV, UVB	Low Noise	0.8	3.5	0.05	4
	High Dynamic Range	3	0.9	0.05	1
	High Dynamic Range (Fast)	3	2.8	3	1
	Electron Multiplying	CRR not possible			
DU49<X>A	High Sensitivity	CRR not possible			
	High Dynamic Range	CRR not possible			

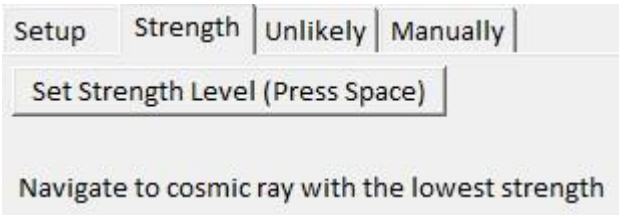
# of Neighbours
Shows the number of neighbor spectra that have a cosmic on the same spectral position.
Neighbour cosmics are normal for image and large area scans that don't use stepwise raster due to interpolation of neighbour spectra (TrueScan Feature).

Clean Neighbour Spectra (Checkbox)
If checked, neighbour cosmics will be automatically corrected.
Should be turned off for stepwise raster scans.

Expected # of CR
Shows the expected number of cosmic rays.
This is calculated using a statistical analysis and should be in the magnitude of the found # of Cosmics.
If much more cosmics were found than this expected number (by around a factor of 2), it might indicate that there are a lot of false positive detections.

List of Cosmics
Shows all detected cosmic rays together with their strength, spectral position, number of events (# cosmics at the same position) and the location in the image (pixel position X / Y).

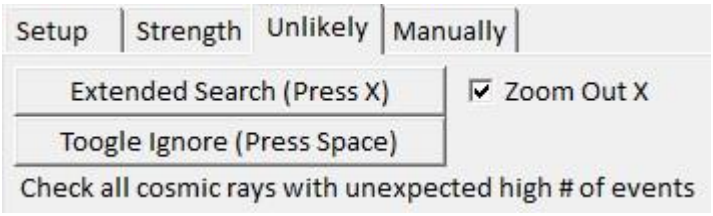
Strength



If this tab is selected, the cosmic list will be sorted by strength.
This allows to deselect false positive cosmics with a too weak cosmic signal.
If the setup was done correctly, then the first real cosmic should have a strength of around 6.

Set Strength Level
Please look at the cosmics and select the first real cosmic ray, then press "Set Strength Level".
This will deselect all cosmics with a lower strength than the selected one.

Unlikely



If this tab is selected, the cosmic list will be sorted by the number of cosmic rays that are on the same spectral position, then by the spectral position itself.
Cosmics that happen on the same spectral position in multiple different spectra / measurement points are unlikely to be a cosmic ray.
Please inspect all cosmics with more then 1 event or 2 events (if clean neighbor pixels is activated) by selecting them in the list.
If the different spectra of cosmics that occur at the same spectral position have similar Raman peaks, the cosmic could be a false positive.

Extended Search
This will calculate the cosmic ray detection by comparing the selected cosmic (and all cosmics of the same spectral position) with all other spectra in the dataset in order to find out, whether there are spectra with a Raman peak at the cosmic position.
If there are other spectra having a Raman peak at this position, the cosmics for this spectral position will be automatically unselected.

Zoom Out X
If turned off, the preview graph viewer will zoom into the spectral position of the cosmic ray in order to have a closer look at the cosmic itself.
If turned on, you can see the whole spectrum in order to compare the Raman peaks of different spectra that have cosmics on the same spectral position. If spectra look similar, the cosmics might be false positives.

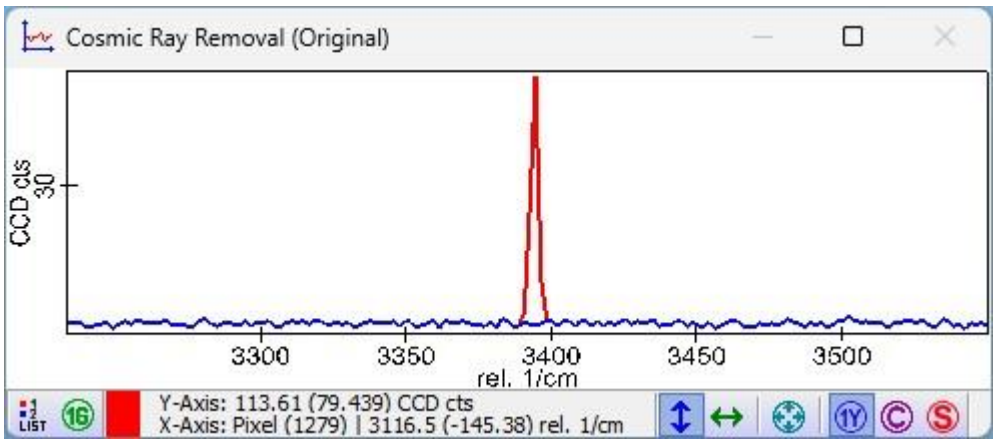
Toggle Ignore
Press to toggle the ignore state of the selected cosmic ray.

Manually

After having removed all cosmics that are too weak and all unlikely cosmics, you should look at each remaining cosmic ray to find out if there is still a false positive. This can be the case e.g. for Raman peaks that occur only once in the whole dataset.

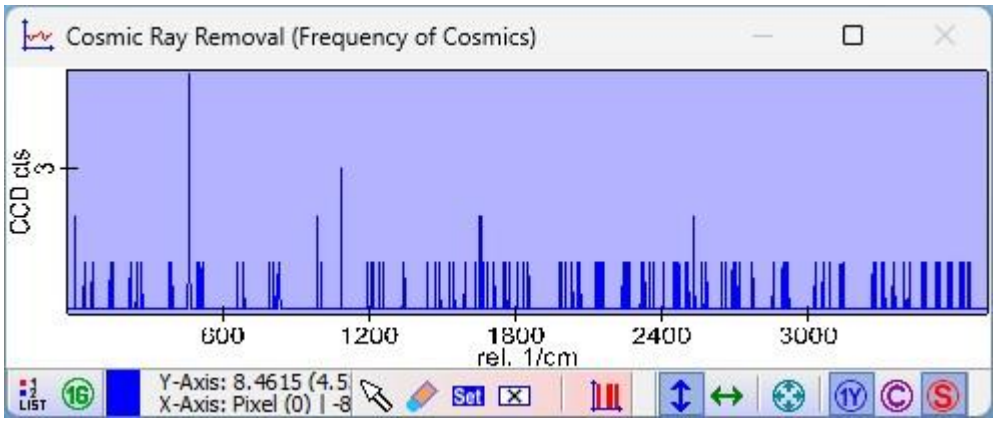
Preview Windows

Cosmic Preview



Shows the original spectrum (red) and the cosmic ray removed spectrum (blue) of the currently selected cosmic ray.

Frequency of Cosmics



Shows the frequency of all found cosmic rays, that means how much cosmics were found at each spectral position.
If multiple cosmics were found at the same spectral position, this might be an indicator for false

positives.
Already excluded events are shown in red color.

Graph Background Subtraction Dialog

Description

With this dialog a background can be subtracted from one or multiple spectra using various algorithms.
If used on a multi-spectral data set, all spectra can be corrected at once using the same mask and algorithm.

Input and Results

Input:
One graph object that can be a single spectrum or a multiple spectra object (e.g. Image Graph).

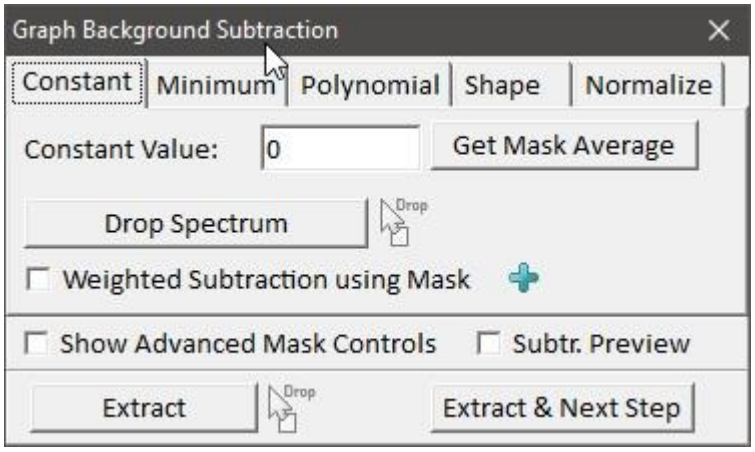
Results:
A copy of the input graph object, background subtracted.
Suffix: *(Sub BG)*

User Interface

- Constant Tab
- Minimum Tab
- Polynomial Tab
- Shape Tab
- Normalize Tab
- Advanced Mask Controls
- Preview Window

Constant Tab and General UI

If the "Constant" tab is selected, the background is subtracted using a static constant user definable value and/or using a single spectrum which is subtracted from all input spectra.



Constant Value (Float Edit)
The constant value will be subtracted from all pixels and all spectra.

Get Mask Average (Button)
Press this button to set the constant value to the average value of all pixels that are currently set in the mask using the current preview spectrum.

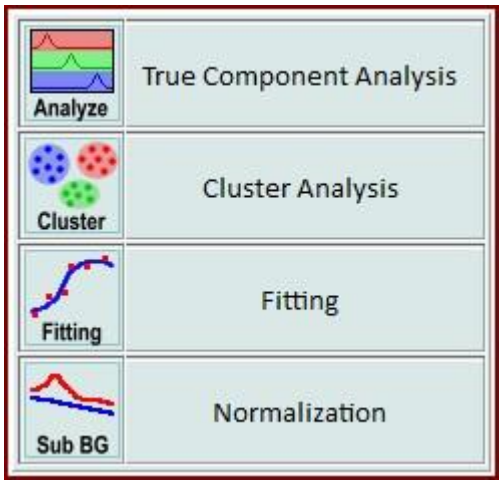
Drop Spectrum (Button & Drop Zone)
You can drop a single spectrum onto this button in order to subtract this spectrum from all your input spectra. Click the button if you do not want to use the single spectrum for subtraction anymore.
The Constant Value will be set to zero if you drop a single spectrum; however, but you can enter a constant offset afterwards for an additional constant value subtraction.

Weighted Subtraction using Mask (Check Box)
If checked, you can change the mask in order to define which spectral area should be used for calculating a weighting factor for subtracting a dropped single spectrum. This is a plus feature.

Subtr. Preview (Checkbox)
If checked, the blue preview graph shows the subtracted spectrum preview instead of the line, that will be subtracted.

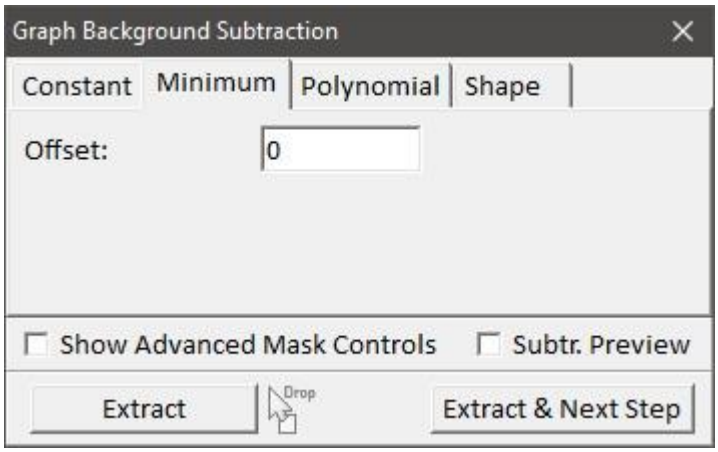
Extract (Button & Drop Zone)
performs the background subtraction for all spectra of the input graph data object and adds the result to the current project.
You can drag and drop multiple other graph Data Objects onto this button for batch processing.

Extract & Next Step (Button)
Wizard Feature: With this Button, you can choose whether you would like to use the result of this dialog and do a TrueComponent analysis, a cluster analysis, peak fitting or a normalization



Minimum Tab

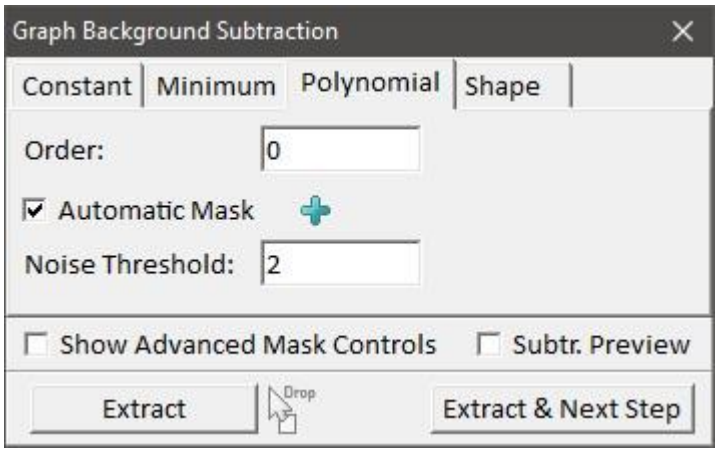
If the "Minimum" tab is selected, the background is subtracted using a horizontal line at the minimum value of all spectral pixels in the marked area.



Offset (Float Edit):
Adds an offset to the found minimum value.

Polynomial Tab

If the "Polynomial" tab is selected, the background is subtracted by fitting a n-order polynomial to the masked spectrum.
If this background subtraction mode is used, all Raman peak areas should be removed from the mask - unless you use the automatic mask mode.



Order (Integer Edit):
The order of the polynomial for fitting the background. An order of 0 will subtract a horizontal line, an order of 1 will subtract a slope, e.c.

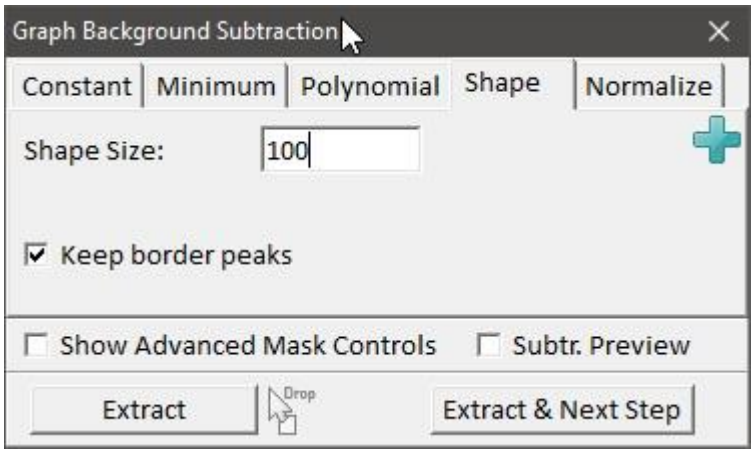
Automatic Mask (Check Box):
If checked, the algorithm tries to ignore peaks by automatically use spectral noise pixels only. Thus you can also set the mask at pixels where you have Raman information.
This is a WITec Project Plus feature.

Noise Threshold (Float Edit):
The noise threshold for the automatic polynomial mode. You can shift the background level horizontally by changing this parameter.
It is only available, if the "Automatic Mask" mode is enabled.

Shape Tab

If the "Shape" tab is selected, the background is subtracted using a rounded shape which is

approached to the spectrum from below pixel by pixel of the spectrum. The shape method is very effective for subtracting fluorescence areas and it is quite easy to use.
It is a WITec Project Plus feature.



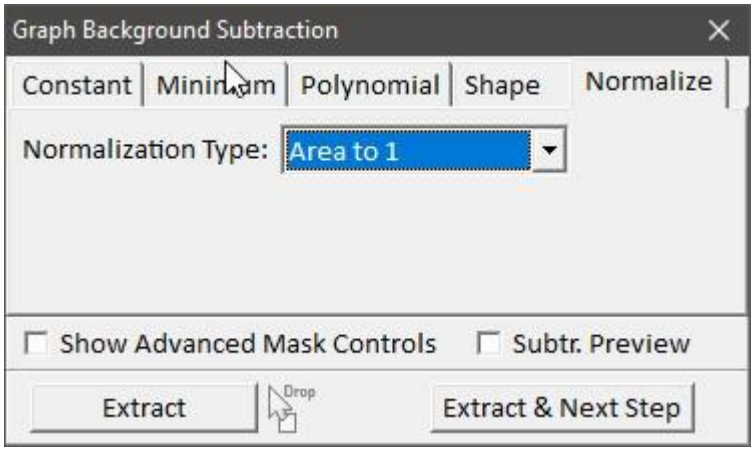
Shape Size:
The size of the rounded shape. A smaller size will subtract more details from the spectrum, whereas a larger size will subtract more rough shapes.

Keep border peaks:
If not checked, then peaks at near the border pixels are subtracted.

Mask:
Use the blue mask for a linear interpolation of pixels.

Normalize Tab

If the "Normalize" tab is selected, the spectrum will be normalized to a desired value.



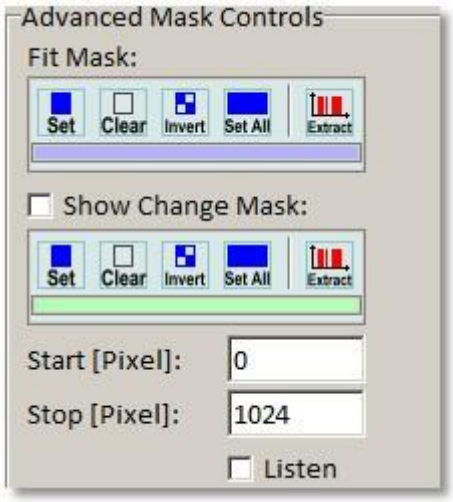
Normalization Type:

Area to 1	– The area within the mask will be 1
Area Absolute to 1	– The area within the mask will be 1, using an absolute value for negative values
Max Peak to 1	– The maximum spectrum value within the mask will be normalized to 1
Max Peak to 100	– The maximum spectrum value within the mask will be normalized to 100

Mask:
Use the blue mask to define which part(s) of the spectrum should be used for normalization.

Advanced Mask Controls

Show Advanced Mask Edits (Check Box):
If checked, the advanced mask edits are shown. These edits are optional, you can change the masks directly in the preview graph viewer.

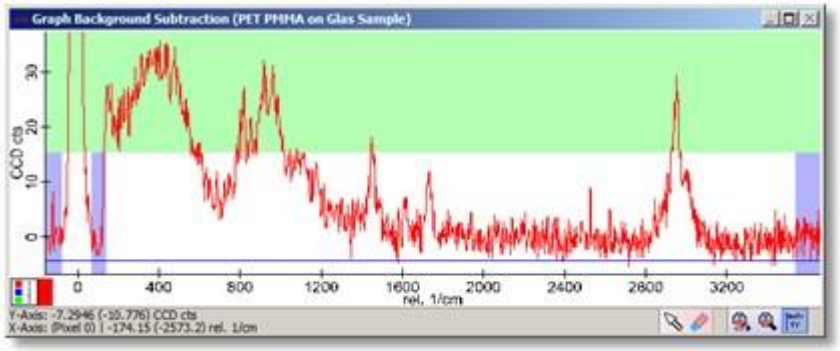


Fit Mask (Mask Tool Bar):
With those tool buttons you can modify the blue fit mask (using the Start / Stop [Pixel] Edits).

Show Change Mask (Check Box):
if checked, there is an additional, green mask in the graph preview window that can be manipulated. With the tool buttons below you can modify the change mask (using the Start / Stop [Pixel] Edits).
Clearing pixels in the change mask will flatten the result spectrum at this position using the value of the last masked pixel. This could be useful e.g. if a polynomial fit has very extreme values at the borders of a spectrum.

Start / Stop [Pixel], Listen (Edits, Check Box):
see Mask Manipulation Tools.

Preview Window



The Preview Window shows the original graph object in red as well as a blue preview curve that will

be subtracted from the original.

If a multi-spectral graph object (line spectrum, image spectrum) is used, you can click on any pixel in an image to see the preview of the selected spectrum.

True Component Analysis Dialog

Description

The TrueComponent Analysis Dialog is the successor of the Basis Analysis Dialog. It creates intensity distribution images that show the distribution of different components. In the WITec Project Plus Version, the dialog finds components automatically, creates average component spectra and supports demixing of spectra.

See also Basis Analysis (Math).

Input and Results

Input:

- One spectral Raman image data set OR
  One spectral line graph data set (e.g. spectra along a line) and
- <n> Component Spectra (can be added via drag drop or using the find component feature of the dialog)

Results:

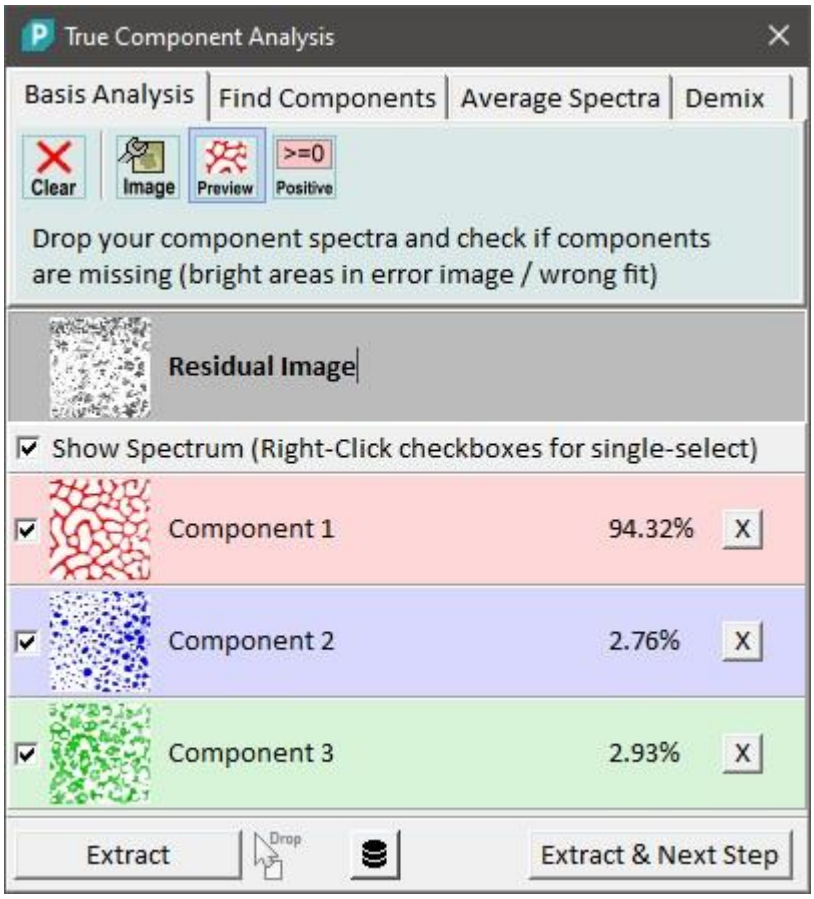
- One image for each component spectra showing the intensity distribution for this spectrum or
  One single spectrum for each of the single spectra if a line graph data set is used.
- Average Component Spectra if added using the find component feature.

User Interface

- Basis Analysis Tab
- Find Components Tab
- Average Tab
- Demix Tab

Basis Analysis Tab and General UI

The Basis Analysis Tab is only visible, if the WITec Project PLUS Version is not active.
It allows to do the normal Basis Analysis like in prior Versions of WITec Project.



Clear (Tool Button)
Press clear to delete all component spectra.

Image (Tool Button)
Shows options for changing the preview thumbnail size.

Preview (Tool Button)
Turns the preview calculation on or off. If turned on (default), the basis analysis is calculated for the whole image graph data object and shows a preview image for the currently selected component or the residual image.
For very large datasets, this feature is turned off by default and can be turned on by the user.

Positive (Tool Button)
If checked, negative weighting factors will be set to zero. This way you can e.g. check whether you have mixed spectra.

Residual Image (First List Entry)
Shows a preview thumbnail for the residual components. You can click on this entry to select the residual image as a large preview in the image viewer. In this image bright areas show you which components are missing so you can find and add them to your list of components.
In the demix mode, this image shows bright areas on components that might be mixed so you can demix it easily.

Show Spectrum (Check Box)
Here you can show or hide all components from the preview graph window.

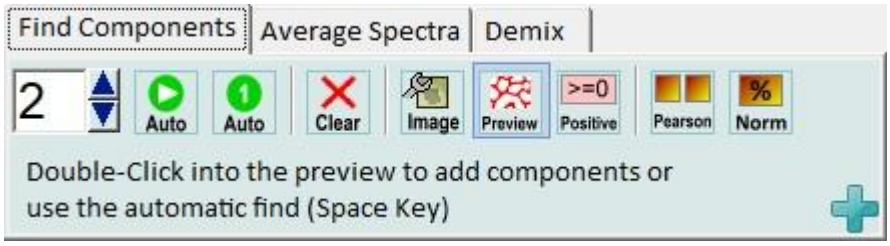
Component 1, 2, ... (Further List Entries)
The check boxes can be used to show or hide each individual component spectrum in the preview graph window.
Shows a preview thumbnail of the intensity image for this component.
You can edit the name just by clicking on the label "Component 1".
The percentage label shows you how much of each component is mixed to describe the currently selected spectrum.
The X button simply lets you delete the component from your list.

Extract (Button)
Extracts the intensity distribution images and average component spectra (if created by the dialog) in your project.
You can drop multiple image graph data objects on this button for batch processing.

Export to Database Application (Button)
Exports all component spectra to the database application, e.g. to WITec TrueMatch or ACDLabs

Extract & Next Step (Button)
Wizard Feature: you can choose whether you would like to use the result images of this dialog as an image combination or do some image smoothing:

Find Components Tab



This is a WITec Project Plus Feature!

Number Edit and Up/Down Buttons
Sets the number of spectra for the automatic component finder. Just select a number of components and press "Auto".

Auto (Tool Button)
Starts the automatic component finder. Automatically finds and adds a desired number of spectra.

Auto 1 (Tool Button)
Automatically adds one component using the current residual image.

Clear (Tool Button)
Deletes all your component spectra.

Image (Tool Button)
Shows Options for adding and finding components, see Advanced Options.

Preview (Tool Button)
Starts the automatic component finder.

Positive (Tool Button)
If checked, negative weighting factors will be set to zero. This way you can e.g. check whether you have mixed spectra.

Pearson (Tool Button)
Exports all component images using the Pearson correlation coefficient. Uses the spectral mask for calculation.

Norm (Tool button)
Exports all component images with having normalized percentage values.
See Percentage Images (Math).

Average Spectra Tab

This is a WITec Project Plus Feature!

Auto (First Tool Button)
This will calculate the average spectrum for all your components.

Auto (Second Tool Button)
Automatically calculates an image mask for the currently selected component.
This mask can be edited or directly used for calculating an average spectrum (just press the Average Tool Button).

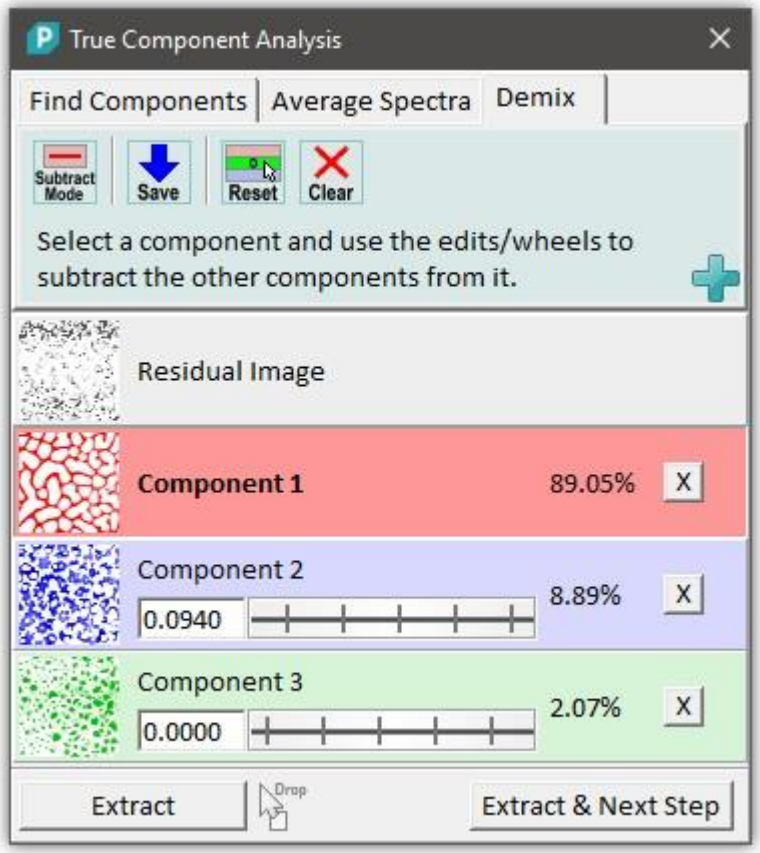
Level
This opens the threshold mask tool of the image viewer in order to define which pixels should be set in the image mask.
This mask can be edited or directly used for calculating an average spectrum (just press the Average Tool Button).

Average
This will calculate the average spectrum for the selected component using the current image mask.
You can use the Automatic Mask or level/threshold tool to define the mask or use the custom image viewer mask tools to define your own mask.

Reset
Resets the average operation of the currently selected spectrum.

Clear
Resets all average operations of all components.

Demix Tab



This is a WITec Project Plus Feature!

Subtract Mode (Tool Button)
If checked, the selected component can be subtracted from all the other components by changing the weighting factors.
If not checked, the selected component will be changed by subtracting the other components using the weighting factors.

Save (Tool Button)
Saves the current demix in order to subtract a demixed spectrum from the other spectra.

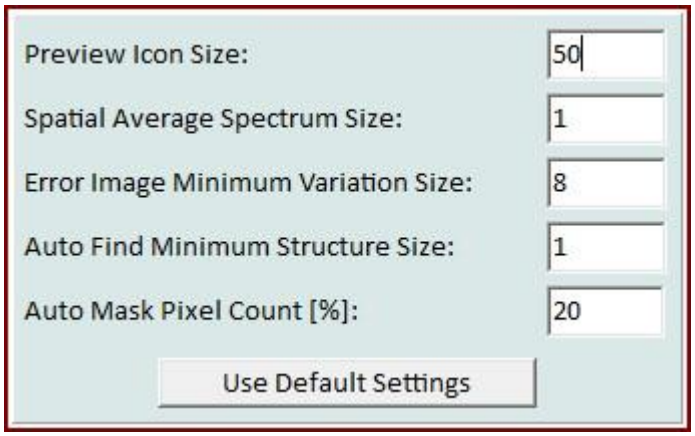
Reset (Tool Button)
Resets the demix of the currently selected component (sets all weighting factors to zero). This will not reset saved demixes.

Clear (Tool Button)
Resets all demixes.

Weighting Factor (Number Edit and Wheel Control)

You can simply turn the wheels or edit the number to define a weighting factor for subtracting the spectra from each other.

Advanced Options



Preview Icon Size
This will change the size of your preview icons / thumbnails in your component list.

Spatial Average Spectrum Size:
If a spectrum is added using the double-click or the auto find algorithm, your component spectra will be an average of the nearby pixels. This parameter defines the spatial average size of this spectrum.

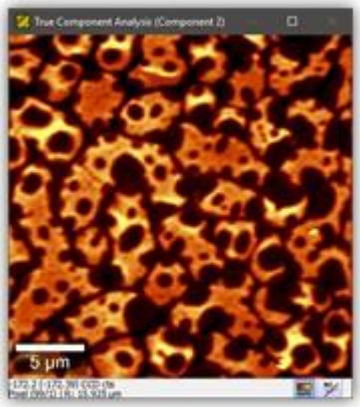
Error Image Minimum Variation Size:
A parameter defining how noise is treated when calculating the error image. A higher value avoids noise.

Auto Find Minimum Structure Size:
The structure size of an automatically found spectrum must be larger than this value.

Auto Mask Pixel Count:
In the Average Tab, you can automatically calculate an image mask for the selected component. This parameter gives you the chance to only select the most "important" pixels, avoiding to select pixels on edges of a structure leading to mixed spectra.

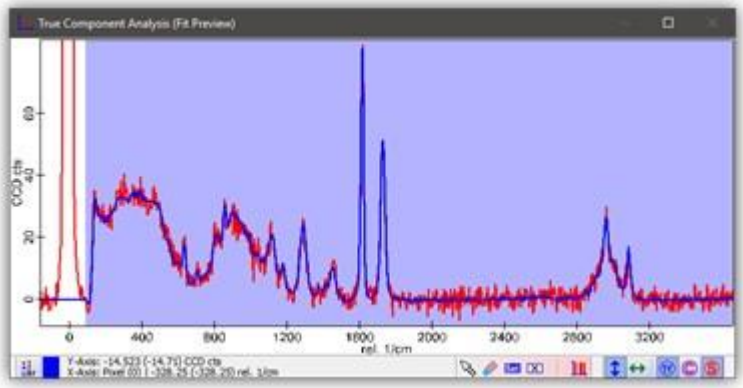
Preview Windows

Residual Image / Intensity Image



This image preview window shows the residual image or one of the component distribution images. In the WITec Project PLUS Version, you can double click into this image to add a selected spectrum as a new component. The residual image will show you the missing components.

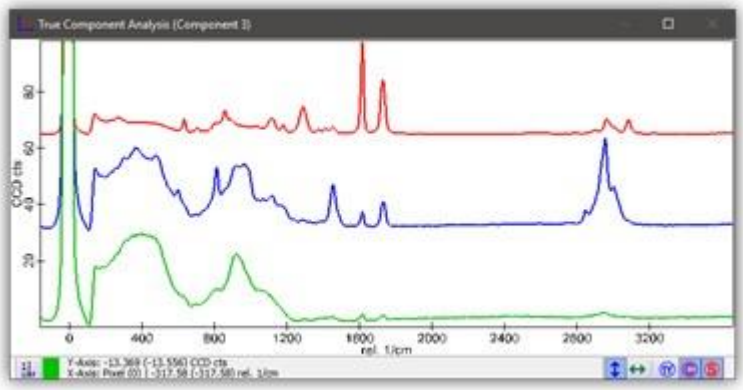
Fit Preview and Mask



The fit graph preview window shows the original spectrum in red and the fit preview in blue. This preview is the linear combination of the all component spectra that fits the original spectrum the best.
Just click somewhere in the preview image to see the selected spectrum and the fit curve using your components.

Use the mask to define which spectral area should be considered for fitting your component spectra combination.

Component Spectra



This graph preview window shows the component spectra which were dropped by the user or added using the dialog.

Advanced Fitting Tool Dialog

Description

With the Advanced Fitting Tool it is possible to do simple as well as more advanced curve fitting. The tool enables peak fitting as well as other curves like exponential or polynomial curves or even a user defined functions.

Input and Results

Input:
One graph object (single spectra or hyper spectral data object).

Results:

- For input single graph objects: a text object containing all fit parameters.
- For input line graph objects: one spectrum for each fit parameter.
- For input image graph object: one image for each fit parameter.

How do I ... ?

Save my fit function parameters to use it again later:

1. Change your function parameters in the Fit Parameters Tab.
2. Open the Fit Function Tab and press "Add" above the Fit Function List. This will add the currently selected function and all its current parameters to the category "User".
3. Select the newly added function in the "User" category. Now you can change its name as desired.

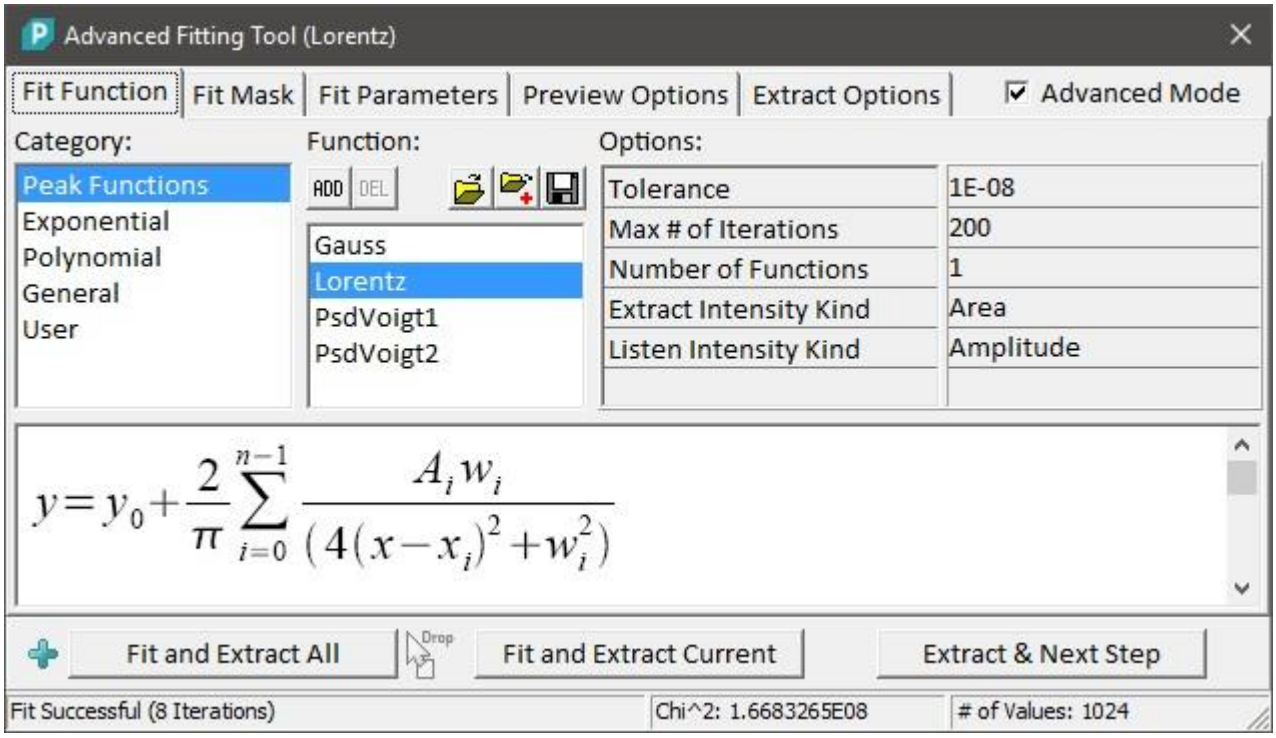
Use my own fitting function formula:

1. Open the Fit Function Tab and select the category "General". Select the fitting function "User Defined".
2. Change the number of fit parameters.
3. You can define each fit parameters' name and unit by setting the parameter number first (Parameter #) and then change the rest of the parameters.

User Interface

- Fit Function Tab
- Fit Mask Tab
- Fit Parameters Tab
- Preview Options Tab
- Extract Options Tab
- Preview Window
- Fit Function Options

Fit Function Tab



Category (List Box):
Select a fit function category here. The "Function" list will be updated upon selecting another category.

Function (List Box):
Select a fit function here.

Add (Tool Button):
Adds the currently selected function and all its current settings and parameters as a user fit function. An additional category "User" will be created where the added functions can be found. All functions added by a user will be saved automatically when closing the dialog ("per user" setting).

Del (Tool Button):
Removes a user defined function from the user function list.

Open (Tool Button):
Opens a WITec User Fit Functions file and replaces the current user fit functions.

Append (Tool Button):
Opens a WITec User Fit Functions file and adds the loaded fit functions to the current user defined fit

functions.

Save (Tool Button):
Saves the current user fit function list into a file.

Options (Parameter List):
Some standard fit algorithm parameters can be changed here (like tolerance and number of iterations). Each fit function may also have its own parameters like extraction kind for some parameters or the number of curves e.g. for multiple peak fitting.
See Fit Function Options.

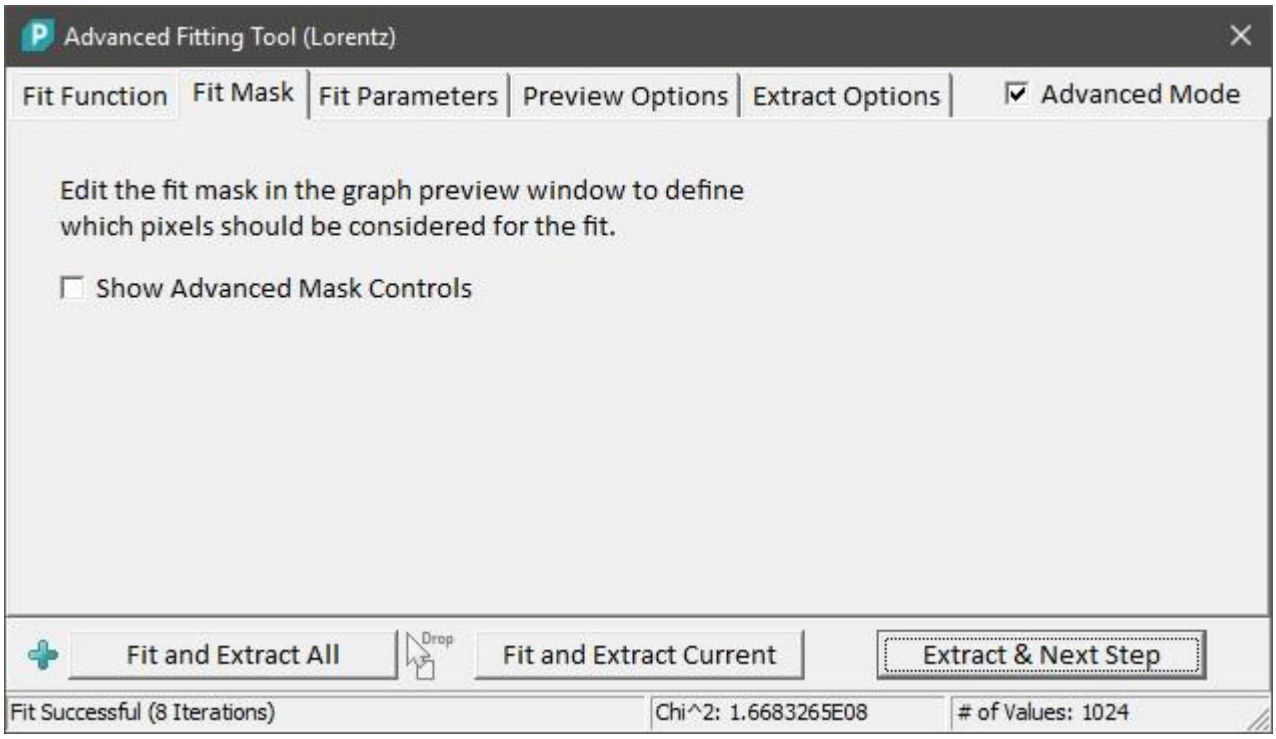
Description (Graphic Box):
Shows the fitting formula and all its parameters. You can move the graphic by clicking and moving it or by using the scroll bars.

Fit and Extract All (Button, Drop Zone):
Starts the fitting calculation for all spectra in the current hyper spectral data set. You can also drop (multiple) data objects from the project manager onto this button in order to do batch processing. This is a WITec Project Plus feature.

Fit and Extract Current (Button):
Starts the fitting calculation for the currently selected spectrum and extracts the results.

Extract & Next Step (Button):
Wizard Feature: you can choose whether you would like to use the result images of this dialog in the image statistics dialog or do some image smoothing:

Fit Mask Tab

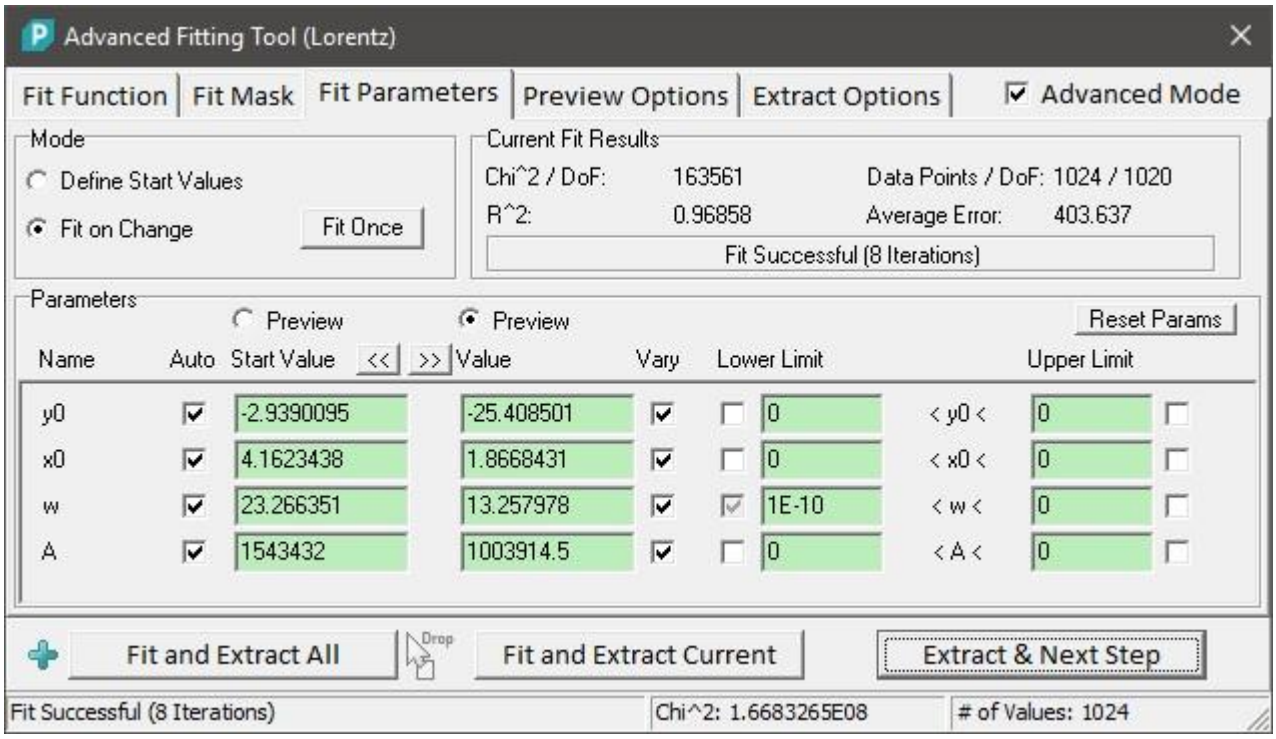


You can change the fit mask in order to define which pixels should be considered for the fitting algorithm.
These edits are optional, you can also change the masks directly in the preview graph viewer.

Advanced Mode

If the advanced mode is turned on, there is access to all fit parameters and preview/extract options.
This is necessary if you e.g. do a multiple peak fit or a user defined fit function in order to define start values for all fit parameters.

Fit Parameters Tab



Mode

Define Start Values (Radio Button):
Select this mode in order to define the start values of the fit. This will show a fit function using the start values as a blue preview curve in the preview graph viewer in order to facilitate the finding of good start values.
Also select this mode if you would like to change any parameter or limit without doing an automatic fit: The values in the "Start Value" column (or "Value" column resp.) are preserved.

Fit on Change (Radio Button):
Select this mode if the fit should automatically be calculated if any parameter or limit is changed, or even if another spectrum is selected by clicking to another position in e.g. an image.
This is the default mode.

Fit Once (Button):
Click this button to execute the fit algorithm once.

Current Fit Results

Indicates whether the last fit was successful or not and shows typical fit result information.

Parameters

Show all the fit function parameters. Note that all green edits can be double-clicked in order enable the listening to a mouse action done in a viewer window.

Reset Params (Button):
Resets all parameters, values, start values, limits and all check boxes to their default values.

Name (Label Column):
Shows the name for each function parameter. Drag over the label with the mouse in order to get a more detailed information.

Auto (Check Box Column):
If checked, the start value for the function parameter will be calculated automatically when doing a fit.
If using multiple peak functions or user defined functions, this feature is not possible.

Start Value (Float Edit Column):
Shows the start values for all parameters.

Preview (Start Value Column Radio Button):
If this Radio Button is checked, the blue graph in the preview graph viewer will show the current fit function using parameters from the start value column (automatically estimated or defined by the user).

Value (Float Edit Column):
Shows all parameters of the current fit result.

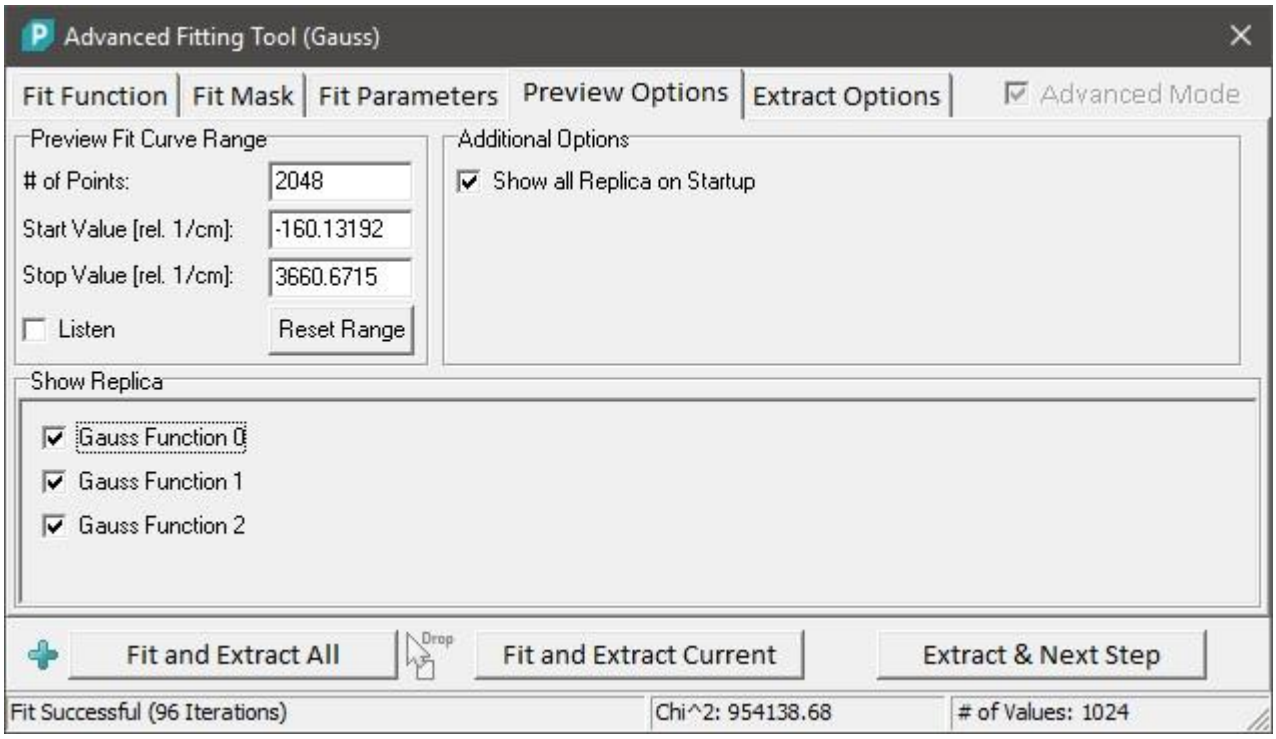
Preview (Value Column Radio Button):
If this Radio Button is checked, the blue graph in the preview graph viewer will show the current fit function using parameters from the value column (typically the current fit result).

Vary (Check Box Column):
If checked, the parameter (value column) is used for fitted and therefore changed by a fit process - Don't vary a parameter if you want it to be static (e.g. a known peak position).

Lower Limit (Check Box and Float Edit Column):
Enables defining a lower limit for each parameter. The limit will only be used if the check box is checked.

Upper Limit (Check Box and Float Edit Column)
Enables defining an upper limit for each parameter. The limit will only be used if the check box is checked.

Preview Options Tab



Preview Fit Curve Range

# of Points (Integer Edit):
Sets the number of supporting points for the fit preview curve (it usually should be around twice the number of spectral pixels of your input graph).

Start/Stop Value (Float Edits):
Set a range that should be shown as a preview fit curve.
With this feature, you can hide "unwanted" areas (e.g. if a curve like polynomial has extreme values).

Listen (Check Box):
It can be listened to a range from graph viewer (using the mark region mouse mode) in order to set the range.

Reset Range (Button):
Resets the range to the complete input spectral range.

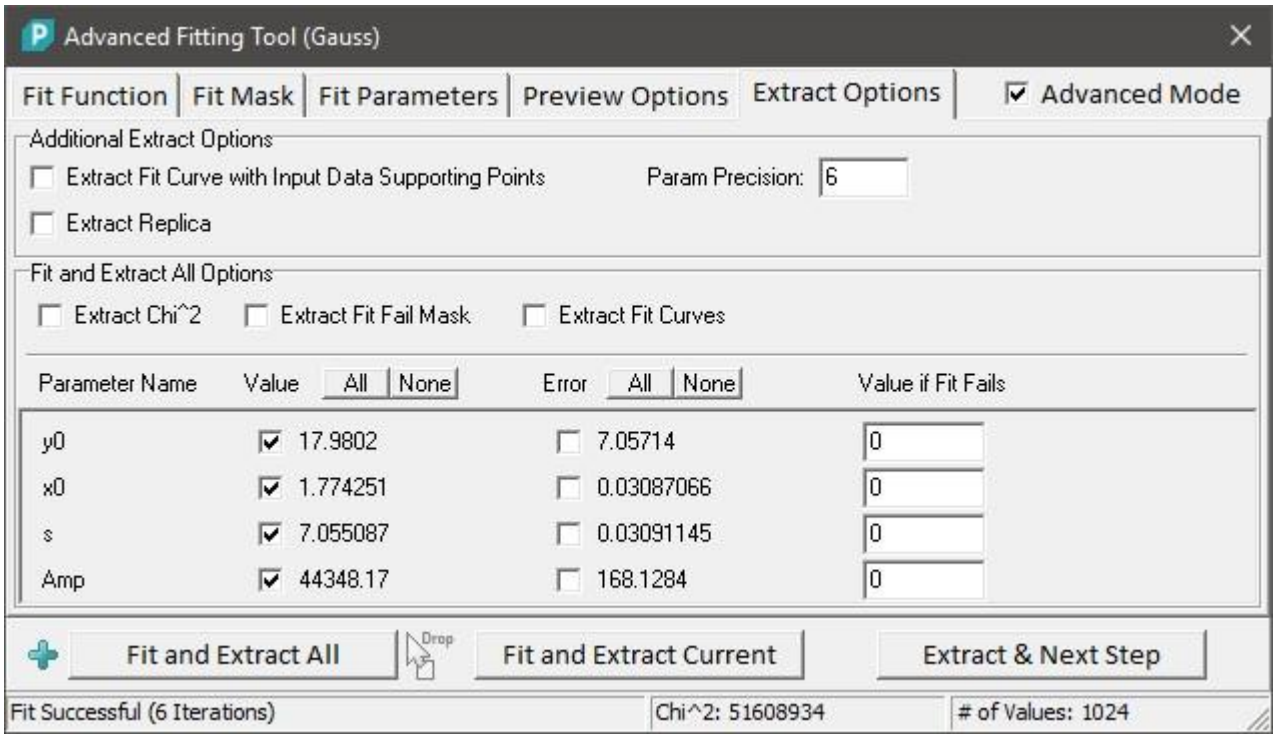
Additional Options

Show all Replica on Startup (Check Box)
If checked, the replica of a multiple peak curve will be shown as green graphs in the preview graph window.

Show Replica (Check Box List)

You can toggle the visibility of each single replica curve of a multiple peak fitting function in the preview graph window.

Extract Options Tab



Additional Extract Options

Extract Fit Curve with Input Data Supporting Points (Check Box):
If checked, the extracted fit curve uses the exact supporting points and the same x-transformation as the input data object. This enables to use the extracted fit curve together with the spectral objects in other drop action dialogs (e.g. Calculator).

Extract Replica (Check Box):
If checked, each replica fit curve is extracted separately when executing the extract action.

Param Precision (Integer Edit):
Defines the precision of floating point numbers in text objects when using the "Fit and Extract Current" Button.

Fit and Extract All Options

Extract Chi^2 (Check Box):
If checked, the Chi^2 (which describes the fit error) is extracted together with the fit results.

Extract Fit Fail Mask (Check Box):
If checked, a fit fail mask is extracted together with the fit results. All pixels that could **not** be fitted (due to an fitting error) are set to 1 in this mask.

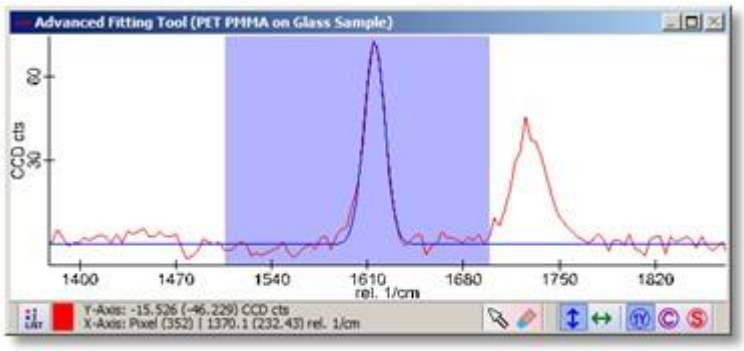
Extract Fit Curves (Check Box):
If checked, the fit curves are extracted together with the fit results.

Value (Check Box Column):
You can select whose function parameter's fit result should be extracted as a result data object.

Error (Check Box Column):
You can select whose function parameter's fit error should be extracted as a result data object.

Value if Fit Fails (Float Edit Column):
Enter a value that should be used as the parameter's fit result if the fit fails.

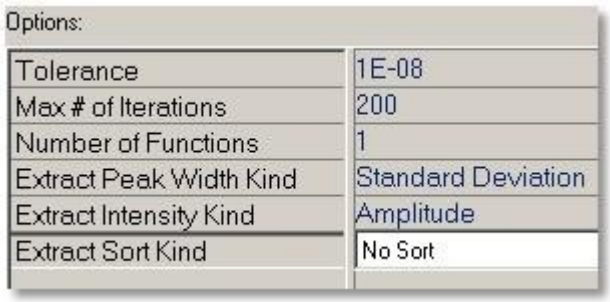
Preview Window



The graph preview window shows the original graph as a red graph and the fit preview as a blue curve.
In Advanced Mode it's possible to show the start value column as a preview curve. In this case, the blue curve shows the fit function using start values.

Fit Function Options

Options for peak fitting



Tolerance:
If this error threshold is reached, the fit algorithm stops.

Max # Number of Iterations:
If the the number of fit iterations specified in this edit is reached the fit algorithm also stops - even if the Tolerance error is not reached yet.

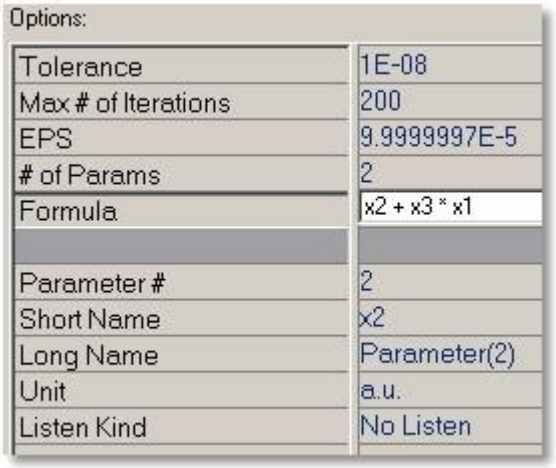
Number of Functions:
Some peak fit functions (Gauss, Lorentz) allow multiple peak fitting; the number of functions can be changed here.

Extract Peak Width Kind:
Some peak functions allow different peak width kinds (like "Standard Deviation" or "FWHM"). Only the extracted results contain these width kinds.

Extract Peak Intensity Kind:
Some peak functions allow the different peak intensity kinds "Area" and "Amplitude". Only the extracted results contain these intensity kinds.

Extract Sort Kind:
The Gauss fit function allows to sort the extracted results when using multiple peak fitting, e.g. by peak center/position. That means that the first result has the lowest peak position, the second result has the second lowest peak position and so on.

Options for user defined fit functions



# of Params:
Defines the number of variables for the fit function.

Formula:
Enter your fit formula here (see Formula Editor):

$$n = Number\ of\ Parameters$$
$$x_1 = x\ (Independent\ Variable)$$
$$x_i = P_{i-1}\ (2 \le i \le n+1)$$

So for example x2 + x3 * x1 is a simple line with x2 being the offset (Parameter Number 2) and x3 being the slope (Parameter Number 3).

Parameter Number:
Enter a parameter index/number in order to define a short/long name, a unit and a listen kind for the parameter.
E.g. parameter number 2 is the first parameter, named x2 in the formula.

Short Name:
The short name for a parameter. Should only be a few characters.

Long Name
A detailed name for a parameter.

Unit:
The parameter unit. You can use the following variables:

- $x represents the X-Axis unit of the input graph.
- $y represents the Y-Axis unit of the input graph.

Listen Kind:

The listen kind of this parameter. E.g. if the parameter represents the peak position, the listen kind "X-Axis" can be used in order to find good start values using the listen mechanism in the Fit Parameters Tab.
The following listen kinds are available:

- X-Axis (listens to an absolute X-Axis position).
- Y-Axis (listens to an absolute Y-Axis position).
- X-Diff (listens to the difference of a clicked and moved cursor position in the X-Axis).
- Y-Diff (listens to the difference of a clicked and moved cursor position in the Y-Axis).
- X-Diff Absolute (same as X-Diff but always positive values).
- Y-Diff Absolute (same as Y-Diff but always positive values).
- Area (listens to the calculated area from the clicked and moved cursor position in the X-Axis).

Cluster Analysis Dialog

Description

The Cluster Analysis Dialog automatically finds similar spectra in an image spectrum data object and creates the corresponding cluster average spectra as well as the cluster distribution maps.

See also
Average Spectrum (Math)
Cluster Analysis K-Means (Math)
Cluster Analysis K-Means (Geometric View)
Creating Average Spectra using Masks

Input and Results

Input:
One hyper spectral data object.

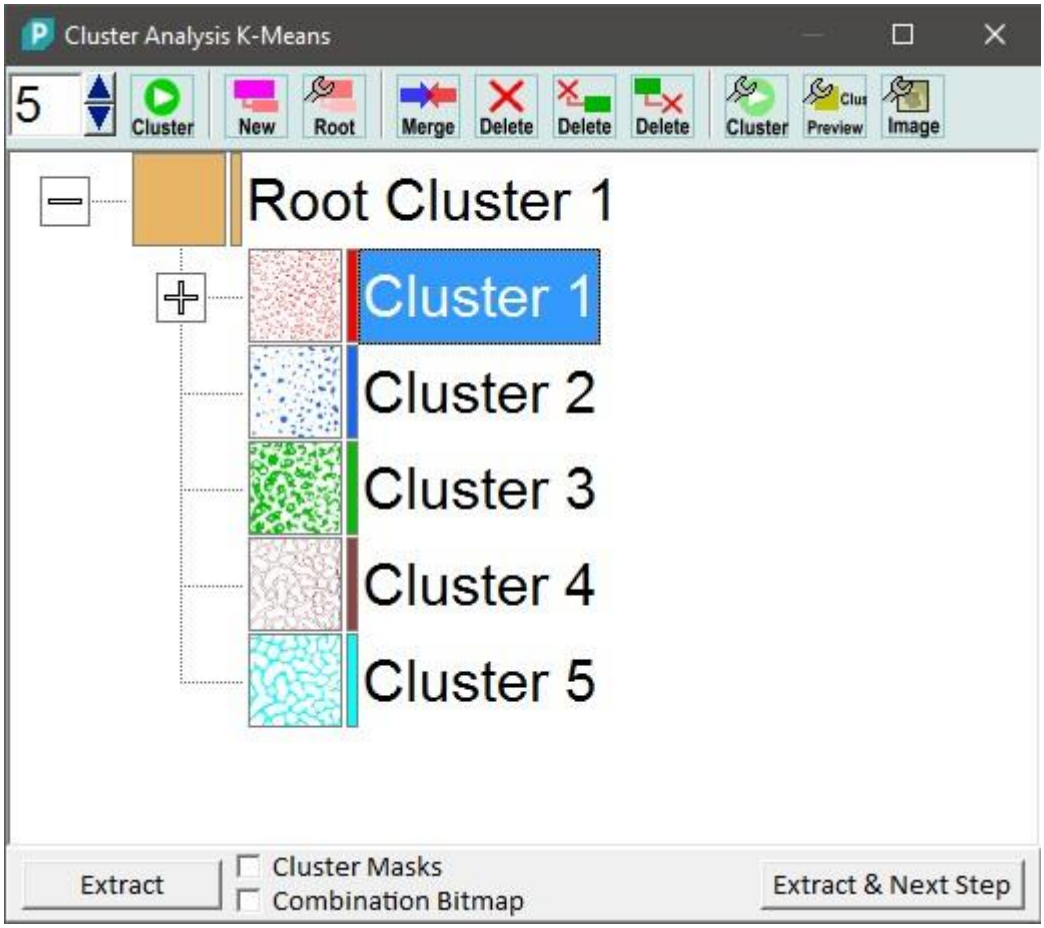
Results:

- Cluster Average Spectra
- Cluster Distribution Masks
  or
  Relative Cluster affiliation
  (when using intensity images, the result image shows the "distance" from each spectrum/pixel to its cluster center)
- Cluster Color Combination Bitmaps

User Interface

- Main Window
- Context Menu
- Root Cluster Options
- Cluster Algorithm Options
- Preview Options
- Intensity Image Options
- Preview Windows

Main Window



Number of Clusters (Integer Edit / Up/Down Buttons):
Sets the number of clusters to be used for the following cluster analysis.

Cluster (Start Cluster Analysis):
Starts the Cluster Analysis using the cluster node that is currently selected.
If no root cluster was created before, a new root cluster is created and clustered automatically (using the current root cluster creation options).

New (Create New Root Cluster):
Creates new root cluster using the current root cluster creation options.

Root (Show Root Cluster Options):
Shows the Root Cluster Options.

Merge (Merge Selected Clusters):
Merges the selected clusters into a new cluster using an OR Operation (i.e. the new merge cluster contains all pixels of all merged clusters).

Delete (Delete Selected Clusters):
Deletes all selected clusters recursively.

Delete (Delete and keep Children):
Deletes the selected cluster and keeps the sub-clusters ("**children**"), moving them to the tree level of their deleted original cluster ("**parent**").

Delete (Delete only all Children):

Deletes all children of the selected cluster while preserving the existence of the selected parent cluster.

Cluster (Show Cluster Algorithm Options):
Shows the Cluster Algorithm Options.

Preview (Show Preview Options):
Shows the Preview Options.

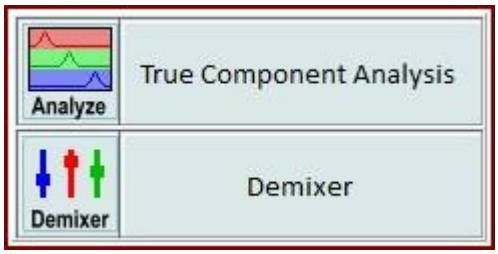
Image (Show Intensity Image Options):
Shows the Intensity Image Options.

Extract (Button):
Extracts the average spectra of all selected clusters.

Cluster Masks (Checkbox):
If checked, the cluster masks / distribution images are additionally extracted upon pressing the extract button.

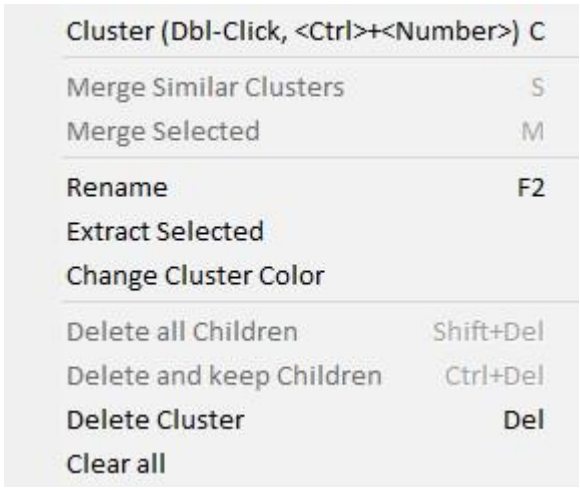
Combination Bitmap (Checkbox):
If checked, the current combination preview bitmap is additionally extracted upon pressing the extract button.

Extract & Next Step (Button):
Wizard feature: you can use the cluster result spectra for the TrueComponent analysis or for demixing:



Context Menu

You can right-click on a cluster result to open the context menu:



Cluster
This will execute the cluster analysis on the currently selected root cluster / cluster result / sub cluster.

Merge Similar Clusters
Merges two sub clusters, that are most similar to each other. Only works on a cluster that has sub cluster results.

Merge Selected
Merges all selected clusters into one bigger cluster and adds the selected clusters as sub clusters.

Rename
Renames the selected cluster. The name is used in extracted data objects.

Extract Selected
Extracts the selected clusters into the project (Average Spectrum + Cluster Distribution Map).

Change Cluster Color
Changes the color for a cluster (Cluster Distribution Map and Average Spectrum).

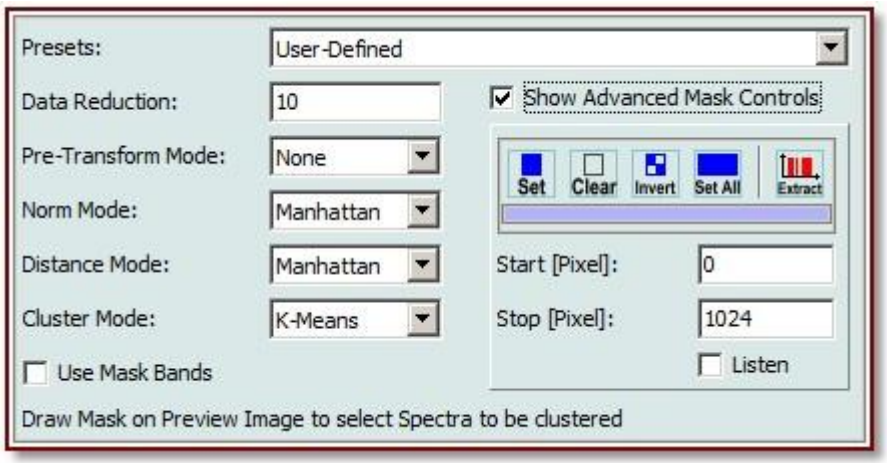
Delete all Children
Deletes all children / sub clusters of the selected parent cluster but keeps the parent cluster.

Delete and keep Children
Deletes the selected cluster and moves the sub clusters one level up.

Delete Cluster
Deletes the selected cluster and all sub clusters.

Clear all
Deletes everything, all root clusters with sub clusters.

Root Cluster Options



Presets (Combo Box):
One of the pre-defined root cluster options can be chosen:

- K-Means: Raman Spectra w/o Background
- K-Means: Raman Spectra with Background
- Fuzzy: Raman Spectra w/o Background

Data Reduction (Integer Edit):
Data Reduction means that the given number of pixels of a spectrum are averaged and used as one property for the cluster analysis algorithm instead of all single pixels.

Pre-Transform Mode (Combo Box):
Transforms the input spectra before using them in the cluster analysis:

- None (No Pre Transformation)
- Derivative
- SG (6, 6, 4, 1) (Uses a Savitzky-Golay smoothed Derivative of the data)

Norm Mode (Combo Box):
Normalizes the input spectra before using them in the cluster analysis:

- None
- Manhattan
- Euclidean

See also Spectrum Normalization (Math).

Distance Mode (Combo Box):
Defines a distance mode for calculating the similarity/distance of spectra:

- Manhattan
- Euclidean

See also Spectrum Normalization (Math).

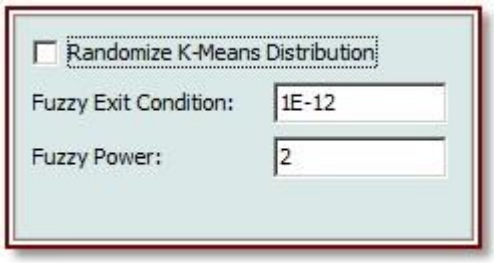
Cluster Mode (Combo Box):
Defines the cluster mode:

- K-Means (Creates Boolean Cluster Maps, Sub-Clustering is allowed, see also Cluster Analysis K-Means (Math).
- Fuzzy (Creates Intensity Cluster Images, Sub-Clustering is not allowed.

Use Mask Bands (Check Box):
If checked each single mask band in the preview window is used as one property for the cluster analysis algorithm (instead of the single pixels in the spectra).

Advanced Mask Controls (Edits and Tool Buttons):
You can change the cluster mask in order to define which pixels should be considered for the cluster analysis algorithm. These edits are optional, you can change the masks directly in the preview graph viewer.

Cluster Algorithm Options



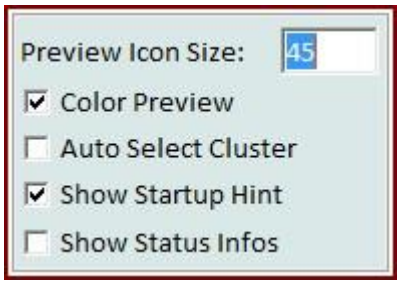
Randomize K-Means Distribution (Check Box):
If checked, the K-Means Start-Iteration begins with a real random cluster distribution.
If not checked, the pixels are sequentially sorted into the clusters (modulo number of clusters: 1, 2, 3, 1, 2, 3, ...). The latter will lead to the same results for the same root cluster options and mask.

Fuzzy Exit Condition (Float Edit):

Defines an exit condition for the iterative fuzzy cluster algorithm.

Fuzzy Power (Float Edit):
Affects the fuzzy cluster algorithm results.

Preview Options



Preview Icon Size (Integer Edit):
Defines the icon size of the cluster tree preview thumbnails.

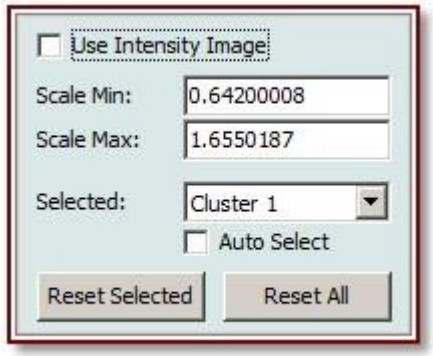
Color Preview (Check Box):
If checked, the cluster tree preview thumbnails use their cluster color.
If not checked, a standard black-yellow color-scheme is used.

Auto Select Cluster (Check Box):
If checked, a cluster node is automatically selected if the mouse cursor clicks and moves in an image viewer.
Note that if you have multiple root clusters, only the sub-clusters or siblings (clusters on the same level) of the currently selected cluster are selected.

Show Startup Hint (Check Box):
If checked, the Cluster Analysis Dialog shows a small startup hint / wizard.

Show Status Infos (Check Box):
If checked, some status infos about the current root cluster / selected cluster are shown in a status bar at the bottom.

Intensity Image Options



Use Intensity Image (Check Box):
If checked, the K-Means Cluster preview images - and also extracted images - don't show boolean cluster distribution maps but intensity images showing the "distance" of the spectra to their cluster average spectra (see "Distance Mode" in Root cluster Options). I.e. the brighter a single pixel in the

intensity image is, the higher is the similarity of the selected spectrum to its average cluster spectrum.
This enables to find structures within the same cluster which could be a hint for another component.

Scale Min/Max (Float Edit):
It's possible to select more than 1 cluster in the cluster tree view, showing them together in the preview image viewer. You can change the intensity scale of each of those selected clusters separately.

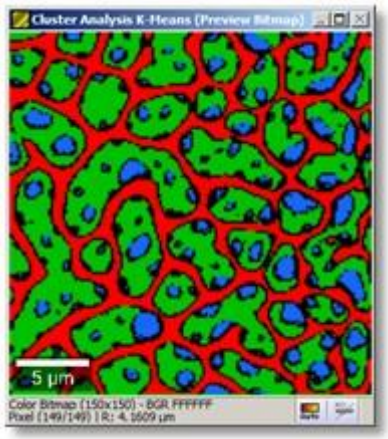
Selected (Combo Box):
The Scale Min/Max Edits act on the selected cluster from this window.

Auto Select (Check Box):
If checked, the selected cluster from this window is changed by clicking in an image.

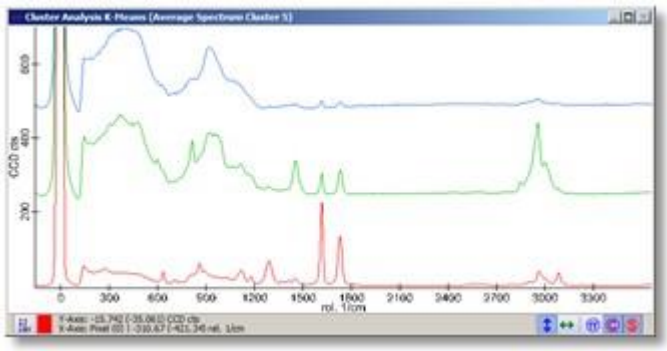
Reset Selected (Button):
Resets the scale of the selected cluster from this window.

Reset All (Button):
Resets all scales of all selected cluster of the cluster tree.

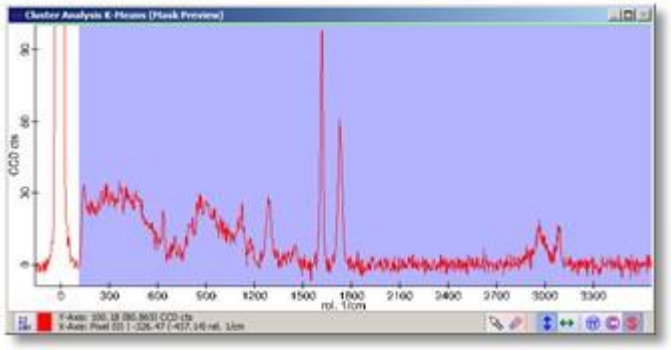
Preview Windows



The preview image viewer shows the selected cluster distribution maps.



The first preview graph viewer shows the average spectra for all selected clusters.



The second preview graph viewer shows the original spectrum and the cluster mask.
Before you start the cluster analysis, you can change the cluster mask here in order to define which part of the spectra (or mask bands) should be used for the cluster analysis.

Filter Viewer

Description

The Filter Viewer creates images from image-graph objects (or graph objects from line-graph objects or single result values from single spectra) using defined filters, e.g. a sum filter.

See also
Creating Images using the Filter Viewer
Filter List
Filter Viewer (Math)

Input and Results

Input:
Use can use any graph object with this dialog.

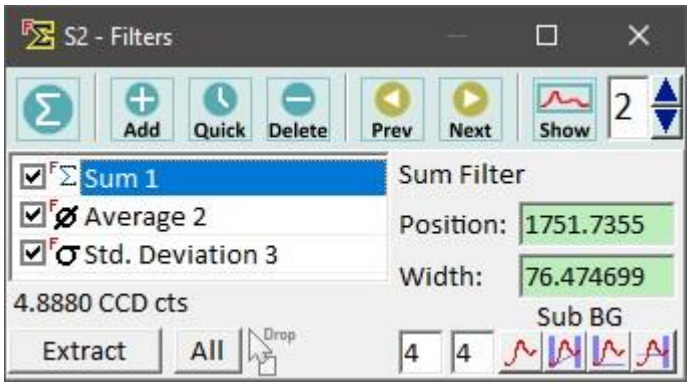
Results:
The result is depending on the dimensionality of the input graph object.
The Filter Viewer creates the following results for each defined filter:

- image objects for image graph input data objects
- single graph objects for line graph input data objects
- text object containing result as text for single graph input data objects,

Special Features

- Some measurements (especially multi-spectral measurements) will create a Filter Viewer upon starting a measurement.
- In contrast to other Drop Actions or Data Analysis Features it is possible to use the Filter Viewer during the measurement.
- The Filter Viewer together with the contained filters is saved with the project. Preview Windows as well as the input graph preview is not saved but can be reopened after loading (see Saving a Project).

User Interface



Create Sum Filter (Button):
This creates a new sum filter.

Create Filters (Button):
You can simply create a new filter by pressing "Create Filters" and selecting one of the available filters.
See Filter List for a short description for each filter and its purpose.

Quick Filters (Button):
If you would like to save your filters to the hard drive and reload them later on, you can use the "Quick Filters" menu.
You can save **selected filters** using the context menu in the white Filter List Box. **All filters** can saved using the quick filters button.
Filter that are saved in the Quick Filter directory are automatically shown in the Quick Filter Menu.
You can open the directory and organize your quick filters using subdirectories which are shown as sub menus in the quick filter menu.

Delete (Button):
Deletes all selected filters.

Previous / Next (Buttons):
Switches to the next or previous possible graph object in the project manager to use it as the new input graph object.
Shortcut: Page-Up or Page-Down in a preview image viewer (showing the results of a filter).

Show Input Graph (Check Button):
Shows or hides the graph viewer window showing the (averaged) input graph.

Spatial Average Size (Integer Edit):
Defines the average filter size of scanned spectra, that will be averaged and shown as a preview in the graph viewer mentioned above.

Extract (Button):
This will create new data objects for the selected filters.

All (Button):
This will create new data objects for all your filters.

Filter List Box:
Shows all filters

- Click on the "Filter Name" column heading to select or deselect all filters.
- Click on the "Show" check boxes to toggle the filter preview (affects all selected filters).
- Double-click on any filter to show the preview only for this filter (shortcut: return key).
- Filter parameter changes will affect all selected filters.
- Click on the "Start" or "Stop" column header to turn on/off the listen mechanism.

"4.8880 CCD Cts." (Label)
Shows the result of the selected filter for single spectrum input graph objects.

Position (Float Edit):
Defines the position of the selected filter(s).
Double-click to listen to a position from any graph viewer window; if listening is enabled the edit is colored red. The listening is disabled again by double-clicking once more.

Width (Float Edit)
Defines the width of the selected filter(s). The filter range will be from <Position - Width/2> to <Position + Width/2>.
Double-click to listen to a position from any graph viewer window; if listening is enabled the edit is colored red. The listening is disabled again by double-clicking once more.

Background Subtraction (Edits and Buttons)
Changes the background subtraction used with the current filter:

- The left and right edit field will define the number of pixels on the left and right of the filter area that is used as an average value for the background subtraction.
- A value of 0 for both edits indicates no background subtraction.
- If both edits are not 0, a slope that is connected from the average of the left side pixels and the average of the right side pixels is subtracted
-  Click on the tool buttons for defining:
  - no Background Subtraction
  - 4 and 4 (both sides, subtracts a slope)
  - 4 and 0 (left side, subtracts a line)
  - 0 and 4 (right side, subtracts a line).

See Filter Viewer (Math)

Drop Zones / Batch Processing

The both following options are possible if the dropped graph has the same dimensionality and x-transformation kind:

- drag & drop a graph object onto the form caption bar to set a new input graph object
- drag & drop one or more graph objects onto the "Extract" or "Extract All" button to do batch processing.

Filter List

Purpose of different Filters of the Filter Viewer

For a mathematical description of all filters, see Filter Viewer (Math)

Sum
Calculates the overall intensity of the selected range.
Is typically used to make structures visible, that are described by a (Raman) peak.
Can also be used to suggest the overall number of electrons/photons.

Average
Can be used e.g. to show the background (if the filter is set to a range where there is no raman signal).

Average minus Minimum
The minimum of the selected range is subtracted from the average.
Can be used e.g. to do some kind of "background subtraction" if a Raman peak is on a fluorescence offset.

Standard Deviation
Shows the changes within a range.
Can be used to determine the amount of noise or to show variations in the spectral range.

Center of Mass (Weighted Position)
Calculates the peak position (roughly). Use peak fitting tools for more accurate peak position fitting.

Peak Width
Calculates the peak width (roughly). Use peak fitting tools for more accurate peak width fitting.

Maximum
Calculates the maximum of the range. Shows the position of the maximum intensity in the result image.
Can also be used e.g. to find a specific cosmic ray that couldn't be removed by the Cosmic Ray Removal tool. For this, calculate the total average of all spectra in an spectral image, then do the maximum filter on the area of the "small cosmic ray peak" that could be visible in this total average spectrum. The result image then could show the position of the cosmic ray.

Raman TV Dialog

Description

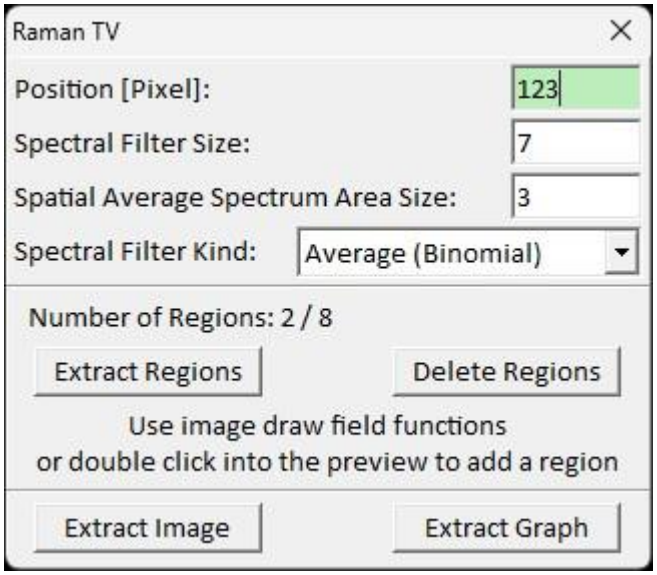
This dialog creates images very quickly from image spectrum data objects using different filters; it ts also possible to see a local average spectrum for a selected image pixel. This combination allows a very easy and fast overview on your complete Raman image measurement.

Input and Results

Input
One image spectrum data object.

Results
An image calculated using a selected spectral position or the local average graph for a selected image position.

User Interface



Position (Integer Edit)
The current spectral pixel position for image creation.

Spectral Filter Size (Integer Edit)
The spectral filter size for image calculation. The total window size is 2*<filter size> + 1.

Spatial Average Spectrum Area Size (Integer Edit)
The spatial window size for the local average graph calculation. The total window size is (2*<filter size> + 1)^2.

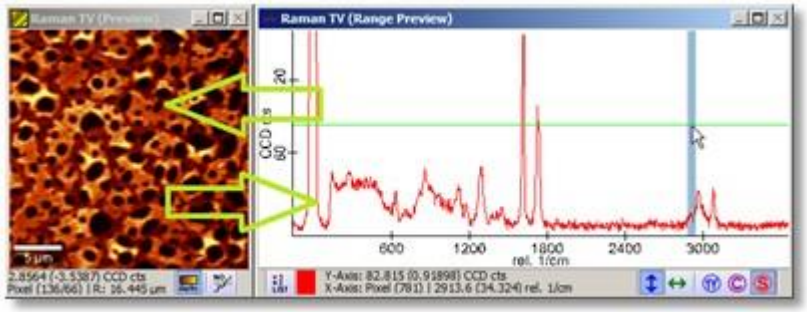
Spectral Filter Kind (Combo Box)
Chose one of those filters:

- Average (Binomial): averages the spectral pixel window using binomial distributed weighting factors.
- Average (Box): averages the spectral pixel window without weighting factors.
- Sum: calculates the sum of the spectral pixel window.
- Standard Deviation: calculates the standard deviation of the spectral pixel window.
- Average - Minimum: subtracts the minimum from the average of the spectral pixel window.

Extract Regions
For each connected region in the draw field of the preview image window, an average spectrum will be created, along with a color bitmap showing the regions and a text object containing region information.
You can add regions by drawing a mask in the draw field of the preview image window or by simply double-clicking into the preview image viewer (A mask with the size defined in <Spatial Average Spectrum area Size> will be drawn around the selected pixel).

Delete Regions
Clears the draw field of the preview image window and removes all region preview graphs.

Preview Windows



The preview image viewer shows the image which is calculated from the current selected spectral position.
The preview graph viewer shows the local average spectrum of the current selected image position.

Graph Demixer Dialog

Description

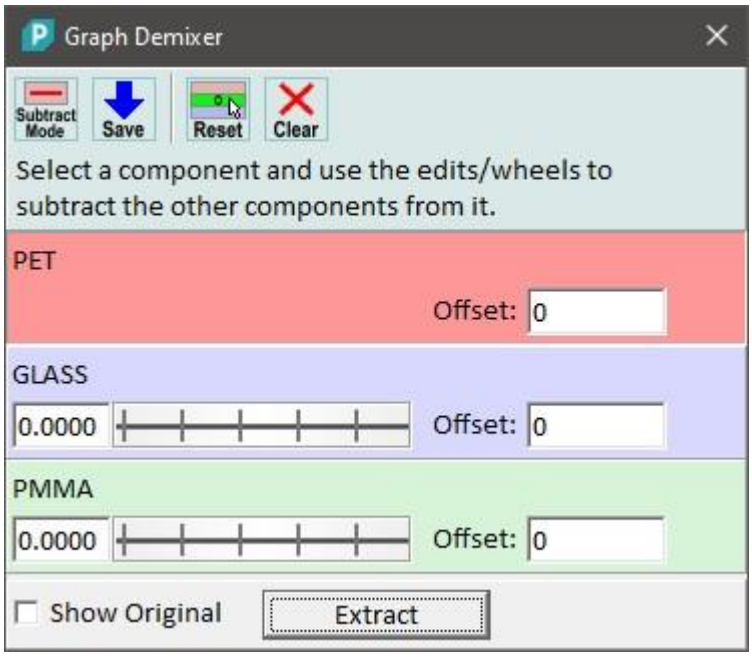
The Graph Demixer Dialog allows to subtract several single spectra from each other in order to create pure component spectra from mixed spectra (very often you have average spectra that are mixed).

Input and Results

Input:
<n> single spectra data objects (at least 2)

Results:
<n> single spectra (demixed)

User Interface



Subtract Mode (Tool Button)
If checked, the selected component can be subtracted from all the other components by changing the weighting factors.
If not checked, the selected component will be changed by subtracting the other components using the weighting factors.

Save (Tool Button)
Saves the current demix in order to subtract a demixed spectrum from the other spectra.

Reset (Tool Button)
Resets the demix of the currently selected component (sets all weighting factors to zero). This will not reset saved demixes.

Clear (Tool Button)
Resets all demixes.

Show Original
If checked, the preview graph viewer window will show the original (dropped) graph objects.

Extract:
Extracts the demixed spectra.

Preview Windows

The preview graph window shows the current mix graphs.
Zooming the baseline makes it easier to find good weighting factors.

Graph Repair Dialog

Description

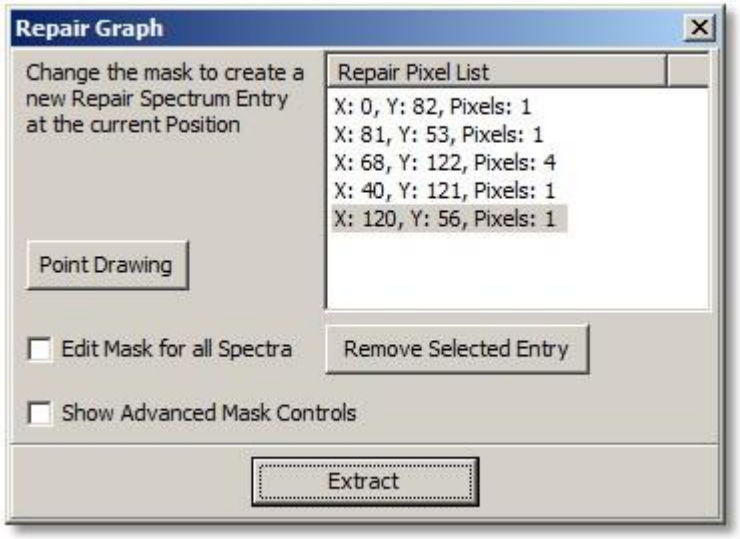
The Graph Repair Dialog can be used to repair a selection of pixels like cosmic rays, hot or dark pixels of the CCD camera or other defects using simple interpolation.

Input and Results

Input:
One graph data object.

Results:
One repaired graph data object.

User Interface



Repair Pixel List (List Box):
If you change the mask for a selected spectrum (select a spectrum by clicking e.g. into an image), this part of the spectrum automatically is added to the repair pixel list, the pixel coordinates as well as the number of pixels that were repaired are listed. Click on any repair pixel to show the r in the preview. Additionally, the repair mask for this pixel will be shown.

Remove Selected Entry (Button):
Deletes the Repair Pixel item that is selected in the Repair Pixel List above.

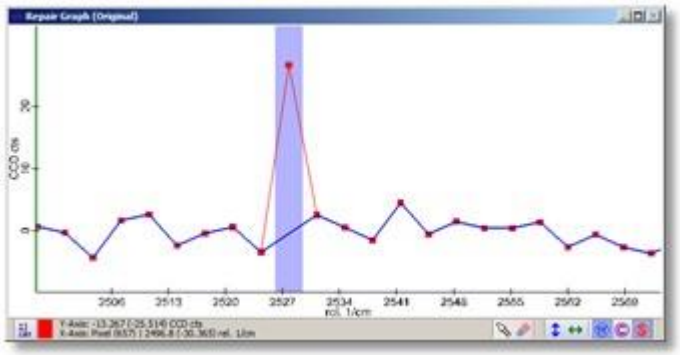
Point Drawing (Button):
Toggles the point drawing of the original graph in the preview graph window.

Edit Mask for all Spectra (Check Box):
If checked, you can define global repair mask which will be applied to all spectra in a hyper spectral data set.
Uncheck if you would like to repair a single pixel again.

Show Advanced Mask Controls (Check Box):
Shows the mask controls for mask export or for changing the mask via listen mechanism.
You can also directly change the repair mask in the preview graph window.

Extract (Button):
Creates a new graph data object containing the repaired / interpolated pixels.

Preview Windows



The preview graph window shows the original spectral data object as a red graph and the preview repaired graph as a blue graph.

Graph Smoothing Dialog

Description

With this dialog it is possible to apply several spectral smoothing filters on spectra or to do a cosmic ray removal, which is the first step of the spectra preprocessing.

Input and Results

Input:
one or multiple Graph Objects (single spectra, line or image Graph Objects).

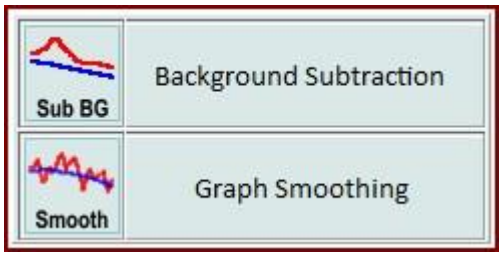
Results:
a smoothed or cosmic ray removed graph Data Object.

User Interface

- Savitzky-Golay Tab
- Median Tab
- Average Tab
- Preview Window

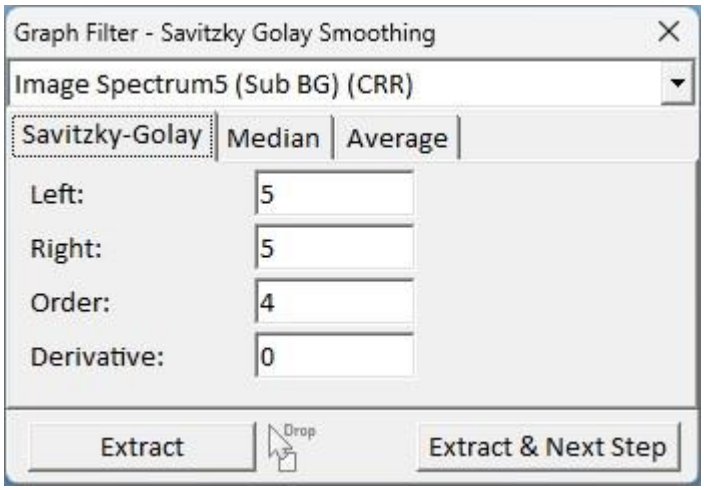
Extract (Button & Drop Zone)
Press the "Extract" button in order to start the currently selected smoothing or cosmic ray removal algorithm on all spectra of the input graph object.
You can also drag and drop multiple graph objects from the Project Manager onto this button in order to do a batch processing using the same algorithm and settings.

Extract & Next Step (Button)
Wizard Feature: you can choose whether you would like to do a background subtraction or further graph smoothing algorithms with the result of the current calculation:



Savitzky-Golay Tab

If the "Savitzky-Golay" Tab is selected, the graph is smoothed using the Savitzky-Golay Filtering algorithm.



Left (Integer Edit):
defines the window size of the smoothing algorithm (left from the current pixel)
The total window size is Left + Right + 1.

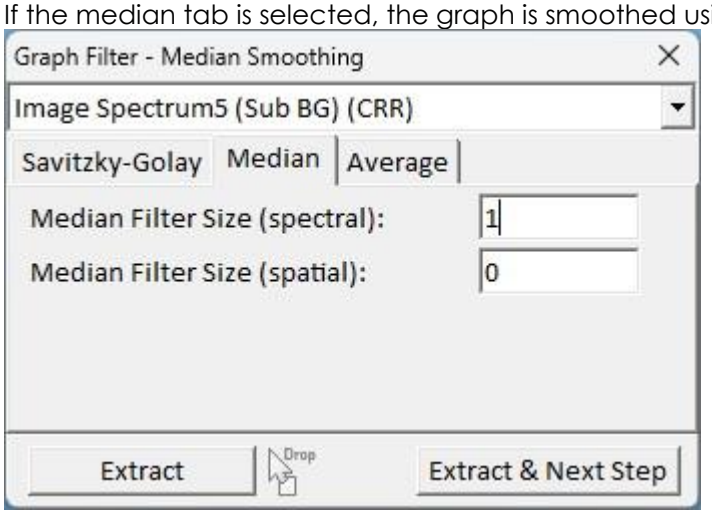
Right (Integer Edit):
defines the window size of the smoothing algorithm (right from the current pixel)
The total window size is Left + Right + 1.

Order (Integer Edit):
the order of the polynomial which is fitted to the smoothing window.

Derivative (Integer Edit):
defines the order of derivative of the filter. A value of 0 means no derivation, i.e. "only" a smoothing is done.
If a value of 1 is entered the calculated result will be the first derivative; the yielded data can be used e.g. not to be being sensitive to fluorescence when doing a basis analysis.

Median Tab

If the median tab is selected, the graph is smoothed using a median filter algorithm.



Median Filter Size (spectral):
the spectral filter size for the median filter. The total spectral window size is 2*<Filter Size> + 1.

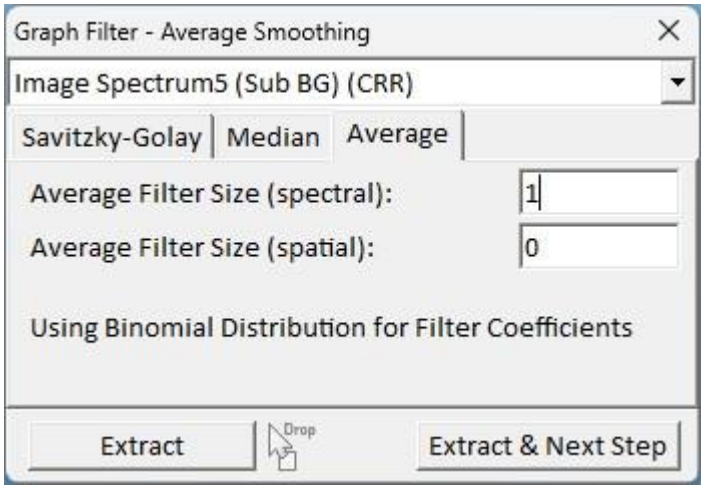
Median Filter Size (spatial):

the spatial filter size for the median filter. If larger than zero, the spectra of neighbored scan pixels are used for smoothing.

The total window size is <Filter Size * 2 + 1>^2 multiplied by the total spectral window size.

Average Tab

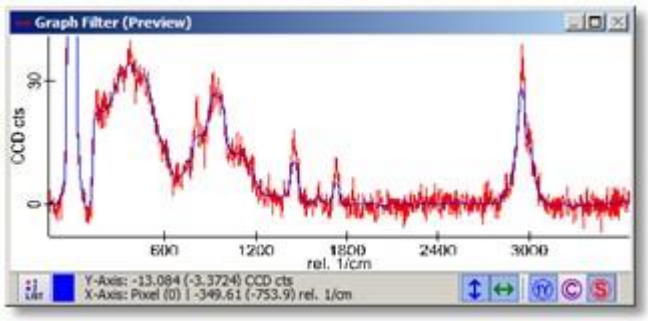
If the average tab is selected, the graph is smoothed using a average filter algorithm that uses binomial distributed filter coefficients.



Average Filter Size (spectral):
the spectral filter size for the average filter. The total spectral window size is 2*<Filter Size> + 1.

Average Filter Size (spatial):
the spatial filter size for the average filter. If larger than zero, the spectra of neighbored scan pixels are used for smoothing.
The total window size is <Filter Size * 2 + 1>^2 multiplied by the total spectral window size.

Preview Window



The preview graph viewer window shows the original graph object as a red graph and the result preview as a blue graph.

Average Spectrum

Description

Average Spectrum calculates the average spectrum of a multidimensional graph data object

(optionally using weighting factors).
To average a couple of single spectra data objects the Calculator Dialog has to be used.

See also
Creating Average Spectra using Masks
Average Spectrum (Math)

Input and Results

Input:

- You can use any kind of multidimensional graph object with this dialog.
  Additionally, you can **combine** this graph **with one or multiple images** that have a corresponding size. The additional objects can be used to define weighting factors for each spectrum that shall be averaged. Thus you can use Image Masks to average only those pixels that are set in the mask (weight = 1.0 or 0.0).
- You can use any number of single spectrum data objects to create the average of all those spectra.
- You can use multiple multidimensional graph objects for batch processing.

Results:
Depending on the number <n> of additional weighting factor objects, the result data object is one or <n> average graph objects.
If no additional weighting factor object is used, only one single graph is created: the total average of all spectra in the dropped data object.

Advanced Graph Average Dialog

Description

With this dialog image masks can be created using thresholds and calculate an average spectrum for the resulting mask in real time.

See also
Creating Average Spectra using Masks
Average Spectrum (Math)

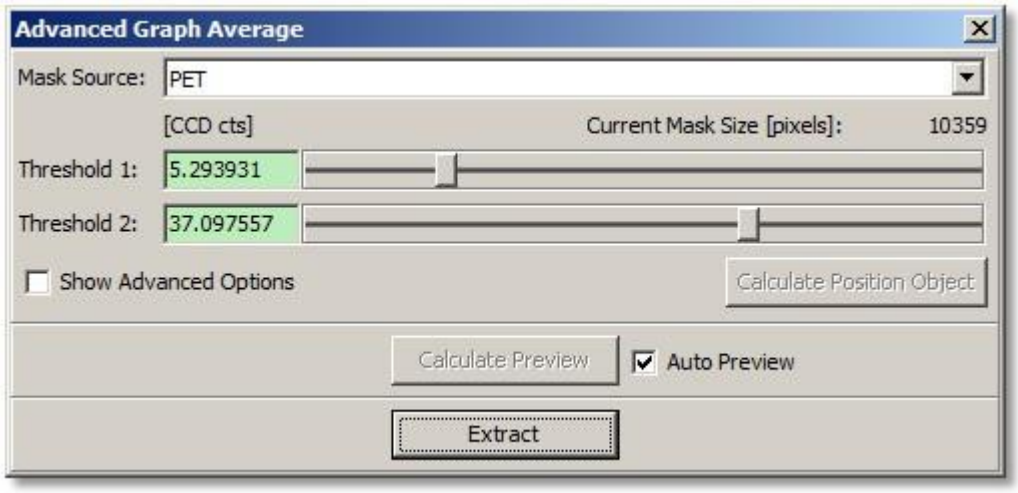
Input and Results

Input:

- Image Graph and optionally corresponding images (of no images are used, an image can be created using a sum filter mask) OR
- Line Graph (e.g. from a line scan) and a corresponding single spectrum (which is e.g. the result of the Filter of the line graph)

Results:
One average spectrum for each mask object.

User Interface



Mask Source (Combo Box):
you can toggle to each of the dropped images in order to use it to create a mask using thresholds.

Threshold 1/2 (Float Edits and Sliders):
these are the two thresholds that will define the image mask. An image pixel is marked, if the pixel value is within the range of the two thresholds.

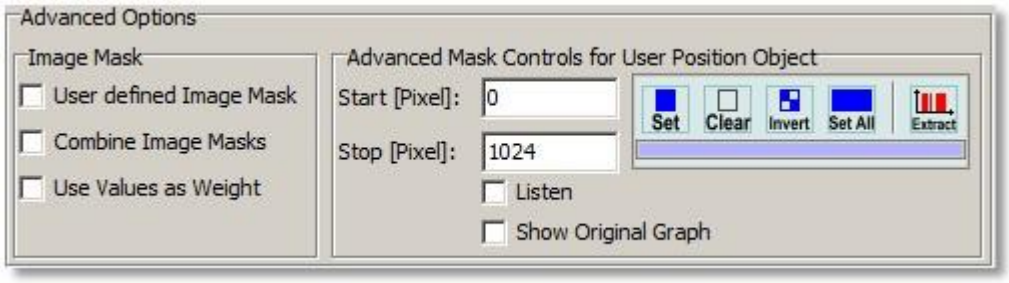
Calculate Position Object (Button):
calculates a new image object using the sum of all pixels in the graph preview mask. This image can be used to define a mask using thresholds. The button is only enabled if the "Mask Source" is set to "User Position Object".

Calculate Preview (Button):
calculates the preview average spectrum once if "Auto Preview" is enabled.

Auto Preview (Check Box):
turns on or off the automatic preview average spectrum calculation.
For very large data sets, this is turned off automatically and you have to press the "Calculate Preview" Button in order to see a preview average spectrum.

Extract (Button):
extracts the average spectra, one for each mask/additional image.

Show Advanced Options (Check Box):
shows advanced options:



User Defined Image Mask (Check Box):
if checked, the Threshold Edits/Sliders are disabled. Will be automatically checked, if the user changes the image mask in the image viewer preview window with the image viewer draw tools.

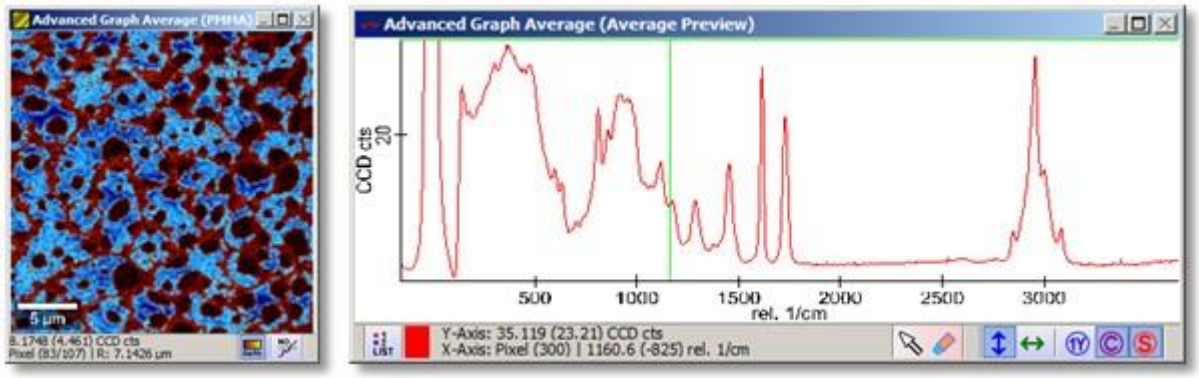
This behavior will prevent the user from accidentally destroying the user defined mask.
If the check box is unchecked by the user, the Thresholds are used in order to overwrite the current mask.

Combine Image Masks (Check Box):
if checked, all masks from all additional images will be combined using an AND-operation.

Use Values as Weight (Check Box):
if checked, the image mask will be ignored; instead, the image values itself define weighting factors for each spectrum in the averaging process.

Advanced Mask Controls for User Position Object:
These edits are optional.
You can define a mask in the graph preview window in order to create a user position object (sum image).
This is interesting if you don't have any images and dropped only a graph object onto this dialog.
Check the "Show Original Graph" Check Box in order to see a valid spectrum for defining the mask.

Preview Windows



The Image Viewer shows the current selected image and its mask.
The Graph Viewer shows the average spectrum preview calculated with the current image mask.
It is also possible to define a spectral mask in the graph viewer in order to create a user defined position object (e.g. sum image) for mask creation.

Calculator Dialog

Description

The calculator can be used to convert or combine values of image or graph objects by typing a custom formula.
Each single floating point value of an image or a (multidimensional) graph object is converted using this formula.

See also
Creating Image Masks using the Calculator
Formula Editor

Input and Results

Input:
You can either use a single graph or image object with this dialog or combine multiple input data

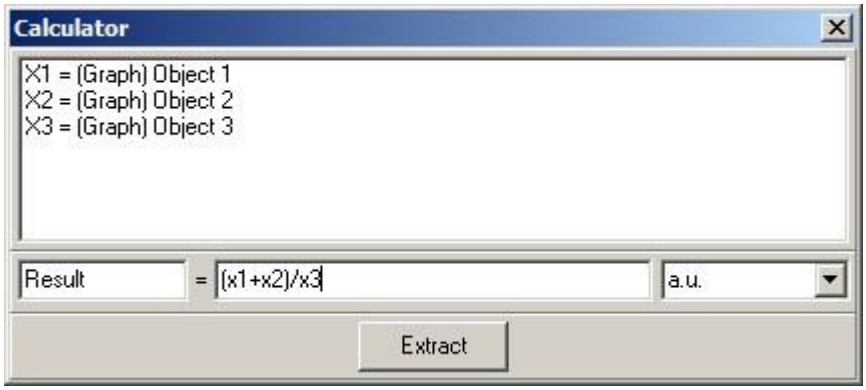
objects to create a new data object.

The following combinations are allowed:

- Multiple Image (and Image Graph data) objects that have the same size and space transformation
- Multiple Graph data objects that have the same spectral size and x transformation
- Multiple Line Graph objects and optionally corresponding single graph objects that

Results:
Depending on the dimensionality of the input data, the result data object is an image, a single graph, a line graph or an image graph. If graph objects with a different dimensionality are dropped, the result will be a graph with the dimensionality of the largest-dimensioned input graph.

User Interface



Object List View:
This list shows the input data objects. Each object defines a variable (x1, x2, ...). These variables can be used in the formula.

Result (String Edit):
Enter the caption/name for the new data object (the formula string will be appended to this caption).

"(x1+x2)/x3" (String Edit):
The desired formula can be entered here (see Formula Editor for possible operators, functions and examples).

"a.u." (String Edit + Pre-Selection):
Enter the desired unit of the result or select one of the predefined names (a.u. = arbitrary unit).

Extract (Button):
Pressing that button will start the calculation / conversion.

Preview Windows

If there are only image data objects used, there is a preview Image Viewer window showing the result image.

If there is at least one graph object in the input data list, there is a preview Graph Viewer showing the result graph.
In case of a multidimensional input graph (e.g. an Image Graph), the Dialog listens to the respective cursor and shows the corresponding preview graph.

Formula Editor

The Formula Editor (formula parser) converts an algebraic expression (text) into a stack of operations which can be understood by the computer. This formula parser can process several mathematical operators and functions (see below).

The order of execution depends on the priority of the operator. Although the formula parser uses floating point numbers, some operators need boolean or integer values to operate. In this case, the float value is converted prior to operation. A float to integer conversion truncates the decimal places.

A float to boolean conversion is given by:

$$\text{Boolean}(x) = \begin{cases} \text{true} & \text{for} \quad x <> 0 \\ \text{false} & \text{for} \quad x = 0 \end{cases}$$

The result is converted back into a floating point number. A boolean value of true is converted to 1.0, a boolean value of false is converted to 0.0.

All functions have at least 10 significant digits after the decimal point other than the besselj1() and the airysqr() function, which deal with only 7 significant digits after the decimal point.

Depending on the usage, the formula parser accepts a fixed number N of input variables. These variables are addressed by X1, X2 ... Xn.

Example Formulas

x1+500	Result is the original value plus 500
x1>250	Result is 1, if the original value is larger than 250; result is 0 if it is smaller
x1+x2	Result is the sum of x1 and x2
x1*(x2>250)	Result is the original value of x1, if x2 is larger than 250, otherwise zero

Operators

Priority	Operator	Description
1	|	Or (Boolean)
2	&	And (Boolean)
3	!	Not Equal (Boolean)
4	=	Equal (Boolean)

5	>	Larger (Boolean)
6	<	Smaller (Boolean)
7	-	Minus (Float)
7	+	Plus (Float)
8	%	Modulo (Integer)
9	*	Times (Float)
9	/	Over (Float)
10	- (algebraic sign)	Change Sign (Float)
11	^	Power (Float)
12	( )	Bracket (Float)

Functions

Function	Description
sin()	Sine (Radian)
asin()	Arc Sine (Radian)
cos()	Cosine (Radian)
acos()	Arc Cosine (Radian)
tan()	Tangent (Radian)
atan()	Arc Tangent (Radian)
cotan()	Cotangent (Radian)
sinh()	Hyperbolic Sine
asinh()	Hyperbolic Arc Sine
cosh()	Hyperbolic Cosine
acosh()	Hyperbolic Arc Cosine
tanh()	Hyperbolic Tangent
atanh()	Hyperbolic Arc Tangent
loge()	Natural Logarithm
log10()	Logarithm of Base 10
log2()	Logarithm of Base 2
exp()	Exponential
abs()	Absolute
sqrt()	Square Root
sinc()	sin(x)/x (Radian)
sincsqr()	(sin(x)/x) ^ 2
heavyside()	0 for x < 0 | 0.5 for x = 0 | 1 for x > 0
sign()	-1 for x < 0 | 0 for x = 0 | 1 for x > 0
besselj1()	J1(x) bessel function of first kind
airysqr()	((2J1(x)) / x) ^ 2
pi()	Mathematical constant PI (use "pi(0)")

Used by:

- Calculator Dialog
- Advanced Fitting Tool Dialog

Data Cropping and Reduction Dialog

Description

With the Data Cropping and Reduction dialog it is possible to cut away parts of the data and to reduce the number of pixels / the resolution.

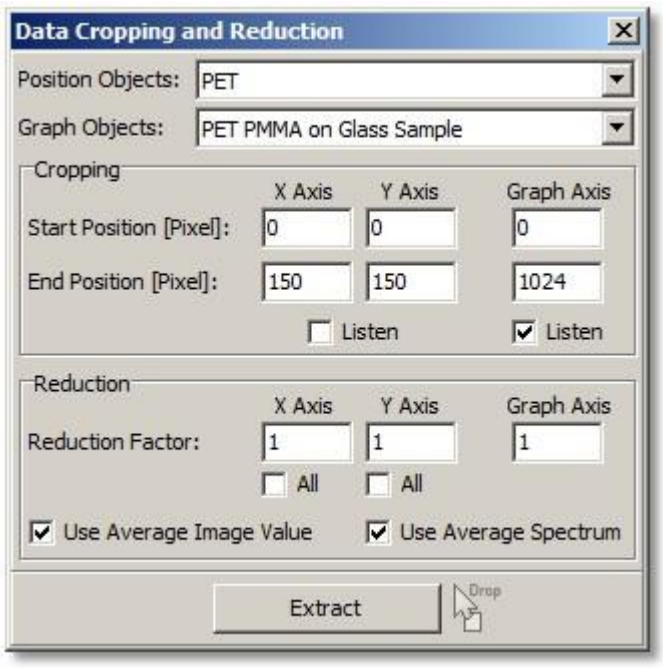
Input and Results

Input:

- one image spectrum data object and any number of belonging images or
- one line spectrum data object and any number of belonging graph objects or
- one or a number of images that have the same spatial transformation or
- one or a number of single spectrum data objects that were created from the same Raman image scan, e.g. average spectra

Results:
Cropped and/or reduced data objects.

User Interface



Position Objects (Combo Box):
Shows the input data objects that either

- define a spatial position for images/image spectrum objects or

- define other units like a time line for time series.

The selected object is shown in the preview windows.

Graph Objects (Combo Box):
Shows input data objects like an image spectrum, line spectrum or single spectrum object (if it's not defining a space or other position). Is used to switch the preview if multiple objects are dropped.

Cropping

Start/Stop Position X/Y Axis (Integer Edits):
Can be used to crop images or image spectrum objects.

Start/Stop Position X/Y Axis Listen (Check Box)
If checked, a rectangle in an image viewer can be simply marked in order to crop image or image spectrum data objects.

Start/Stop Position Graph Axis (Integer Edits):
Can be used to crop a part of the spectral axis.

Start/Stop Position Graph Axis Listen (Check Box):
If checked an area in an graph viewer can be simply marked in order to crop spectral objects.

Reduction

Reduction Factor X/Y Axis (Integer Edits):
Can be used to change the number of pixels respectively the resolution of images or image spectra data objects.
Example:
A factor of 1 means no reduction. A factor of 2 means the result contains only half the number of pixels by using every second pixel of the original data in the result. A factor of 3 means the result contains only a third of all original pixels using every third pixel of the original data in the result.
If the "Use Average Image Value" (for the spatial axes) or "Use Average Spectrum" (for the spectral axis) check box is selected, the data is reduced using the average value/spectra of 2, 3, 4, .. pixels, see below.

Reduction Factor Graph Axis (Integer Edit):
Can be used to change the number of pixels / resolution of spectral data objects.

Reduction X Axis All (Check Box):
Does a data reduction for the complete X Axis. The result is a line graph object (vertical line).

Reduction Y Axis All (Check Box):
Does a data reduction for the complete Y Axis. The result is a line graph object (horizontal line).

Use Average Image Value (Check Box):
If checked, the data reduction is done using the average of all reduced pixels. E.g. if reduction factor for one axis is 2, the result pixel is the average of 2 neighbored original pixels.
If not checked, the reduced original pixels are just skipped and not used in the result.

Use Average Spectrum (Check Box):
If checked, the result spectrum for reduced image spectrum data objects is an average spectrum calculated from the reduced neighbor image pixels. E.g. if reduction factor for one axis is 2, the result spectrum is the average of two spectra from two neighbored pixels.

If not checked, only a single spectrum is used and reduced original spectra are just skipped and not used in the result.

Extract (Button and Drop Zone):
Calculates and extracts the cropped/reduced data. You can do batch processing by dropping a number of data objects on this button.
Note that batch processing only works, if the dropped data objects have the same dimensionality as the data objects in the dialog. Graph batch processing only works if only one graph object is currently in the dialog.

Preview Windows

Depending on the input data objects, there are preview image and/or preview graph viewers. One window always shows the original size and one window shows the preview which is cropped or reduced.

For example if you drop

- only spectral graph data objects you will see two graph viewers (original and preview of spectral / graph axis)
- at least one image or bitmap object you will also see two image viewers (original and preview of spatial axes)
- a hyper spectral line graph (e.g. time series) and a belonging single graph (e.g. time line) you will see 4 graph viewers (original and preview of spectral axis, original and preview of time line axis).

Graph and Image Data Stitching Dialog

Description

The Graph and Image Data Stitching Dialog allows showing multiple images side by side / as tiles in one bigger image.
It is also possible to stitch multiple spectral image data objects into one bigger graph data object. That can be useful if e.g. 3D data consisting of several stacked image scans shall be analyzed using multi-variate algorithms like Cluster Analysis.

Input and Results

Input:

- any number of image data objects or
- any number of bitmap data objects or
- any number of image spectrum data objects.

All objects must have the same pixel size.

Results:
one bigger/stitched image or image spectrum data object.

User Interface

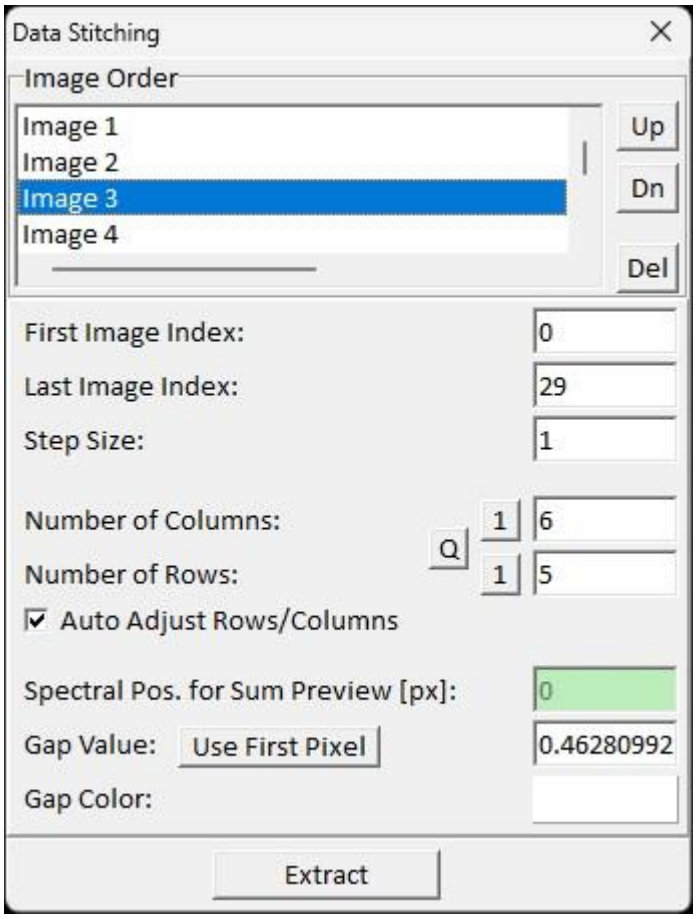


Image Order (Listbox)
Lets you change the image order by simply selecting an image, then pressing "Up" or "Dn".
You can also remove an image from the list by pressing "Del".

First Image Index (Integer Edit):
Define the first image here.
If doing depth stacks it's often the case that the first and last images don't show Raman signal, so you can hide them in the stitching image.

Last Image Index (Integer Edit):
Define the last image here.

Step Size (Integer Edit):
You can skip images with the step size; e.g. if you want to show larger differences in a high-resolution depth stack.

Number of Columns (Integer Edit):
Defines the number of columns for the stitching image.

Number of Rows (Integer Edit):
Defines the number of rows for the stitching image.

Spectral Pos. for Sum Preview (Float Edit):
When stitching image spectrum data objects the preview shows a sum image of a certain spectral position.
Change the position or double-click to turn on the listen mode in order to see another preview image.

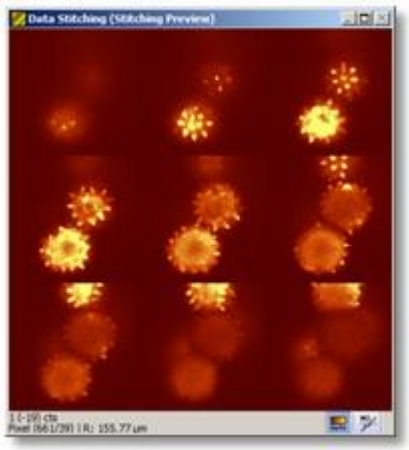
Gap Value (Integer Edit):
If the number of images doesn't fit the number of tiles (rows * columns), there is an empty gap. The gap value will be used in those empty areas.

Use First Pixel (Button):
If you have image gaps it can be useful to have a gap value which is similar to the image data. This button uses the first image pixel value as a gap value.
If a gap value is chosen that is not optimal there can be problems with the image viewer auto scale and it might be difficult to see enough details in the stitched image.

Grap Color (Color Picker):
If color bitmap objects are used for data stitching, then it's possible to define a gap color. Just click on the canvas and define a color in the color picker dialog.

Extract (Button):
Creates the new stitching data object and adds it to the current project.

Preview Windows



The preview image viewer shows the stitched image - possibly with smaller resolution than the real result (in order to have a better preview performance).
If image spectrum data objects are stitched, the preview image viewer shows a sum image for the selected spectral position.

Non Negative Matrix Factorization Dialog

Description

The Non-Negative Matrix Factorization is a multivariate analysis which creates intensity distribution images and their belonging spectra at the same time. For this it uses the fact that both images and spectra must have positive values in order to be a meaningful result. The calculation is an iterative process which consists of three parts:

1. Optimization of the distribution images
2. Optimization of the basis spectra
3. Optimization of the offset

The first iteration starts from random. This dialog is part of the WITec Project Plus package.

See also
Non Negative Matrix Factorization (Math)

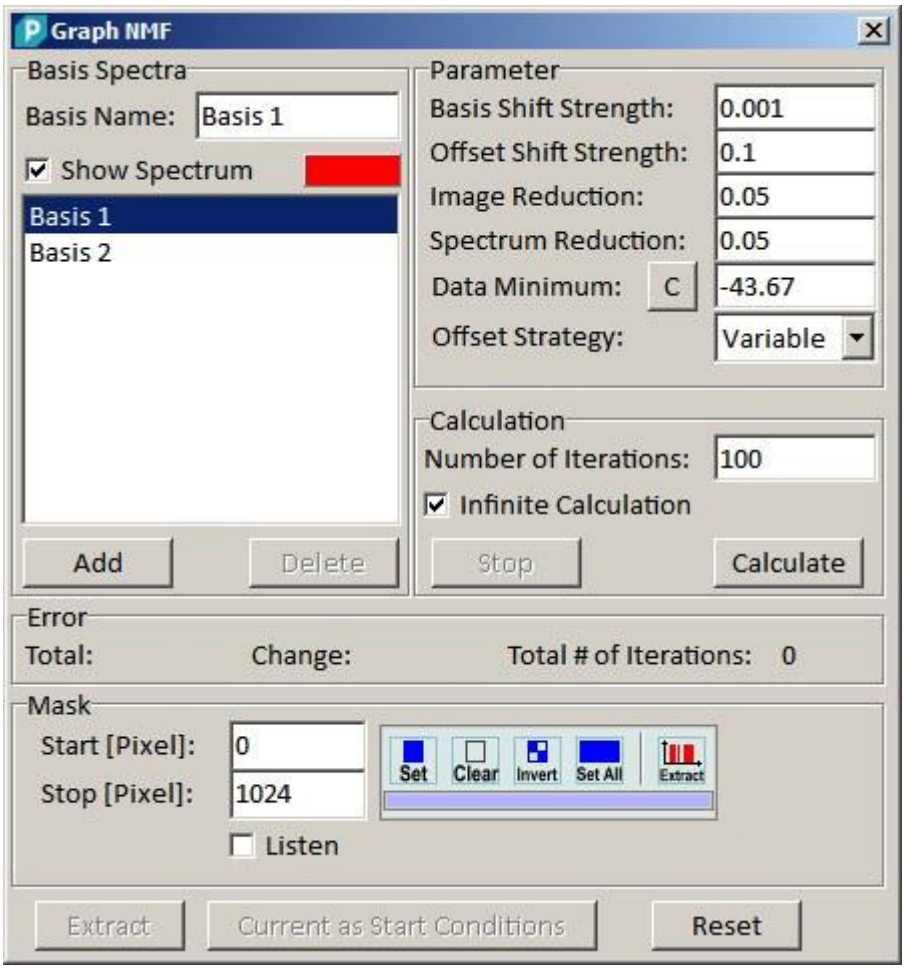
Input and Results

Input:
One spectral Raman image data set

Results:
One image and spectrum for each component

User Interface

- Basis Spectra Group
- Parameter Group
- Calculation Group
- Error Group
- Mask Group
- Buttons



Basis Spectra Group

In this group the number of basis spectra that are needed to describe the dataset can be defined.

Add (Button)
Adds a new basis spectrum to the describing model.

Delete (Button)
Deletes the selected basis spectrum from the model.
The minimum number of basis spectra is two.

List Box
The list box shows the names of all basis spectra. To switch between the different components, click on one entry of the list box.
The belonging information (Name, Show Spectrum and Graph Color) will change and in addition the image preview window will show the distribution map.

Basis Name (Edit Box)
The name of the selected component will be shown in the edit box. Before extracting the data you can change the name of the component. The name will be used as caption for the data object.

Show Spectrum (Check Box)
Unchecking the check box will exclude the basis spectrum from the spectrum preview

Color Box
Here you can define the color for the spectrum.

Parameter Group

The parameters in this group will change the behavior of the algorithm.

Basis Shift Strength
Many solutions with only positive values are possible. In order to get unique and meaningful results it is necessary to push the basis spectra towards the data.
This value is the area which is added to the normalized basis spectrum before an iteration starts.

Offset Shift Strength
Many solutions with only positive values are possible. In order to get unique and meaningful results it is necessary to push the basis spectra towards the data.
This value is an offset which is added to the offset spectrum before an iteration starts.

Image Reduction
Applying the algorithm on hyper spectral datasets is very time-consuming. Besides the image mask the number of pixel which will be used for calculation can be reduced by this factor.
Pixel from the mask are randomly added until all distributions have at least this portion of their total intensity.
At each iteration the selected pixels will change. Set this parameter close to 1, if all basis spectra are found.

Spectrum Reduction
Applying the algorithm on hyper spectral datasets is very time-consuming. Besides the spectral mask the number of spectral pixels which will be used for calculation can be reduced by this factor.
Pixels from the spectral mask are randomly added until all basis spectra have at least this portion of their total intensity.
At each iteration the selected pixels will change. Set this parameter close to 1, if all basis spectra are found.

Data Minimum
For the calculation all values of the dataset must be positive. Therefore an offset is added to the data before calculation. After starting the dialog the absolute minimum value of the data set is searched and copied to the edit box. Due to some preprocessing it can happen that the minimum value is too low (e.g. the background subtraction step did a miscalculation). In this case adapt this value slightly below the noise of the baseline.

Offset Strategy
Two different strategy for offset calculations can be chosen.

- choose "Const" if the offset is a horizontal flat line.
- choose "Variable" if one component always has the same intensity (e.g. Raman spectrum of the substrate)

Calculation Group

Number of Iterations
Defines the number of iterations that will be used.

Infinite Calculation
If this check box is enabled, the calculation runs until the user presses the stop button

Stop (Button)
Stops the the NMF calculation.

Calculate (Button)
Starts the NMF calculation. During the calculation the preview windows are updated regularly.

Error Group

During the calculation these values are updated.

Total
Average error between fit and dataset. If the value decreases the fits gets better.

Change
Difference between the last and the current average error. If this value is positive the fit gets better. If this value fluctuates between positive and negative, increase the value of Image Reduction and Spectrum Reduction.

Total # of iterations
Show the number of iterations.
You can simply turn the wheels or edit the number to define a weighting factor for subtracting the spectra from each other.

Mask Group

This group shows the standard Mask Manipulation Tools.

Buttons

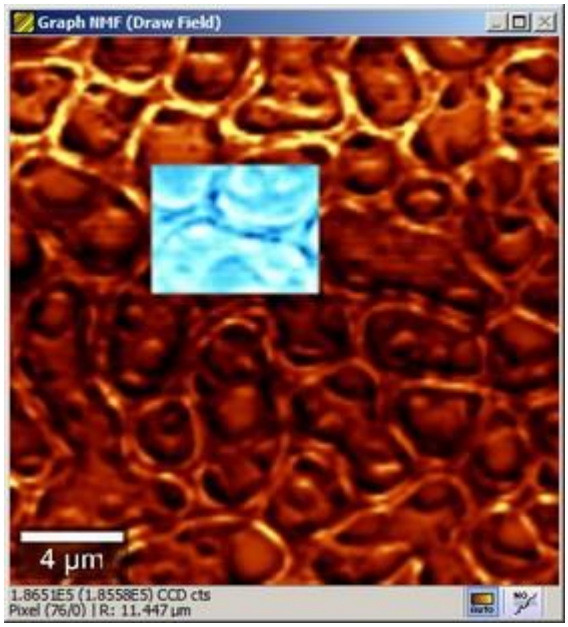
Extract (Button)
Extracts the basis spectra and distribution images.

Current as Start Conditions (Button)
To find the basis spectra only few spectra of the data set are needed. But in this case only the distribution map of the selected pixels are valid. In order to calculate the distribution map of all pixels press this button. After this it is possible to extend the image mask and start the calculation again. The distribution maps are now used as start condition for the algorithm.

Reset
Resets all basis spectra and distribution maps to random. This can be used if a new basis spectrum was added and the calculation ran before and change the basis spectra and distribution maps.

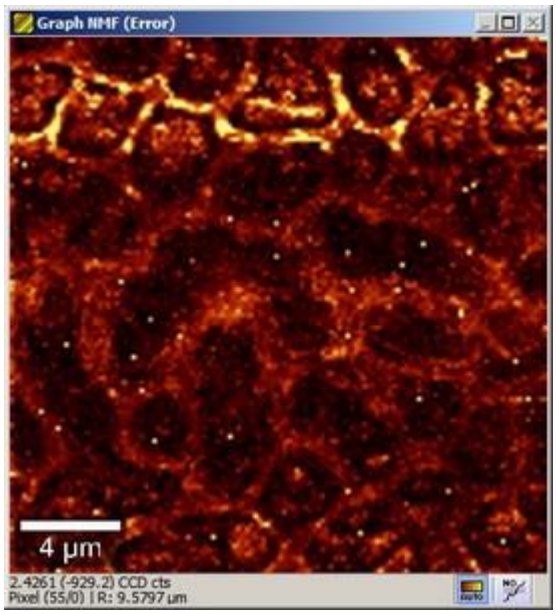
Preview Windows

Graph NMF (Draw Field)



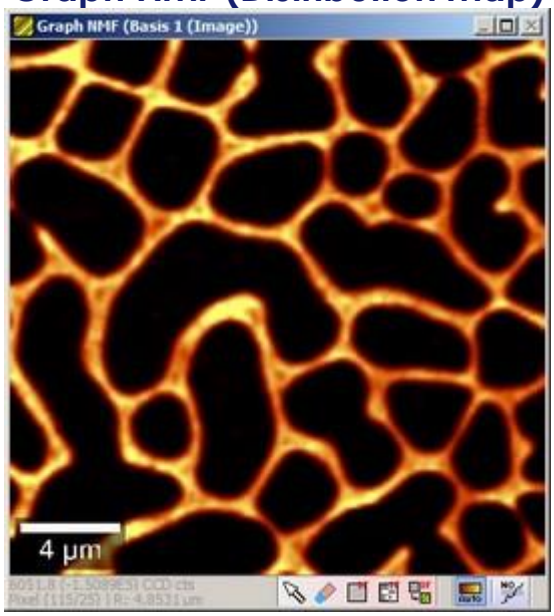
The image data shows the total sum of the spectrum. Use the draw field of this image preview window to select the pixels that should be used for the calculation.

Graph NMF (Error)



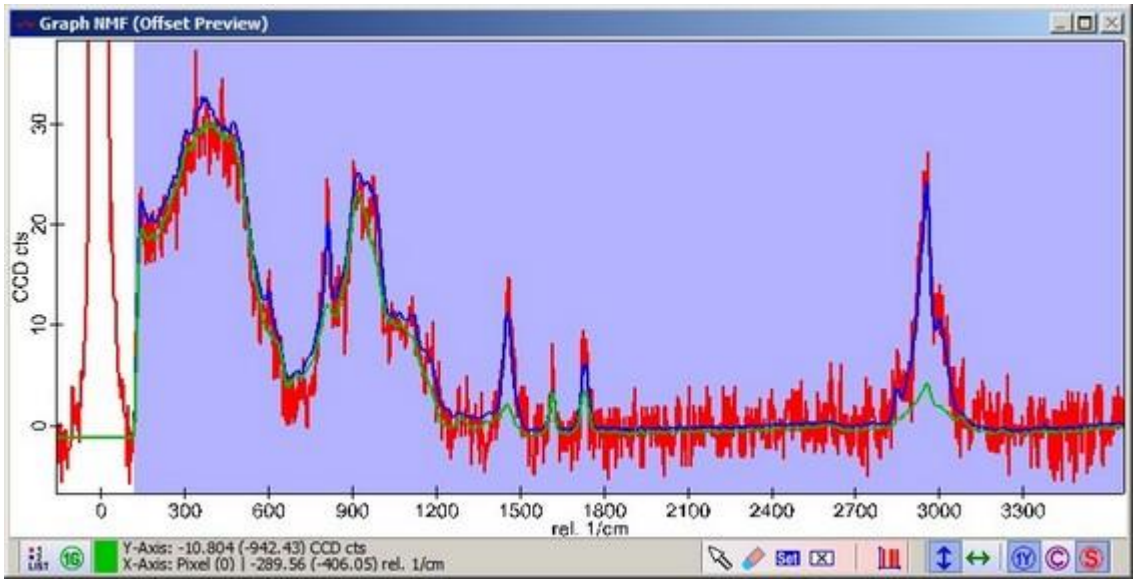
Show the average error between fit and data. A high value might be a hint that a additional basis spectrum is needed.

Graph NMF (Distribution Map)



Shows the distribution map of the currently selected basis spectrum.

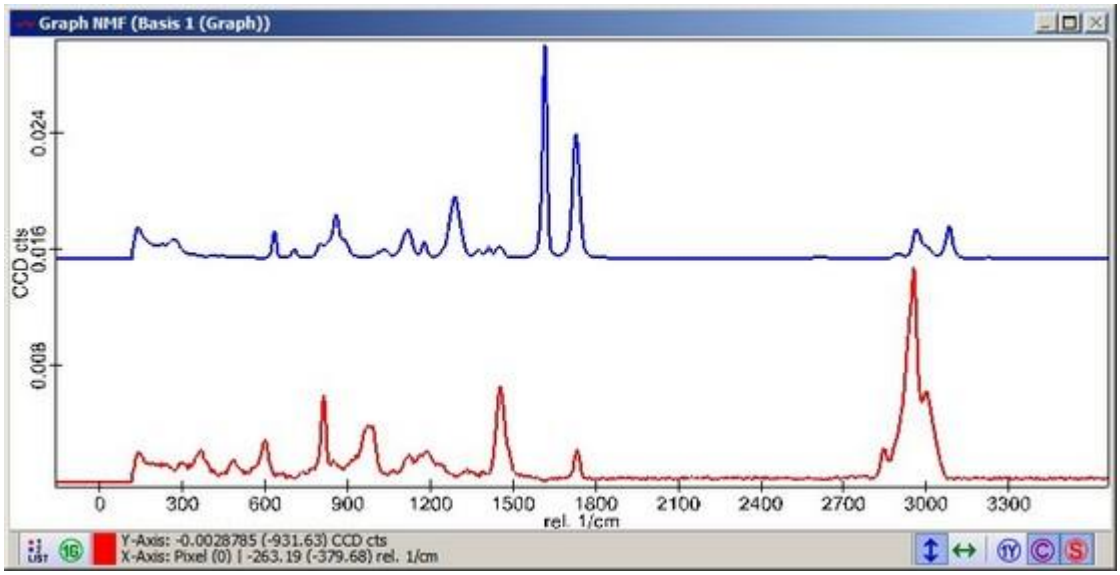
Fit Preview and Mask



The fit graph preview window shows the original spectrum in red, the fit preview in blue and the offset in green.
Just click somewhere in the preview image to see the selected spectrum.

Use the mask to define which spectral area should be considered for NMF calculation.

Basis Spectra



This graph preview window shows the normalized basis spectra.

Principal Component Analysis

Description

The Principal Component Analysis dialog does a principal component calculation and allows to extract its results.

See also
Principal Component Analysis (Math)

Input and Results

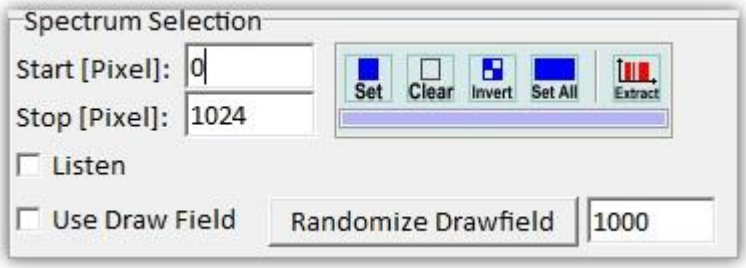
Input:

- One spectral Raman image data set
  or
- One spectral line graph data set (e.g. spectra along a line)

Results:
see "Extract Data" below.

User Interface

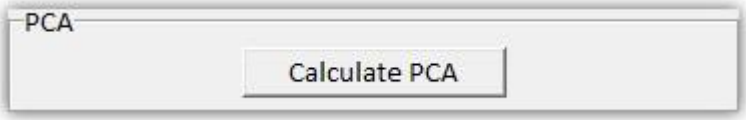
Spectrum Selection



Here you can select

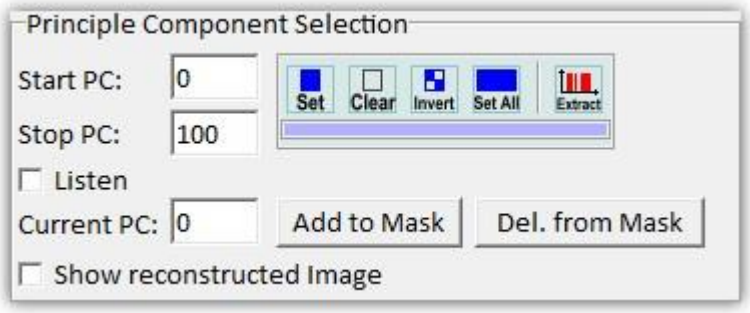
- which spectral range should take part in the PCA, see Mask Manipulation Tools
- which spectra should take part in the principal component calculation:
  If "**Use Draw Field**" is checked, the current mask on the the preview image viewer is used to select the spectra.
  If you click on "**Randomize Drawfield**", then the desired number of pixels are randomly selected.

Calculate PCA



Starts the PCA Calculation.

Principle Component Selection



Here you can add or remove principal components using the graph mask of the "Eigenvalues" preview, see Mask Manipulation Tools.
The selected principal components are used to calculate a reconstructed spectrum preview. Only the selected principal components are used when extracting data.

Show Reconstructed Image
If checked, a reconstructed image will be shown in the preview.
The value "Current PC" defines which component will be reconstructed

Add to Mask / Del. from Mask
Adds or removes the principle component of the "Current PC" value.

Extract Data

Here you can choose with result should be exported, if you press the button "Extract Selected Data".

PCA Result
Three graph objects are added to the project:

- The Eigenvalues
- The corresponding Eigenvectors
- The average spectrum (offset spectrum)

By clicking into the graph of the Eigenvalues it is possible to navigate through the Eigenvectors.

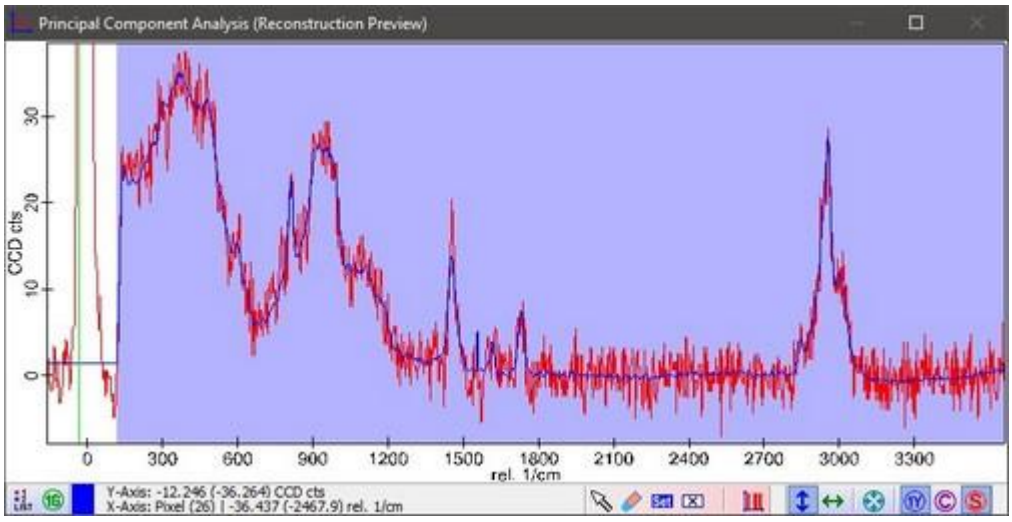
PC Graphs
The Eigenvector of each selected/marked principle component is extracted as a single graph object.

Transformed Spectra
The selected Eigenvectors define a new coordinate system. Each original spectrum can be represented in the new coordinate system. For each selected Eigenvector a new coordinate is calculated.

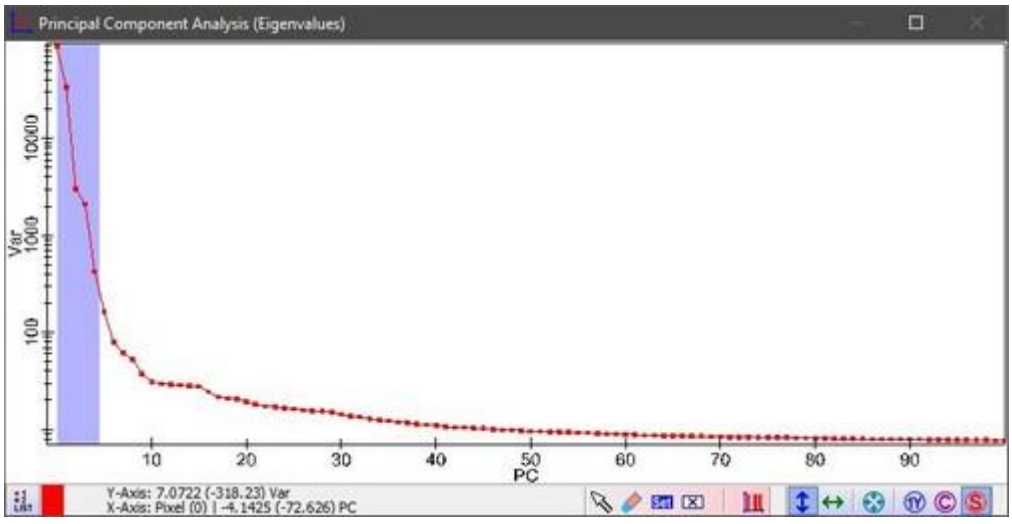
These transformed coordinates are stored into a graph object.

Reconstructed Spectra
It is also possible to do a back transformation of the transformed spectra. The output is equal to the blue spectrum of the Reconstructed Preview window.

Preview Windows



Reconstructed Preview
Shows the original data in red and the reconstructed preview spectrum in blue.
The reconstructed spectrum is calculated using the principal component selection (mask in Eigenvalues preview).
You can manipulate the mask to define which spectral range should take part in the principle component calculation.



Eigenvalues
Shows the sorted Eigenvalues.
You can manipulate the mask to define which principle components should be used for the reconstruction preview and for extracting the data.

Graph Intensity Correction Dialog

Description

In some cases the signal response detected by a CCD is not equal for all pixels. This imperfection can be seen especially if a Raman spectrum contains high fluorescent background.
An optical etaloning effect inside CCD detectors and the optical filters which are used to reduce the Rayleigh light can cause this problem.
In order to remove this imperfection, this dialog uses a reference measurement in order to determine a correction curve.

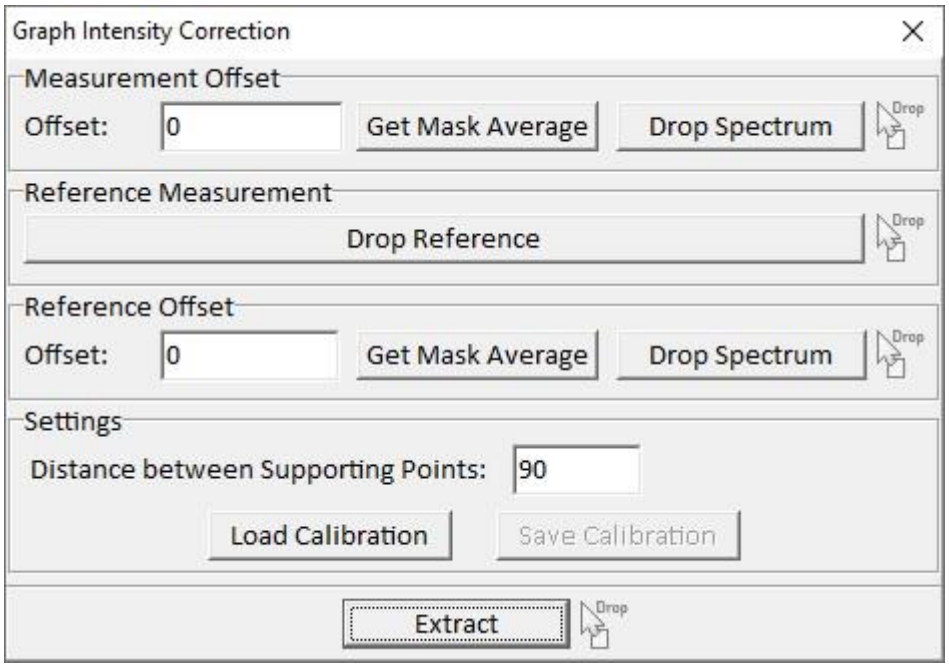
See also
Using Graph Intensity Correction

Input and Results

Input:
One graph object that can be a single spectrum or a multiple spectra object (e.g. Image Graph). This is the object that will be corrected.

Results:
One graph object with the same dimensionality

User Interface



Measurement Offset - Offset (Edit)
Here you can define the offset manually, which will be subtracted before calculation.

Measurement Offset - Get Mask Average (Button)
If you press this button, the current spectrum and the selected mask (blue) is used to calculate an average. This average value is entered into the offset edit.

Measurement Offset - Drop Spectrum (Button)
If a dark spectrum is measured (single spectrum or series), you can drop it here in order to use it.
If you press this button, the offset is deleted.

Reference Measurement - Drop Spectrum (Button)
Drop the reference spectra here (e.g. fluorescence spectra). An average is calculated and displayed inside a preview window.

Reference Offset - Offset (Edit)
Here you can define the offset for the reference spectra manually, which will be subtracted before calculation.

Reference Offset - Get Mask Average (Button)
If you press this button, the average reference spectrum and the selected mask (blue) is use to calculate an average. This average value is entered into the offset edit.

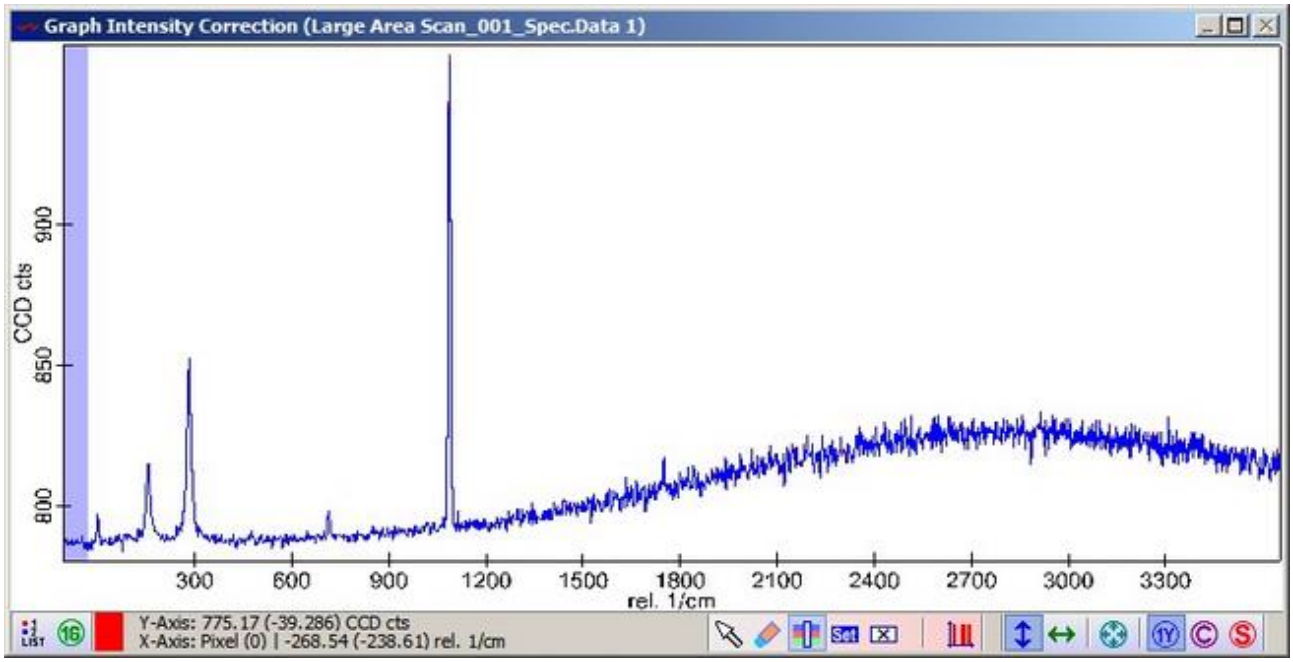
Reference Offset - Drop Spectrum (Button)
If a dark spectrum is measured (single spectrum or series), you can drop it here in order to use it.
If you press this button, the offset is deleted.

Settings - Distance between Supporting Points (Edit)
A smooth spline function is calculated from the average reference spectra. The distance between the spline supporting points can be set with this edit.

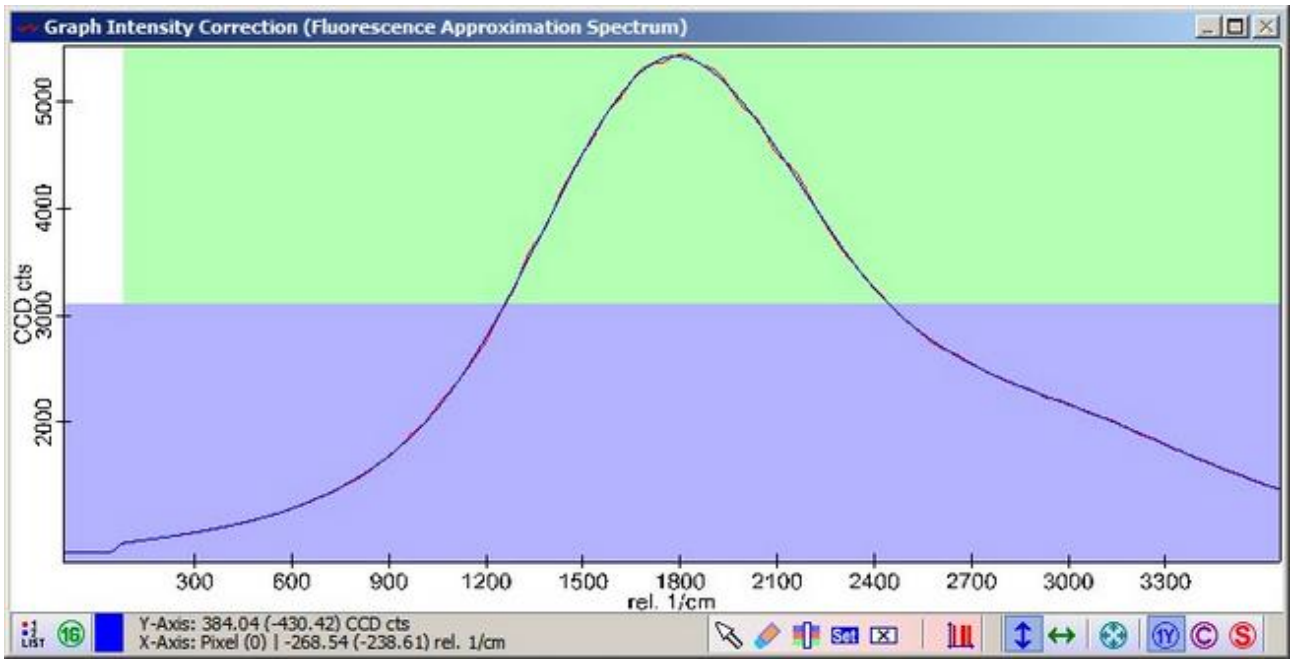
Settings - Load / Save Calibration (Buttons)
Here you can save and load your calibrations to/from the hard drive. You can reuse it for all future measurements that are acquired under exactly the same conditions than the reference spectra (same laser, same spectral position, same CCD settings).

Extract (Button)
The corrected data will be exported to the project.
If more than one measurement have been acquired under same conditions, you can drop all measurements at once on this button.

Preview Windows



In the preview graph above the original and the corrected data is displayed. Normally you won't see any big difference for noisy spectra.



In this preview graph you can see the average reference spectrum and the spline fit. The ratio of both is the correction used for the data.

The green mask defines the region that is used to calculate this ratio. If the green mask is not set, the correction factor will be 1.
Note that the mask will be considered as one big region without gaps in between.

The blue mask can be used to define the offset of the reference using the "Get Mask Average" button.

Image Background Subtraction Dialog

Description

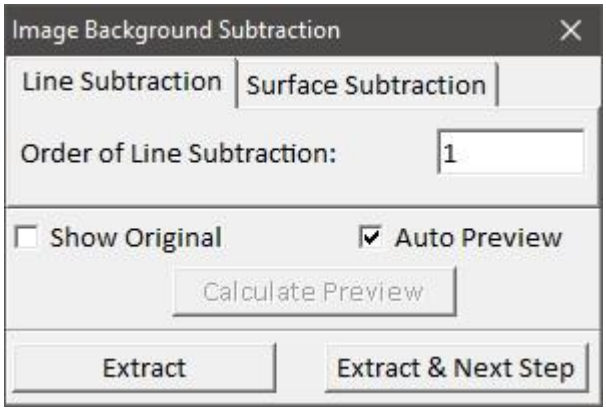
With the Image Background Subtraction Dialog you can subtract the background for each line (one dimensional) or for a whole surface (two dimensional) using polynomial functions with a adjustable order.

Input and Results

Input:
one image data object.

Results:
one background subtracted image data object.

User Interface



Line Subtraction Tab

If the line subtraction tab is selected, the image is being line subtracted (each single line is corrected using a one dimensional polynomial curve).

Surface Subtraction Tab

If the surface subtraction tab is selected, the image is being surface subtracted (a whole surface is subtracted using a two dimensional polynomial function).

Order of Line / Surface Subtraction (Integer Edits):
sets the polynomial order for the line or surface subtraction.

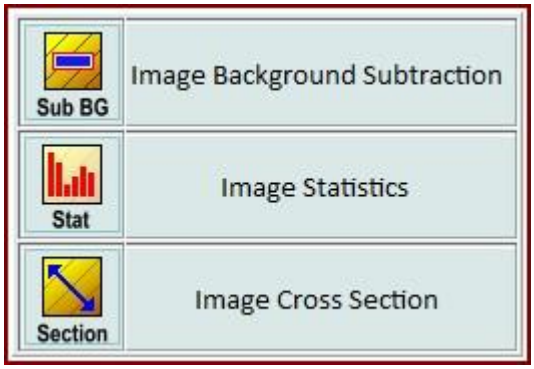
Show Original (Check Box):
If checked, the preview image viewer shows the original image (for a fast comparison).
Don't forget to uncheck this check box afterwards to see the background subtracted image again.

Calculate Preview (Button), Auto Preview (Check Box):
See Automatic Preview.

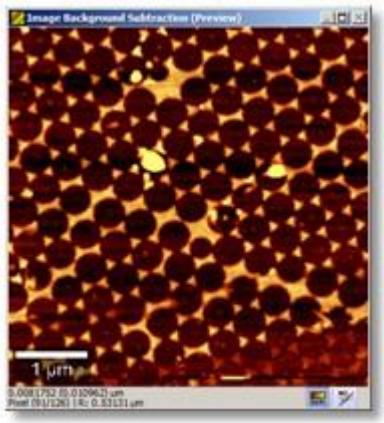
Extract (Button):
calculates and extracts the result to the current project.

Extract & Next Step (Button):

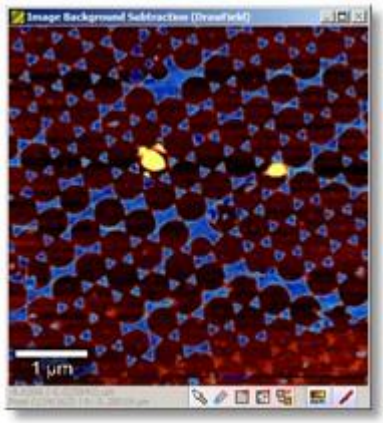
Wizard feature: here you can choose to use the result image for another background subtraction, do some image statistics or an image cross section:



Preview Windows



The first preview window shows the background subtracted image.



The second preview window shows the original image and a draw mask.
Change the mask in order to define which pixels should be considered for calculating the the polynomial background fit function.

Image Statistics Dialog

Description

The Image Statistics Dialog can be used to analyze images by showing histograms and several statistic parameters.

See also
Average Spectrum (Math)
Image Statistics (Math)

Input and Results

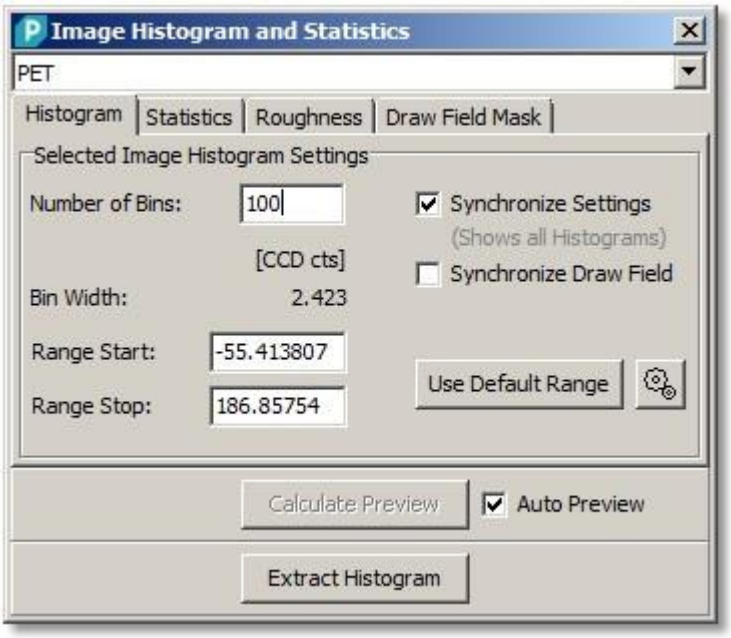
Input:
Any number of images.

Results:
Histograms or text objects containing statistic or roughness information.

User Interface

- Histogram Tab
- Statistics and Roughness Tab
- Draw Field Mask Tab
- Preview Windows

Histogram Tab



Number of Bins (Integer Edit):
Sets the number of bins or "vertical lines" for the histogram.

[CCD cts] (Label):
Shows the value unit of the currently selected image.

Bin Width (Label):
Shows the bin width of one histogram line.

Range Start/Stop (Float Edits):
Define the start and stop range of the histogram.
Both values are calculated automatically upon startup; each dropped image has its own range.

Synchronize Settings (Check Box):
Only enabled if all images share the same value unit kind.
If checked:

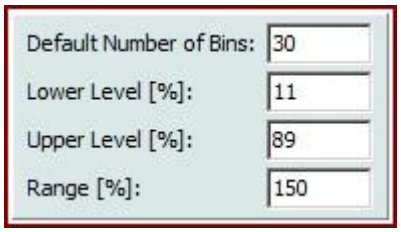
- all histograms of all dropped images are shown together in one graph preview
- all histograms share the same ranges and number of bins.

If not checked, each histogram has its own number of bins and range.

Synchronize Draw Field (Check Box):
Only enabled if all images share the same spatial transformation (same measurement).
If checked, the current draw field is used for all dropped images.
Otherwise each image has its own draw field that defines which pixels are used for the histogram and statistics calculation.

Use Default Range (Button):
resets the start and stop ranges of the current histogram using default settings for range determination (see below).

 **Default Range Settings (Tool Button)**



Default Number of Bins (Integer Edit):
This is the number of bins which is used when the dialog opens.

Lower Level (Float Edit):
The lower level of all sorted image values for default range value determination.

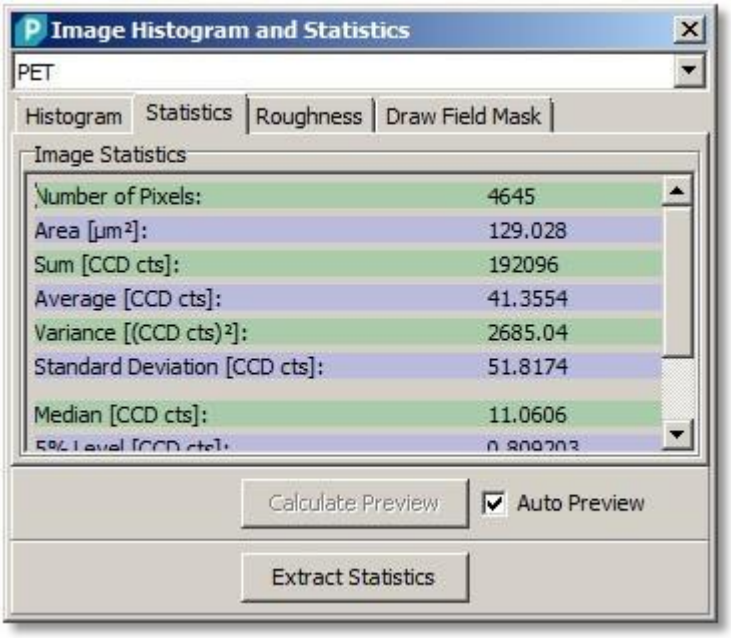
Upper Level (Float Edit):
The upper level of all sorted image values for default range value determination.

Range (Float Edit):
The range which is used to spread the range after using the lower and upper level.

Calculate Preview (Button), Auto Preview (Check Box):
See Automatic Preview.

Extract Histogram (Button):
calculates and extracts all histograms from all images.

Statistics and Roughness Tab



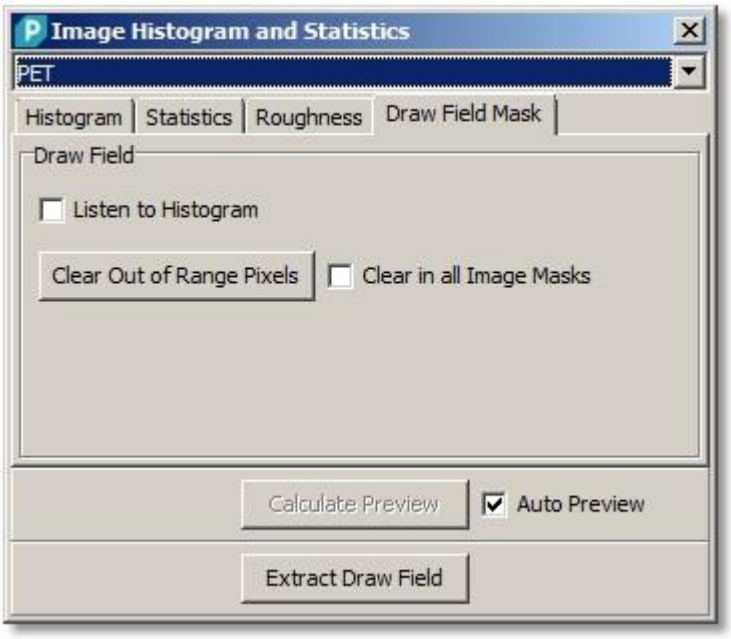
The **Statistics Tab** shows several image statistic values like the Average or Variance.
The values are calculated for all pixels that are set in the current image mask (see preview image viewer).

Extract Statistics (Button)
Creates a new text object containing all statistic parameters.

The **Roughness Tab** is very similar. Instead of standard statistical parameters it shows special roughness parameters (particularly used for topography images).

For a detailed description of the roughness parameters, see Image Statistics (Math).

Draw Field Mask Tab



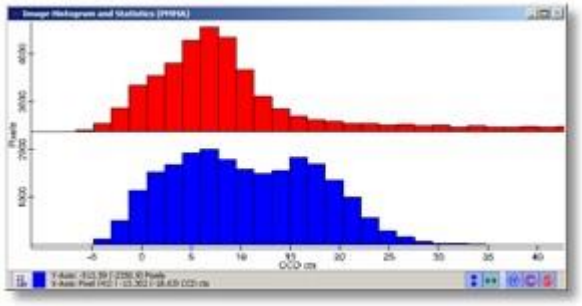
Listen to Histogram (Check Box):
If checked, you can set or clear (hold the shift key down) an area in the histogram in the preview graph viewer in order to set or clear the corresponding pixels from the image mask.

Clear Out of Range Pixels (Button)
This will clear all pixels from the image mask, that have values outside the current ranges of the histogram.

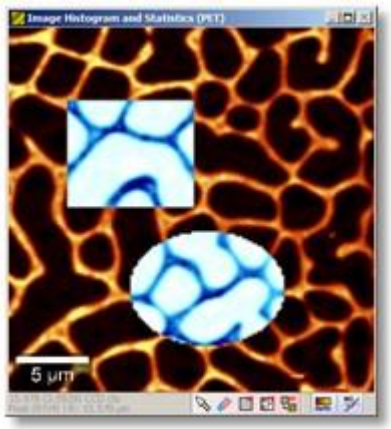
Clear in all Image Masks (Check Box)
If checked, the above button effects all image masks (if the mask is not synchronized anyway).

Extract Draw Field (Button)
Extracts all draw fields as a new boolean image data object to the current project.

Preview Windows



The graph preview window shows the histograms.
Hint: You can turn on or off the cascade mode in order to see the histogram lines side-by-side or cascaded.



The image preview window shows the currently selected image and the mask that defines which pixels are used for the histogram and statistics calculation.

Image Cross Section Dialog

Description

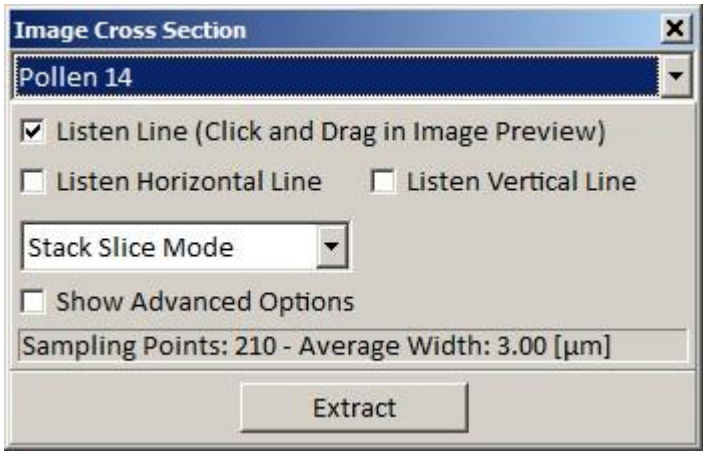
The Image Cross Section Dialog allows to display image intensities along a line as a graph object. This enables to analyze the structure of an image.
Additionally, a complete stack of images can be dropped in order to create a stack slice image.

Input and Results

Input:
Any number of image data objects.

Results:
One cross section graph object for each dropped image.

User Interface



"Pollen 14" (Combo Box):
selects the current preview image here (if you dropped more than one image).

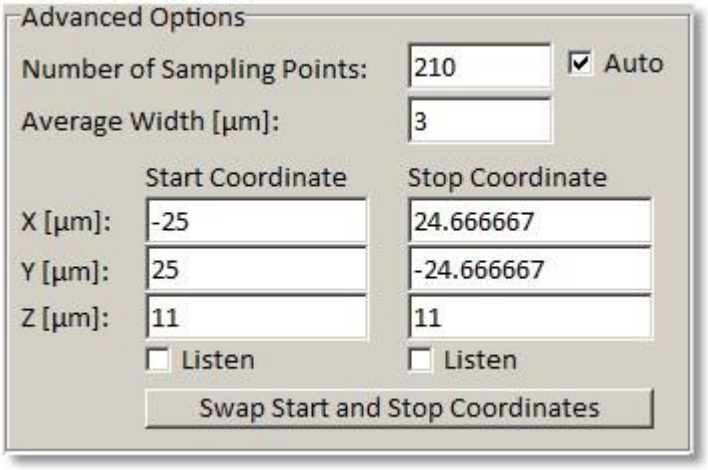
Listen Line (Check Box):
If checked, you just can draw a line in any image viewer in order to set a new cross section line position for this dialog.
This option is automatically checked upon starting the dialog, so you can directly draw your cross section line e.g. in the preview image viewer.

Listen Horizontal Line (Check Box):
If checked, you can click in a image viewer in order to use the clicked image line as a cross section. The dialog automatically sets the number of sampling points to the number of pixels in a line to use the exact supporting points of the image.

Listen Vertical Line (Check Box)
If checked, you can click in a image viewer in order to use the clicked image column as a cross section. The dialog automatically sets the number of sampling points to the number of pixels in a column to use the exact supporting points of the image.

Stack Slice Mode (Combo Box):
Only shown, if a image stack was dropped. Can be used to switch between stack slice mode and cross section mode.

Show Advanced Options (Check Box):
shows the advanced options:



Number of Sampling Points, Auto (Integer Edit, Check Box):
sets the number of pixels of the cross section graph object.
If the Auto Check Box is checked, this value is automatically determined (~3 times image pixel resolution).

Average Width (Float Edit):
If the average width is larger than zero, some pixels perpendicular to the cross section position with a distance of smaller or equal than <average width> are averaged and used for calculating one cross section value.

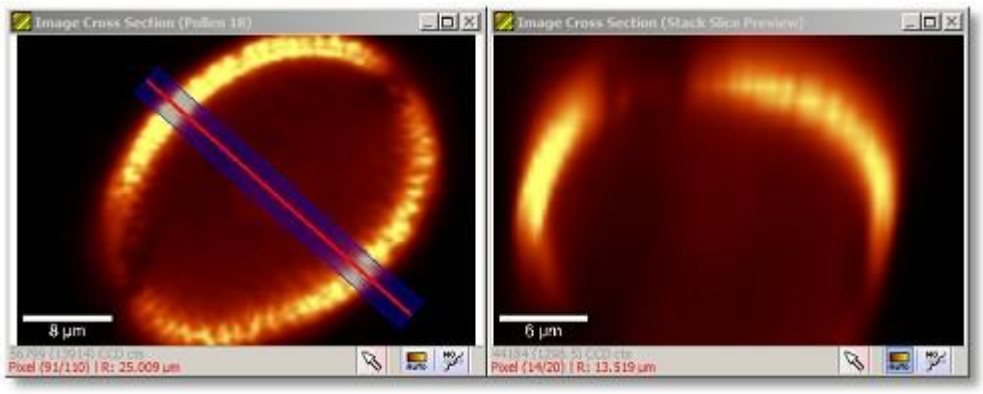
Start/Stop Coordinate (Float Edits):
Here you can exactly define an absolute spatial position for the start and stop coordinate of the cross section.

Listen Start/Stop Coordinate (Check Boxes):
If checked, you can click on an image in order to set the start or stop coordinate using the listen cursor mechanism.

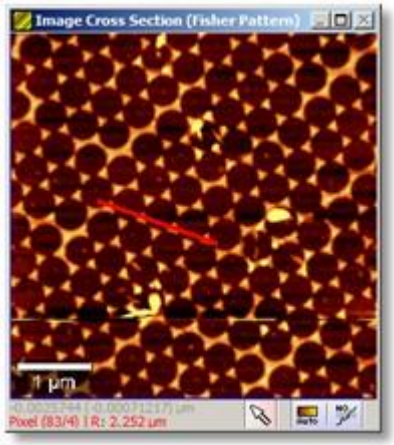
Swap Start and Stop Coordinates (Button):
simply swaps the start and stop coordinates in the result cross section.

Stack Slice Mode

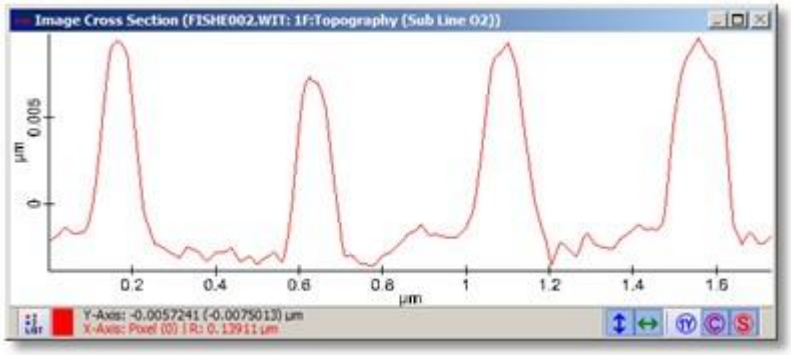
In the stack slice mode, a stack of images can be used to calculate a stack slice image (e.g. a depth image).
You can define a line in the cross section preview image window (lateral image) and see the stack slice preview in the second image window.
Click somewhere in the Stack Slice Preview Window in order to select the current stack image.



Preview Windows



The preview image viewer shows the selected input image and also the cross section line in red. If the average width parameter is larger than zero, the image viewer shows a blue area which is used for averaging.



The preview graph viewer shows the cross section (or multiple cross sections if more than one image is dropped).

Image Filter Dialog

Description

The Image Filter Dialog offers several image smoothing and edge filters as well as a sharpen filter and a user definable custom filter.

Input and Results

Input:
Any number of image Data Objects.

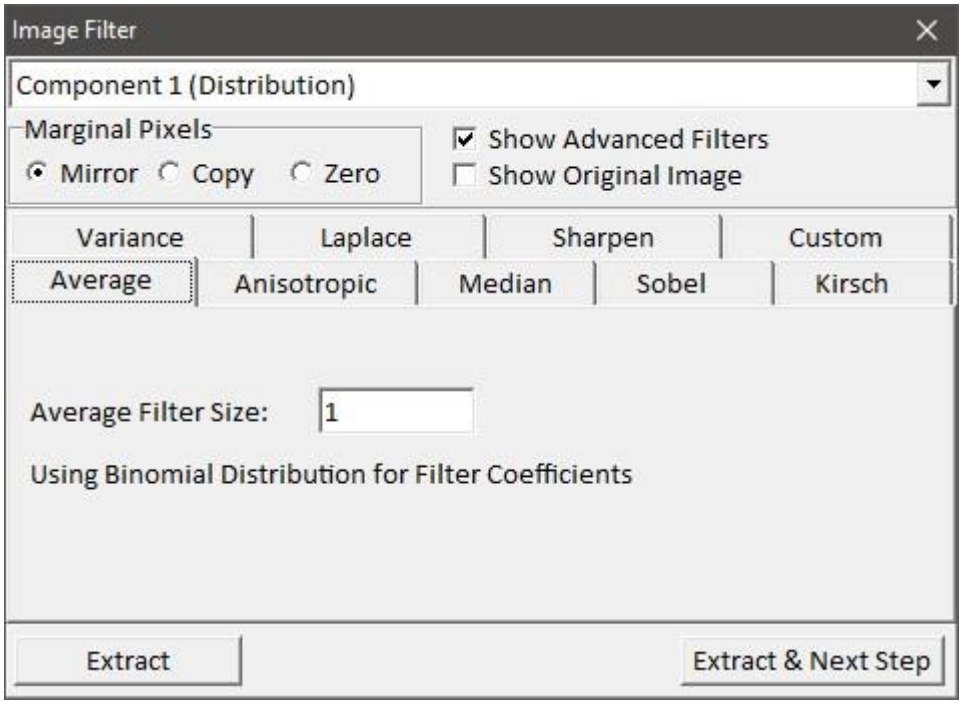
Results:
Smoothed images or edge filter images.

User Interface

- Average Tab and General UI
- Anisotropic Tab
- Median Tab
- Sobel Tab
- Kirsch Tab
- Variance Tab
- Laplace Tab
- Sharpen Tab
- Custom Tab
- Preview Windows

Average Tab and General UI

If the average tab is selected, the image is smoothed by a 2D averaging algorithm using binomial distribution for filter coefficients.



PET (Combo Box):
If multiple images are dropped, you can select the current preview image here.

Marginal Pixels (Radio Buttons):

Most filters use a certain window size using multiple pixels of the input image for calculating the result pixel. For marginal pixels / if the filter window is outside the image, you can select if

- the pixels are mirrored
- the border pixels are copied
- the pixels will be set to zero.

Show Advanced Filters (Check Box):
This will make all edge filters and the custom filter visible to the user.

Show Original Image (Check Box):
If checked, the preview image viewer shows the selected original image instead of the filter preview.
Don't forget to turn off this mode if you would like to see the filter preview again e.g. when changing some filter options.

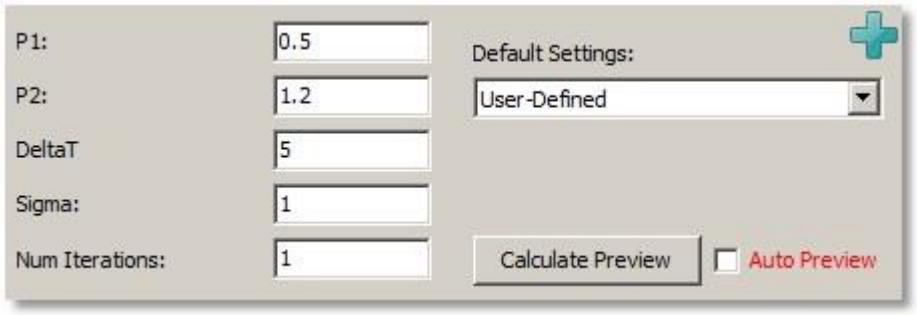
Average Filter Size:
sets the filter size for the average filter. The total average window size is (2 * <filter size> + 1)^2.

Extract (Button):
Calculates and extracts the result image(s) into the current project.

Extract & Next Step (Button)
Wizard Feature: you can use the result images of this dialog as an image combination:

Anisotropic Tab

If the anisotropic tab is selected, the image is smoothed using an anisotropic smoothing filter which also preserves the edges (see D. Tschumperle, Lecture Notes in Computer Science, 3952, 295 [2006]).
This is a WITec Project Plus feature.



P1, P2, DeltaT, Sigma, Num Iterations (Edits):
Change the behavior of the anisotropic image filter.

Default Settings (Combo Box):
Chooses predefined settings for small and large structures.

Calculate Preview (Button), Auto Preview (Check Box):
See Automatic Preview.
The automatic preview is disabled if the dialog opens.

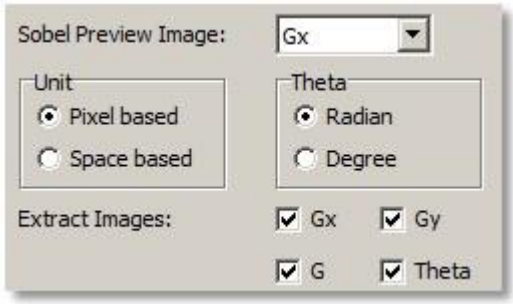
Median Tab

If the median tab is selected, the image is smoothed by a 2D median algorithm.

Median Filter size (Integer Edit):
Sets the median filter size. The total median window size is (2*<filter size>+1)^2.

Sobel Tab

If the Sobel tab is selected, the edges in an image can be detected by searching in different directions using the Sobel operator. Additionally, the angle of maximum gradient can be shown.
This is a WITec Project Plus feature.



Sobel Preview Image (Combo Box):
You can select which Sobel result should be shown in the preview:

- Gx (Shows edges in x-direction)
- Gy (Shows edges in y-direction)
- G (Shows edges in x- and y-direction)
- Theta (Shows the edge angle as intensity)

Unit (Radio Buttons):
Changes the edge unit: pixel or space based (unit µm).

Theta (Radio Buttons)
Changes the angle unit: radians or degrees.

Extract Images (Check Boxes):
Select which of the Sobel results should be extracted when using the Extract Button.

Kirsch Tab

If the Kirsch tab is selected, the edges of an image can be detected by searching in different directions using the Kirsch operator.
This is a WITec Project Plus feature.

Kirsch Filter Direction (Combo Box):
You can change the edge angle of the Kirsch filter (G1 - G8 and GMax which uses all directions).

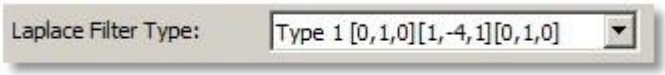
Variance Tab

If the Variance tab is selected, the edges of an image can be detected by calculating the 2D variance.
This is a WITec Project Plus feature.

Variance Filter Size (Integer Edit):
You can change variance filter size. The total filter window size is (2*<filter size>+1)^2.

Laplace Tab

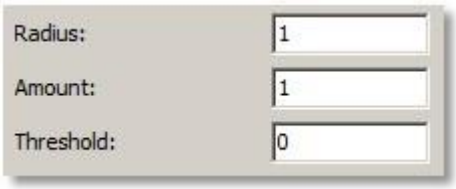
If the Laplace tab is selected, the edges of an image can detected using the Laplace operator.
This is a WITec Project Plus feature.



Laplace Filter Type (Combo Box):
Chooses between predefined Laplace filter coefficients.

Sharpen Tab

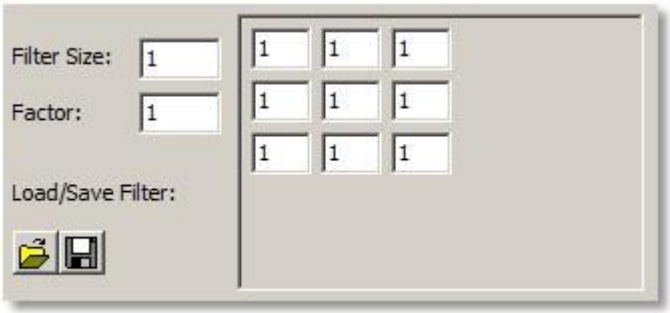
If the Sharpen tab is selected, the image is filtered using the Unsharp Masking technique.
This is a WITec Project Plus feature.



Radius, Amount, Threshold (Edits):
Changes the behavior of the sharpen image filter.

Custom Tab

If the Custom tab is selected, the image is filtered using user defined filter coefficients.
This is a WITec Project Plus feature.



Filter Size (Integer Edit)
Changes the filter size for the user defined filter. The total filter window size is (2*<filter size>+1)^2.

Factor (Float Edit):
A factor all filter coefficients are multiplied with.

Load/Save Filters (Tool Buttons):
You can save the current custom filter and reload it later on.

Filter Coefficients (Float Edit Matrix):
Enter your filter coefficients in the float edit matrix on the right side.

Preview Windows

The preview image viewer shows the preview of the smoothing/sharpen/edge algorithm.
You can toggle the "Show Original" checkbox in order to compare the result with the original image.

Image Repair Dialog

Description

The Image Repair Dialog can be used to replace unwanted pixels or areas in images using different replacement algorithms.

Input and Results

Input:
any number of image data objects.

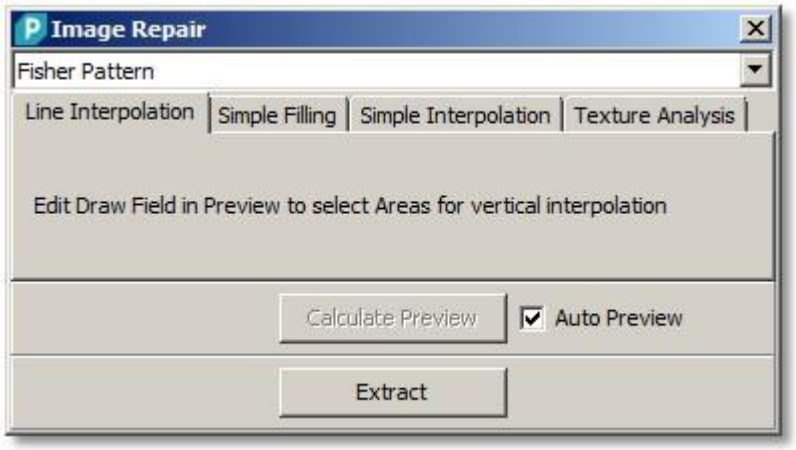
Results:
repaired image data objects.

User Interface

- Line Interpolation Tab and General UI
- Simple Filling Tab
- Simple Interpolation Tab
- Texture Analysis Tab
- Preview Windows

Line Interpolation Tab and General UI

If the line interpolation tab is selected, the area which is set in the mask of the mask preview window will be interpolated using the lines above and below the masked area.



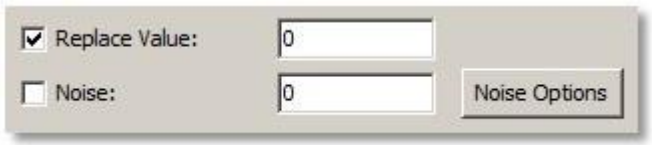
"Fisher Pattern" (Combo Box):
If multiple images are dropped, you can select the current preview with this combo box.

Calculate Preview (Button), Auto Preview (Check Box):
see Automatic Preview.

Extract (Button):
calculates and extracts the repaired image to the current project.

Simple Filling Tab

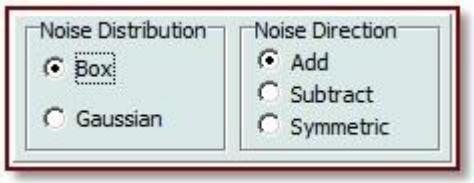
If the simple filling tab is selected, the area which is set in the mask of the mask preview window will be replaced using a given value which is optionally added with some random noise.



Replace Value (Check Box and Float Edit):
If checked, the masked areas are replaced using the replace value.

Noise (check Box and Float Edit):
If checked, the replaced values are furnished with noise of a given noise level/strength.

Noise Options (Button):
opens the noise options window:



Noise Distribution (Radio Buttons):
Here you can choose a noise distribution (Box or Gaussian noise).

Noise Direction (Radio Buttons):
Here you can chose a noise "direction", that means the noise is added or subtracted asymmetric or added symmetric.

Simple Interpolation Tab

If the simple interpolation tab is selected, the area which is set in the mask of the mask preview window will be replaced using a simple interpolation algorithm that uses the borders of the mask areas for interpolation.

Number of Iterations (Integer Edit):
Sets the number of iterations for interpolation. The more iterations, the smoother the transition of image intensities.

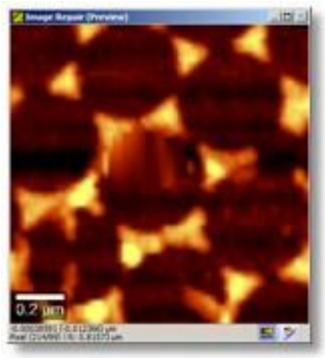
Texture Analysis Tab

If the texture analysis tab is selected, the area which is set in the mask of the mask preview window will be replaced using a texture analysis algorithm that uses areas of the same image that fit best the areas to be replaced.

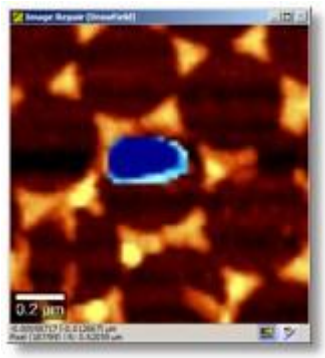
Texture Window Size (Integer Edit):
defines a texture window size which is used to search an appropriate replacement texture.

Number of Rotations (Integer Edit):
defines the number of rotations in order to find a better fitting replacement texture.
Both parameters have a strong influence on the calculation time.

Preview Windows



The first preview image viewer shows the repair preview.



The second preview image viewer shows the original image and the mask, that can be changed to define which pixels should be replaced or repaired.

Image Combination Dialog

Description

The Image Combination Dialog can be used to combine multiple intensity images into a combined color bitmap using a different color table for each image.

Input and Results

Input:
Any number of image data objects.

Results:
One color combined bitmap object.

User Interface

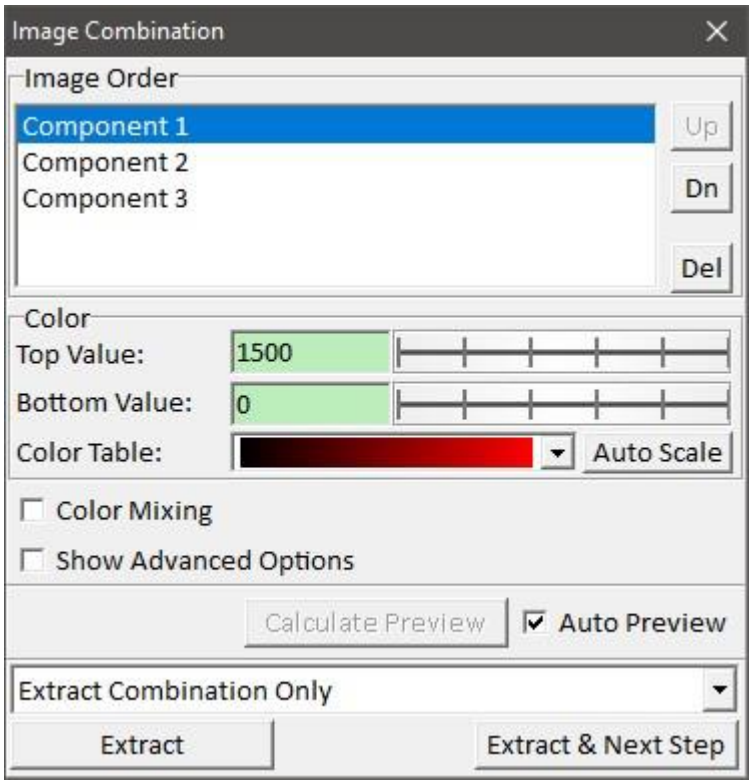


Image Order (List Box)
Shows the image list. You can select an image here in order to define its color scale, color table and - in advanced - mode the transparency thresholds.

Up/Dn (Buttons)
These buttons allow you to change the image order. This is only needed when using top or bottom transparency in the advanced mode.

Del (Button)
Removes the currently selected image (from the dialog only).

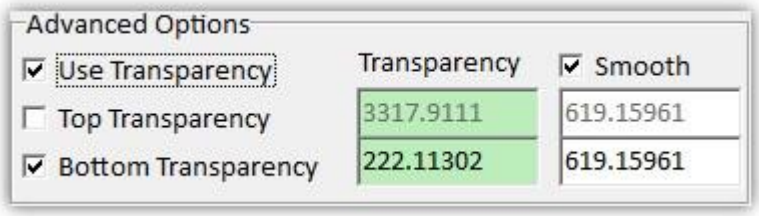
Top/Bottom Value (Edits and Sliders)
Changes the top and bottom color scale of the currently selected image.
In advanced mode, you can also use the preview image viewer ("Transp. Mask") color scale features to set the color scale of the current image.

Color Table (Combo Box)
Changes the color table for the currently selected image.

Auto Scale (Button)
This will do an automatic color scale for the currently selected image.

Color Mixing (Check Box)
If checked, the colors of all images are summed up / mixed for each image pixel.
If not checked, the dialog automatically decides which image will be shown at a certain pixel: the image with the brightest color will be displayed. This can be considered as an automatic mask algorithm.

Show Advanced Options (Check Box)
Shows the advanced options which are used to define a custom transparency:



Use Transparency (Check Box)
If checked, it's possible to use user defined transparency thresholds that define which parts of the images are transparent (letting images that "lay below" shine through the other image).

Use Top Transparency (Check Box)
If checked, all pixels with a higher value than the entered top transparency value are transparent.

Use Bottom Transparency (Check Box)
If checked, all pixels with a smaller value than the entered bottom transparency value are transparent.
Usually only this value has to be increased for each layer to create nice images.

Transparency (Edits)
The thresholds that define which pixels should be transparent.

Smooth (Check Box and Edits)
With these parameters the transparency transitions are drawn more smoothly.
A value of zero means that the border of transparency/no transparency is not smoothed.

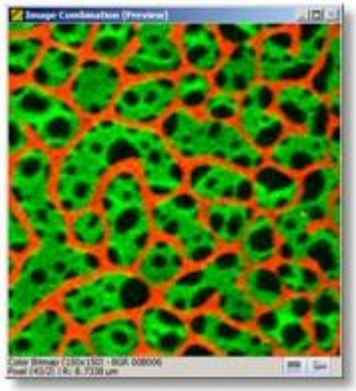
Calculate Preview (Button), Auto Preview (Check Box)
See Automatic Preview.

Extract Mode (Combo Box)

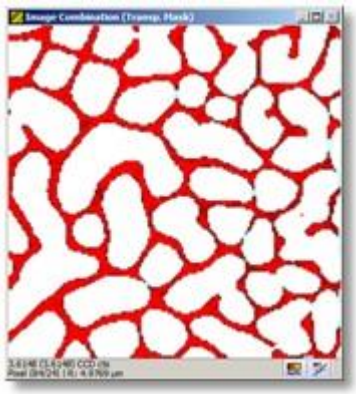
- Extract Combination Only: Extracts the combination bitmap only
- Extract All Images: Extracts the combination bitmap and every single image as a separate bitmap
- Extract All Images using Viewer Export Settings: Extracts the combination bitmap and every single image as a separate bitmap using the Image Viewer Export. This allows you to add a color scale bar next to each image and the "µm scalebar overlay" on the image. The current image graphic export options are used. You can also change those options using the image viewer export circle menu.
  Note that the result images will have no spatial transformation.

Extract (Button)
Calculates the result bitmap (if not already calculated) and adds it to your current project.
Depending on the Extract Mode, also all bitmaps of all images are extracted as well.

Preview Windows



The first preview image viewer shows a preview of the combined color bitmap.



The second preview image viewer shows the selected image and its transparency mask.
This viewer is only visible in the advanced mode or if the extract mode "Extract All Images using Viewer Export Settings" is selected.
You can use the color scale features of this viewer in order to set the color scale of the currently selected image.

Image Fourier Filter Dialog

Description

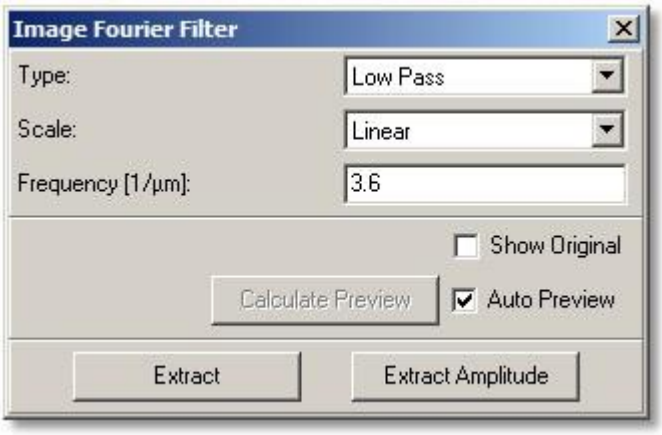
The Image Fourier Filter Dialog transforms an image into Fourier space (showing an amplitude image) and allows to change a mask in order to cut certain frequencies for a back-transformation.

Input and Results

Input:
one image data object.

Results:
the Fourier amplitude image or a back-transformed image.

User Interface



Type (Combo Box):
Depending on this option, you can change the mask in the amplitude image in order to cut frequencies in Fourier space for the back transformation:

- Free Hand: allows to change the mask directly in the image viewer using the draw tools.
- Low Pass: removes high frequencies using the frequency parameter.
- Low Pass Line: removes high frequencies line per line using the frequency parameter; only vertical mask.
- High Pass: removes low frequencies using the frequency parameter.
- High Pass Line: Removes low frequencies line by line using the frequency parameter; only vertical mask.
- Remove Harmonics: Removes harmonics for a given frequency.

Scale (Combo Box):
changes the scale of the amplitude image:

- Linear: linear scale
- Log: logarithmic scale
- Sqrt: square root scale.

Frequency (Float Edit):
defines the frequency for low pass (line), high pass (line) and remove harmonics mask creation.

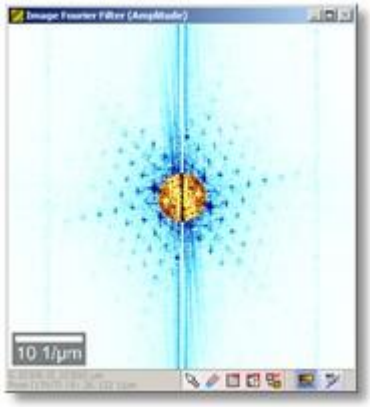
Show Original (Check Box):
if checked, the preview image viewer shows the original image instead of the back-transformed preview (for comparison).

Calculate Preview (Button), Auto Preview (Check Box):
see Automatic Preview.

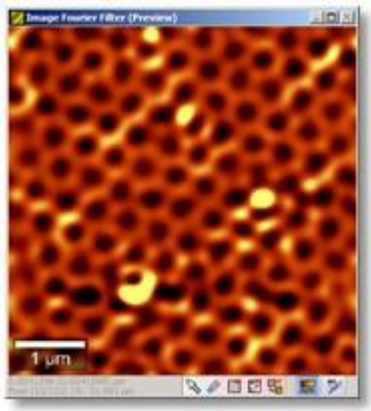
Extract (Button):
calculates and extracts the back-transformed image to the current project.

Extract Amplitude (Button)
extracts the amplitude image to the current project.

Preview Windows



The first preview image viewer shows the Fourier amplitude image. This viewer can be used to define a mask in order to cut frequencies for a back-transformation. Depending on the Type Parameter (Combo Box), this mask is automatically created.



The second preview image viewer shows the back-transformed image using the cut frequencies from the mask in the first preview image viewer.

Image Correlation Dialog

Description

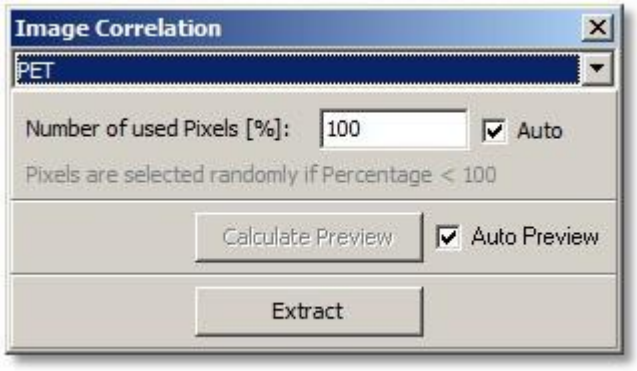
The Image Correlation Dialog creates graph objects containing information about the correlation of the intensity values of two different images.

Input and Results

Input:
at least two image data objects with the same spatial transformation.

Results:
one graph object for each dropped image object that can be shown as a correlation point cloud using the parametric display feature of the graph viewer.

User Interface



PET (Combo Box):
Selects the current image for the preview image viewer (e.g. as a helper for for mask manipulation).

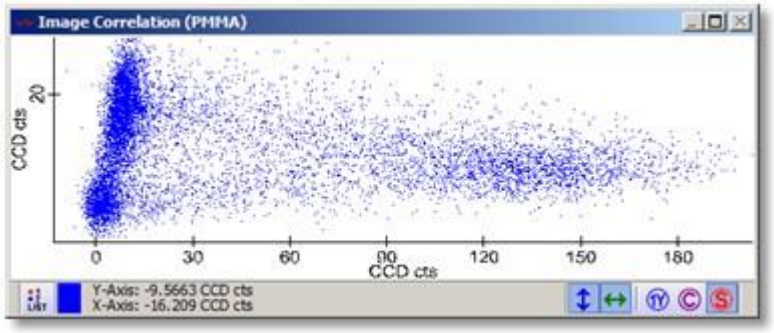
Number of used Pixels (Float Edit), Auto (Check Box):
Sets the percentage of image pixels, that shall be used for the correlation plot / for the graph object.
If not all pixels are used (<100%), the pixels are selected randomly each time the mask changes or the dialog opens.
For very large images, it's not possible to use all pixels (overall limit is 100000 or ~300x300 pixels), therefore the upper limit could be smaller than 100%.

Calculate Preview (Button), Auto Preview (Check Box):
see Automatic Preview.

Extract (Button):
Extracts one graph object for each dropped image.
Open the graph objects in the same graph viewer window in order to show a correlation plot.

Preview Windows

The preview image viewer shows the current selected image and the mask, that can be changed in order to define which image pixels should be used for the correlation plot.



The preview graph viewer shows the preview correlation plot.
Hint: Turn off the automatic zoom features if you don't want the automatic zoom when changing the mask or pixel percentage (e.g. if you zoomed manually because of extreme valued points).

Image Auto/Extended Focus Dialog

Description

The Image Auto/Extended Focus Dialog can be used to combine multiple images from a whole image stack in order to have a nice sum image (Extended Focus) or a maximum image (Auto Focus).

Input and Results

Input:
Any number of images that belong to the same stacked measurement.

Results:
One extended or auto focus image and a position image (for auto focus).

User Interface

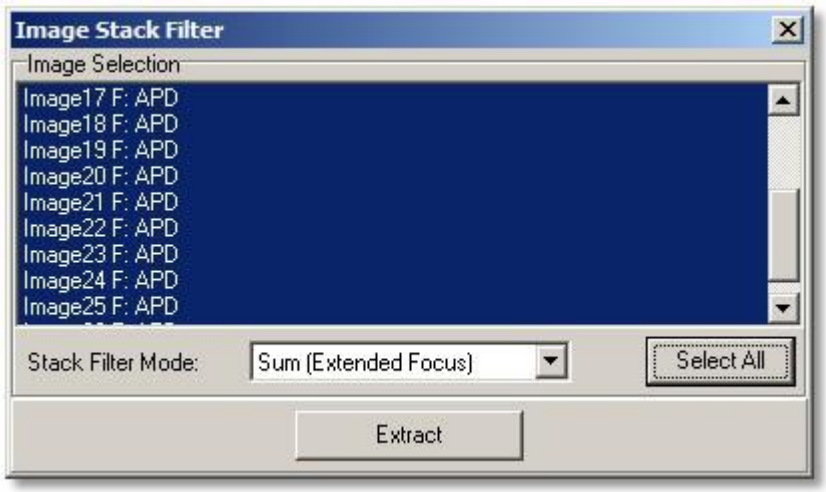


Image Selection (List Box):
You can select which images shall be used for the extended or auto focus operation. Use the shift or control keys to select more than one object.

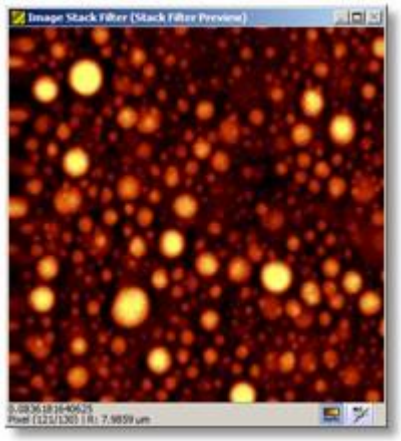
Select All (Button):
Selects all images that were dropped.

Stack Filter Mode (Combo Box):
Select a filter mode here:

- Sum: sums all selected images in order to provide an extended focus image
- Maximum: looks for the maximum intensity among all stacked images for each single xy-position; the found maxima are used in a so called "auto focus" image
- Position of Maximum: uses the spatial z-position of the image which has the maximum value of all images at each pixel

Extract (Button):
Calculates and extracts the results.

Preview Windows



The preview image viewer shows the sum or maximum or position of maximum image.

Image Stack Export Dialog

Description

The Image Stack Export allows to export multiple images as equally scaled bitmap files using a desired color profile and a fixed top/bottom for the color scale.
Stack layers get interpolated automatically in order to ensure cubic pixels - having a XYZ size ratio of 1.

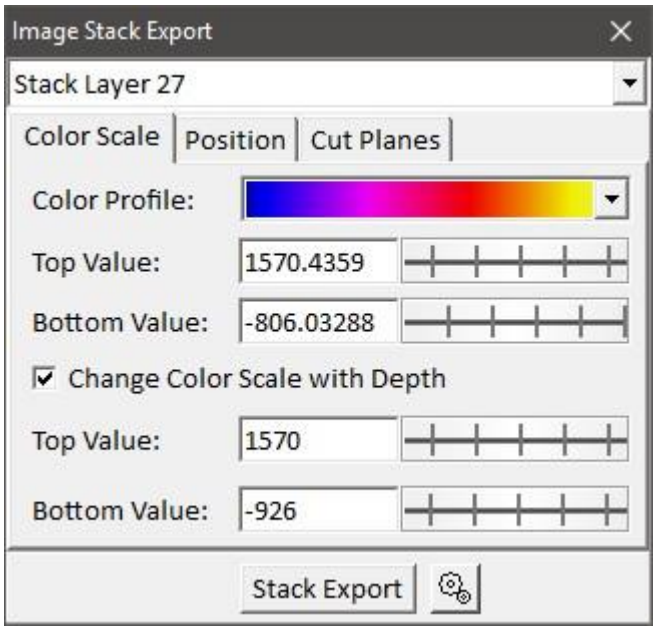
Input and Results

Input:
Any number of images that belong to the same stack measurement.

Results:
TIF image file containing all stack layers or multiple bitmap files on hard drive. Automatic transfer to external program available.

User Interface

Color Scale Tab



"Stack Layer 27" (Combo Box)
Here you can select which of the images should be shown in the XY preview window.

Color Profile (Color Combo Box)
Changes the color profile for all images.

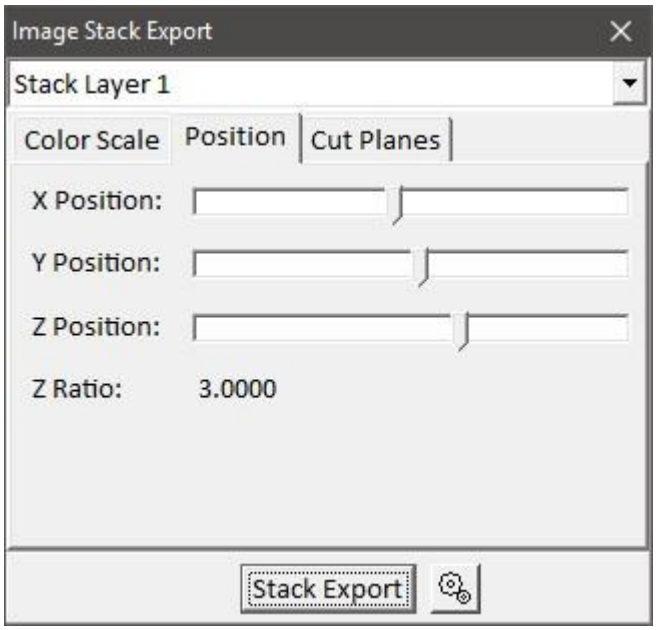
Top/Bottom Value (Edits and Sliders)
Changes the color scale for all images.

Change Color Scale with Depth (Check Box)
If checked, a second pair of Top/Bottom value edits appear and you have the chance to compensate changing intensities along the Z-Axis.
The upper Top/Bottom values define the color scale for the first layer of your stack, the lower Top/Bottom values for the last layer.
Layers between will get interpolated color scales.

Export (Button)
Exports the stack according to the Image Stack Export Options.

Options (Button)
Opens the Image Stack Export Options.

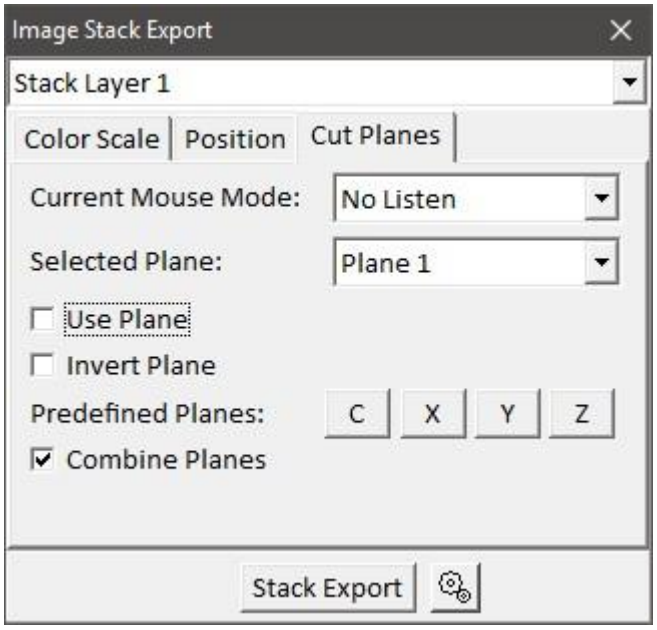
Position Tab



X/Y/Z Position (Slider)
Those sliders let you select the preview position in each direction.

Z Ratio (Label)
Shows the ratio between the xy pixel size and the z layer distance.

Cut Planes Tab



The cut planes tab allows you to "cut away" pixels from the stack (they will get black) in order to make them transparent in a volume or surface presentation.

Current Mouse Mode (Combo Box)

- No Listen: a mouse click into the preview images selects the preview position
- Listen Position: a mouse click into the preview images changes the position of the selected cut plane

- Listen Orientation: a mouse click into the preview images changes the orientation of the selected cut plane (angle)

Selected Plane (Combo Box)
Here you can select up to 3 different planes

Use Plane (Check Box)
If checked, the currently selected plane will be used for cutting away pixels.

Invert Plane (Check Box)
If checked, the currently selected plane will cut away pixels on the opposite side of the plane.

Predefined Planes ("C" Button)
Sets the plane position to the center of the image, keeping its orientation.

Predefined Planes ("X/Y/Z" Buttons)
Configures different default planes.

Combine Planes (Check Box)
If checked, multiple planes are combined which allows you to cut e.g. a "cube" out of your stack.

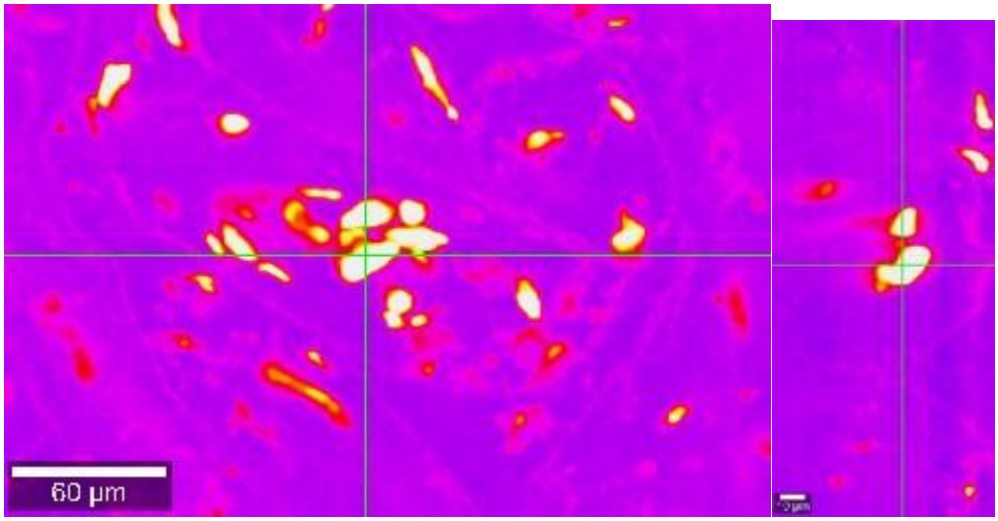
Preview Windows

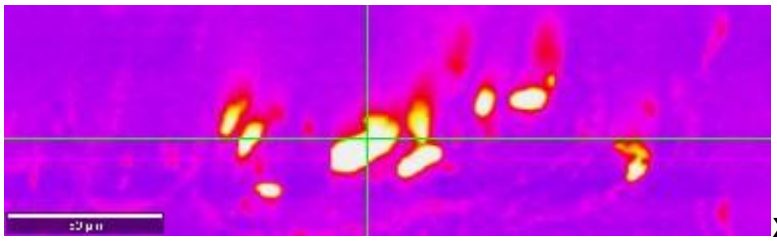
Preview XY
Shows the XY Plane. If you click in this image, the XZ and ZY Images will change.

Preview ZY
Shows the ZY Plane. If you click in this image, the XY and XZ Images will change.

Preview XZ
Shows the XZ Plane. If you click in this image, the XY and ZY Images will change.

XY **ZY**




XZ

Inverse Basis Analysis Dialog

Description

This dialog uses intensity distribution images together with an image spectrum measurement to calculate demixed basis spectra and an offset spectrum.

See also
Inverse Basis Analysis (Math)

Input and Results

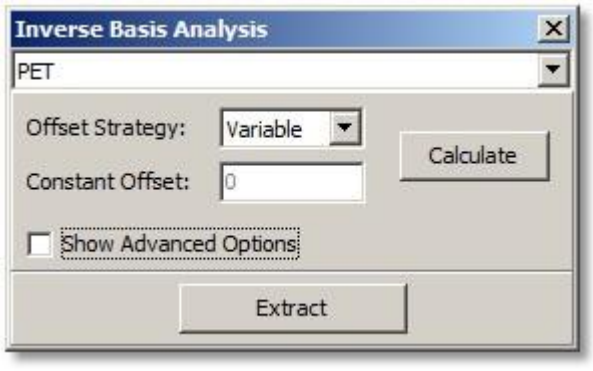
Input:

- One image spectrum data object
- <n> image data objects that belong the the image spectrum.
  Note that the images must be background subtracted and demixed (demixed means that the images must show high/bright values only for 1 component)

Results:

- One basis spectrum for each dropped image and the offset spectrum.
- Optionally, an error image can be extracted.

User Interface



PET (Combo Box):
Here you can select one of the input images. It will be shown in the preview image viewer in order to being able to define an image mask.

Offset Strategy (Combo Box):
Here you can select one of the offset strategies (chose the strategy depending on the offset of your spectra):

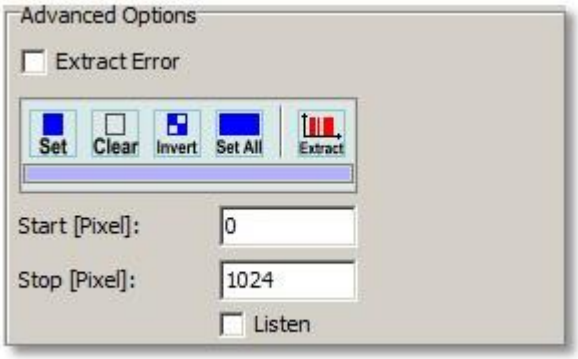
- Variable
- Constant

Constant Offset (Float Edit):
Enter a constant offset value, if your spectra have a constant offset.
Offset Strategy must be "Constant".

Calculate (Button)
This will start the inverse basis analysis and show the result in the preview windows.
Before you can calculate the inverse basis analysis, you have to chose which spectra should take part in the algorithm by drawing a mask in the image preview window.

Extract (Button):
Extract the current results.

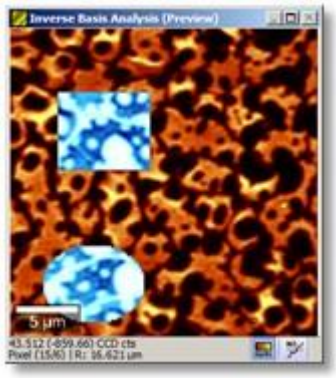
Show Advanced Options (Check Box):
Shows some advanced Options:



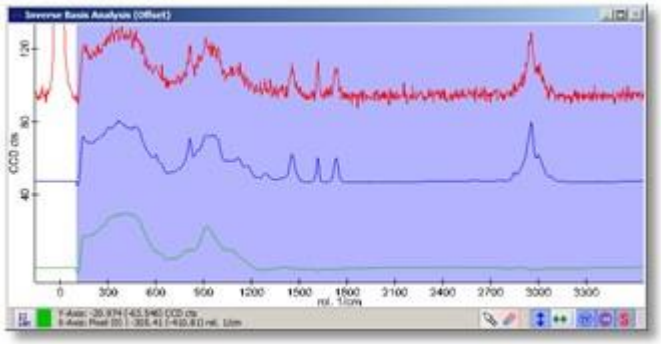
Extract Error (Check Box):
If checked, an additional error image is created when pressing the "Extract" Button.

Mask Controls (Tool Buttons and Edits):
You have to change the mask to define which spectral pixels should be used for the inverse basis analysis.
These edits are optional, you can change the masks directly in the preview graph viewer.

Preview Windows

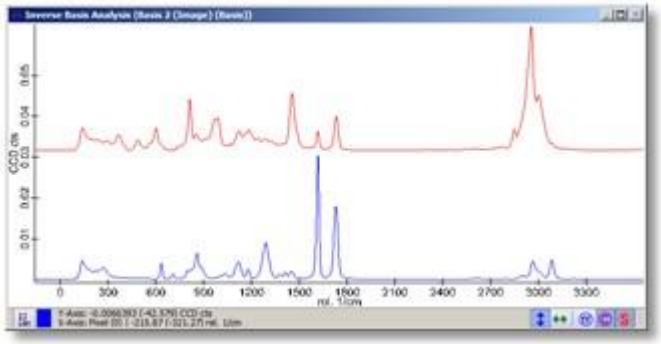


The preview image form shows the currently selected input image. You have to draw a mask here in order to define which image pixels should be used for the inverse basis analysis.



The first graph preview window shows:

- Original spectrum as a red graph
- The fit preview as a blue graph
- The offset preview as a green graph



The second graph preview window shows the preview basis graphs, one for each dropped image.

Image Transform and Overlay Dialog

Description

The Image Transform and Overlay Dialog has the following abilities:

- To create an overlay of two images/bitmaps (e.g. show the chemical Raman image on a video image)
- To change a spatial transformation data object for correcting displacements (Position, Scale, Rotation) or just to change it in the preview for bitmap creation
- Advanced Transformations for bitmap creation: Affine mapping, Bilinear mapping, Multiple-Affine mapping via triangle mesh

See also
Creating Raman Overlay
Transformation Data

Input and Results

Input:

- Background Image (Image or Bitmap Data Object)
- Overlay Image (Image or Bitmap Data Object)

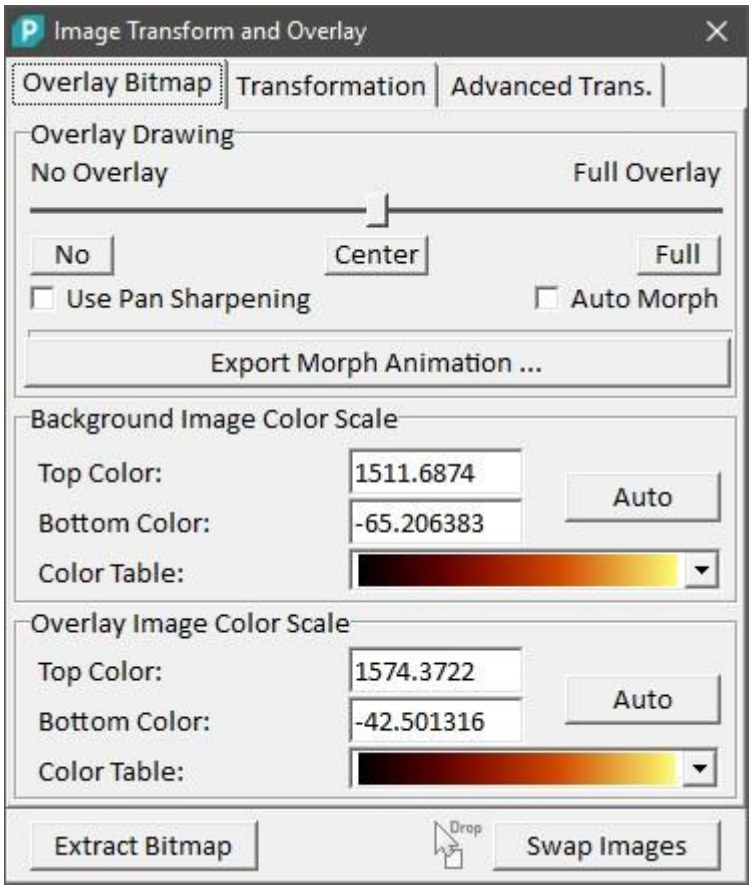
Results:

- Overlay Bitmap
- Changed Space Transformation of an existing data object

User Interface

- Overlay Bitmap tab
- Transformation tab
- Advanced Transformation tab
- Preview windows

Overlay Bitmap Tab



Overlay Drawing (Slider):
With this slider you can change the opacity of the overlay image.

Use Pan Sharpening (Check Box):
If checked, the background image defines the brightness (therefore only black and white) and the overlay image defines the color:

- an overlay slider position from "No Overlay" to "Center" defines the opacity of the overlay color.
- an overlay slider position from "Center" to "Full Overlay" defines the opacity of the overlay intensity.

If not checked, the background image keeps its colors and the overlay image is just overlayed.

Auto Morph (Check Box):
If checked, the overlay image opacity is automatically changed smoothly (this can be helpful e.g. for adjusting the transformation while comparing features in the overlay image with features of the

background image).

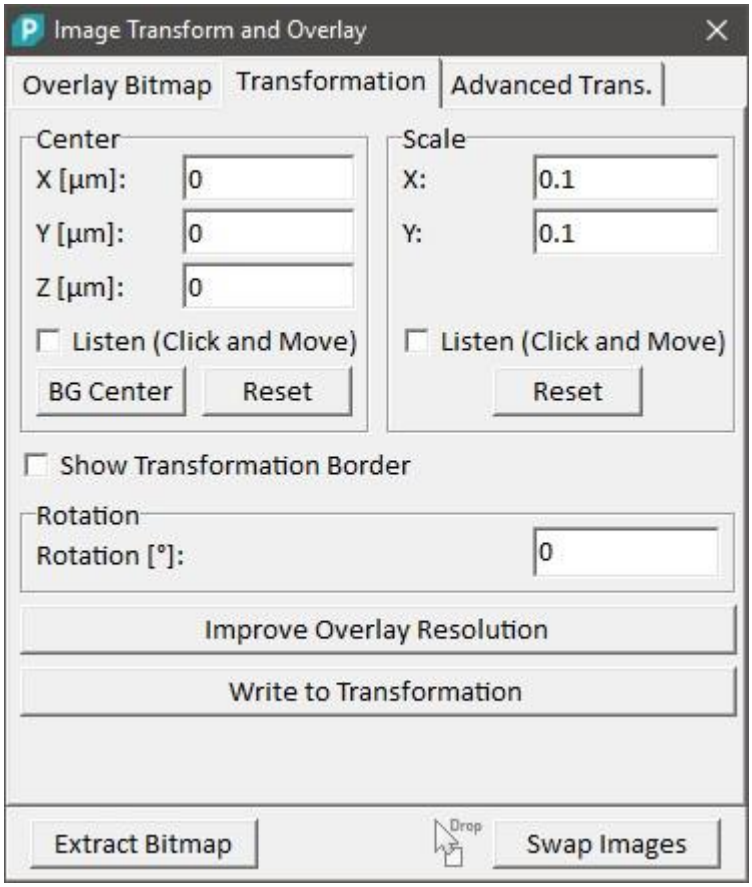
Export Morph Animation (Button):
Opens the Animation Editor for exporting an animation. Uses only the Overlay Parameter for the animation.

Background/Overlay Image Color Scale (group boxes):
The contrast/brightness of a color bitmap or the color scale of an floating point image can be changed - depending on what kind of data objects are used for this dialog.

Swap Images (Button, Drop Zone):
Pressing this button causes the dialog to re-initialize using the current overlay image as the background image and vice versa.
This is also a drop zone. You can drop another overlay image onto this button in order to preserve all settings and transformations. This only works if the dropped image uses the an equal spatial transformation.

Extract Bitmap (Button):
Pressing this button extracts the current preview as a new color bitmap data object and adds it to the current project.

Transformation Tab



Center (Group Box):
With the X/Y/Z edits the current absolute position of the overlay image can be seen and changed.
Note: A much better way to change the position is the following described Listen mechanism.

Listen (Check Boxes):
If checked, the overlay image in the preview image viewer can be clicked and moved in order to change the absolute position or the scale of the overlay image.

BG Center (Button):
Sets the center position of the overlay image to the center of the background image (makes sense if the transformation of the overlay image is completely different, e.g. when importing an image).

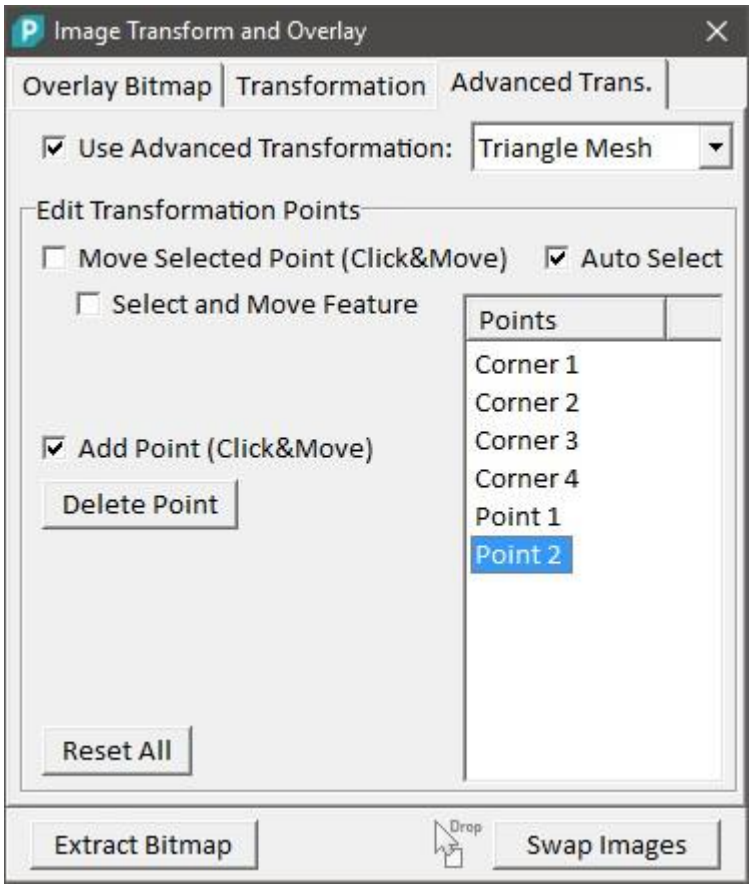
Reset (Button):
Resets the center or scale position of the overlay image using the original transformation.

Rotation (Float Edit):
Changes the rotation of the overlay image (this parameter will only rotate the image on the axis perpendicular to the image plane).

Improve Overlay Resolution (Button):
Creates a new background image or bitmap by increasing its resolution.
This is useful if the overlay image has a higher resolution than the background image (on the overlay region).
After the improvement, the background image has enough pixels such that the overlayed image can be displayed in its full resolution. Note that you will only see a difference in the overlay region.

Write to Transformation (Button):
This will overwrite the spatial transformation data of the overlay image (not only the preview transformation!).
This may affect other data objects that use the same spatial transformation (images and image graph objects). In general this means objects created by the same measurement or were results of an analysis of the same measurement. You'll get a list of all affected data objects before you can confirm or cancel the permanent transformation change.

Advanced Transformation Tab



Use Advanced Transformation (Check Box):
Turns on or off the advanced transformation.

Advanced Transformation Selector (Combo Box):
One can chose between the following advanced transformations:

- Affine (Affine mapping of 3 points, see Affine Transformation Math)
- Bilinear (Bilinear mapping of 4 points, see Bilinear Transformation Math)
- Triangle Mesh (Any number of points that create a triangle mesh; using affine mapping for each triangle, see Triangulation Math)

Move Selected Point (Check Box):
Turns on the listen mechanism to change the position of a single point. If the **Auto Select Check Box** is checked, the point next to the mouse cursor will listen to the cursor position; otherwise, the currently selected point in the Point List will listen.

Select and Move Feature (Check Box):
If checked, the mouse cursor position at the moment of pressing the mouse button down will be used as the source position for distortion. Use this feature if you would like to click on a feature in the overlay image and want to "move" it onto the corresponding feature on the background image.

Add Point (Check Box):
Adds a new point in the Triangle Mesh mode. When pressing the mouse down the point is added using the source position of the clicked position. If you move the mouse afterwards the target of the point is changed.

Delete Point (Button):
Deletes the currently selected point in the point list. Corner points can't be deleted.
Be careful: if the Auto Select Check Box is checked, you might accidentally select another point

before deleting.

Reset All (Button):
Deletes all custom points and resets the corner positions tho the original image corner positions.

Preview Windows

The preview image form shows the background and overlay image.
In Advanced Transformation Mode, it also shows the currently selected point which makes it easy to use customized distortion effects.

Automatic Preview

All data analysis dialogs (drop action dialogs) have preview windows showing a preview of the result(s).
Some of the dialogs need some calculation time if very large data objects are used.
They show the following options:

Auto Preview (Check Box):
With this check box you can turn off the auto preview in order to change settings or masks without being disturbed by a longer running automatic calculation.
This option is automatically turned off if the dialog opens with a very large objects (e.g. images with > 700 x 700 Pixels).

Calculate Preview (Button):
If the auto preview mode is turned off, you can manually do the preview calculation once by pushing that button.

Listen Cursor Mechanism

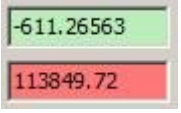
Some parts of the software can listen to a cursor position that was sent from another window, e.g. from the Graph- or Image-Viewer.

There are implicit and explicit listening modes:

Implicit listening happens automatically, e.g. if you click into an image viewer, a graph viewer listens to the spatial position and shows the corresponding spectrum of a image graph data object.

Explicit listening is activated by a dialog or and edit automatically or has to be activated by the user. There are the following possibilities for this explicit listening:

Edits with Listen Capability



All edit controls in the software that have a green background color are "listenable".
That means their value can be changed by sending a cursor position from other windows.
You can **turn on or off the listening mode by double-clicking** into the edit control.
Edit controls that are currently listening have a red background color.

It depends on the value kind to which unit or cursor the edit control will listen.

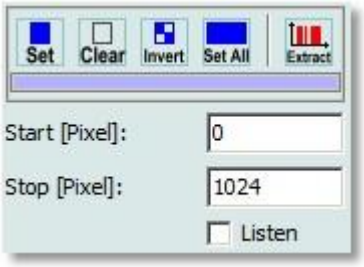
Manipulating Masks via Listen Cursor Mechanism

See Mask Manipulation Tools.

Mask Manipulation Tools

Although masks can be manipulated very fast directly in the Graph Viewer (see Graph Viewer Mask Manipulation), it is possible to set or clear a pixel range exactly defined by the user.

All data analysis dialogs that support mask manipulation offer a belonging mask user control:



Set (Tool Button)
sets a mask at the range given by the Start/Stop Pixel Edits aside.

Clear (Tool Button):
clears the mask at the range given by the Start/Stop Pixel Edits aside.

Invert (Tool Button):
inverts the complete mask.

Set All (Tool Button):
sets all pixels in the mask.

Extract (Most Right Tool Button):
extracts the mask as a single graph object to the project.
You can drag and drop such a mask graph object onto the mask in the graph viewer (see Graph Viewer Drag and Drop) or onto one of the mask tool buttons to reuse it.

Color Bar:
The Color Bar below the tool buttons shows the color in the preview graph window of the mask that is being changed by the buttons above.
If multiple masks are changed, no color bar is visible.

Start [Pixel] (Integer Edit);
the beginning of the range that will be cleared or set in the mask using the Set or Clear Tool Buttons.

Stop [Pixel] (Integer Edit):
the end of the range that will be cleared or set in the mask using the Set or Clear Tool Buttons.

Listen (Check Box):
If the Listen Check Box is checked, the start and stop pixel edits will listen to a spectral range sent by any Graph Viewer window using the Mouse Marker.

See also Listen Cursor Mechanism

Math

Image (Def)

The following symbols are used for 2-dimensional image data:

$I_{i,j}$: *Image value*
i,j : *Pixel position*
$x_{i,j}$: $X - Coordinate$
$y_{i,j}$: $Y - Coordinate$
$z_{i,j}$: $Z - Coordinate$
N : *Pixels per row* $i = 0..N-1$
M : *Pixels per column* $j = 0..M-1$

Average Spectrum (Math)

The following formulas are used to calculate an average spectrum from a set of spectra with given weighting factors:

$$\vec{S}_{Ave} = \frac{1}{W}\sum_{i=0}^{N-1} w_i \vec{S}_i \qquad W = \sum_{i=0}^{N-1} w_i$$

N : *Number of spectra*
$\vec{S}_i$: $i - th$ *spectrum*
w_i : $i - th$ *weighting factor*
$\vec{S}_{Ave}$: *Average spectrum*
W : *Sum of weighting factors*

See also

- Spectrum (Def)

Remarks

No Weighting

The weighting factors equal 1 if no weighting is used. In this case the sum of weighting equals the number of spectra.

Boolean Weighting

In most cases the weighting factors are given by a mask that was set by the user or by some algorithm. In this case the weighting factor equals one if the mask is set, and equals zero if the mask is not set. The sum of the weighting factors equals the number of spectra selected by the mask.

Filter Viewer (Math)

This section describes the math behind the Filter Viewer. This includes the background subtraction which is done before the Filter is calculated.

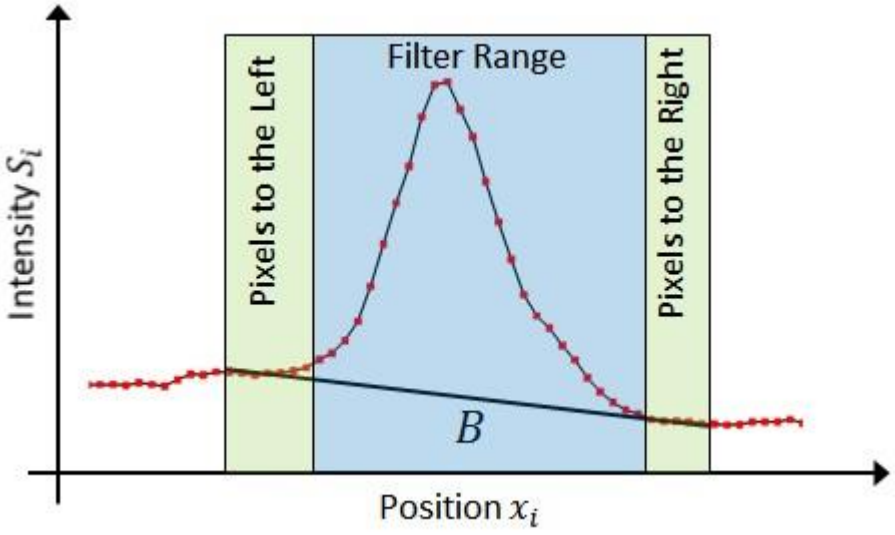
Background Estimation

$$S_i = \tilde{S}_i - B(x_i)$$

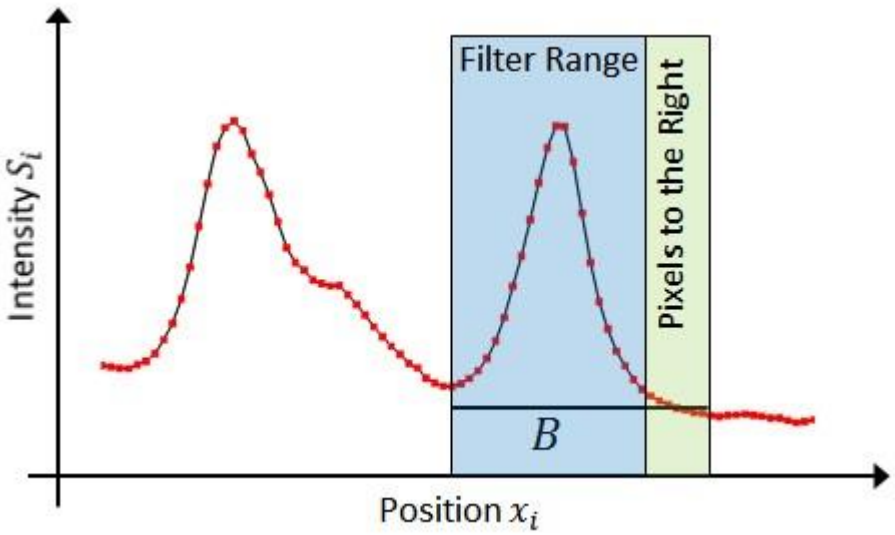
$\tilde{S}_i$: *Spectrum with background*
S_i : *Spectrum without background*
$B(x_i)$: *Background estimation at position* x_i
x_i : *Spectral position at pixel i*

The background B is calculated using the neighbor pixels of the filter range. The number of pixels to the right and to the left can be defined in the Filter Viewer.

If the number of pixels to both sides is larger than 0 a slope is calculated. The average intensity and the average positions on both sides define the coordinate for the slope calculation.



If the number of pixels is zero on one side a horizontal line is used as background estimation. It is calculated by the intensity average of the other side.



If both number of pixels are 0, no background subtraction is performed.

Filter Calculation

The following notation is used for the filter calculations.

n_l : *leftmost pixel inside the filter range*
n_r : *rightmost pixel inside the filter range*

Sum Filter

$$F_{sum} = \sum_{i=n_l}^{n_r} S_i$$

Average Filter

$$F_{ave} = \frac{1}{n_r - n_l + 1} \sum_{i=n_l}^{n_r} S_i$$

Average minus Minimum Filter

$$F_{ave-min} = F_{ave} - F_{min}$$

Standard Deviation Filter

$$F_{\sigma} = \sqrt{\frac{1}{n_r - n_l + 1} \sum_{i=n_l}^{n_r} (S_i - F_{ave})^2}$$

Center of Mass Filter

$$F_{CoM} = \frac{1}{F_{sum}} \sum_{i=n_l}^{n_r} x_i S_i$$

Peak Width Filter (FWHM)

Starting from the F(pos of max) the algorithm searches to the right and the left direction at which the signal falls below 0.5*F(max). The neighbor pixels of these two positions are used in a linear model to calculate the FWHM.

Minimum Filter

$$F_{min} = min\{S_i : n_l \leq i \leq n_r\}$$

Maximum Filter

$$F_{max} = max\{S_i : n_l \leq i \leq n_r\}$$

Position of Minimum Filter

$$F_{pos\ of\ min} = first\{x_i : S_i = F_{min}, n_l \leq i \leq n_r\}$$

Position of Maximum Filter

$$F_{pos\ of\ max} = First\{x_i : S_i = F_{max}, n_l \leq i \leq n_r\}$$

Line Correction (Math)

Each line of an image is subtracted by a k-order polynomial. The coefficients of the polynomial are calculated by fitting the polynomial to the line data.

$$I_i^{sub} = I_i - P_k(\vec{a}|i)$$

$$P_k(\vec{a}|i) = \sum_{m=0}^{k} a_m(i)^m$$

$$\sum_{i=0}^{N-1} w_i(I_i - P_k(\vec{a}|i))^2 = Minimum$$

I_i : *Image value*
I_i^{sub} : *New image value*
$P_k(\vec{a}|i)$: *Polynomial of order k*
$\vec{a}$: *Fit parameter*
i : *Pixel position*
w_i : *Weighting factor*

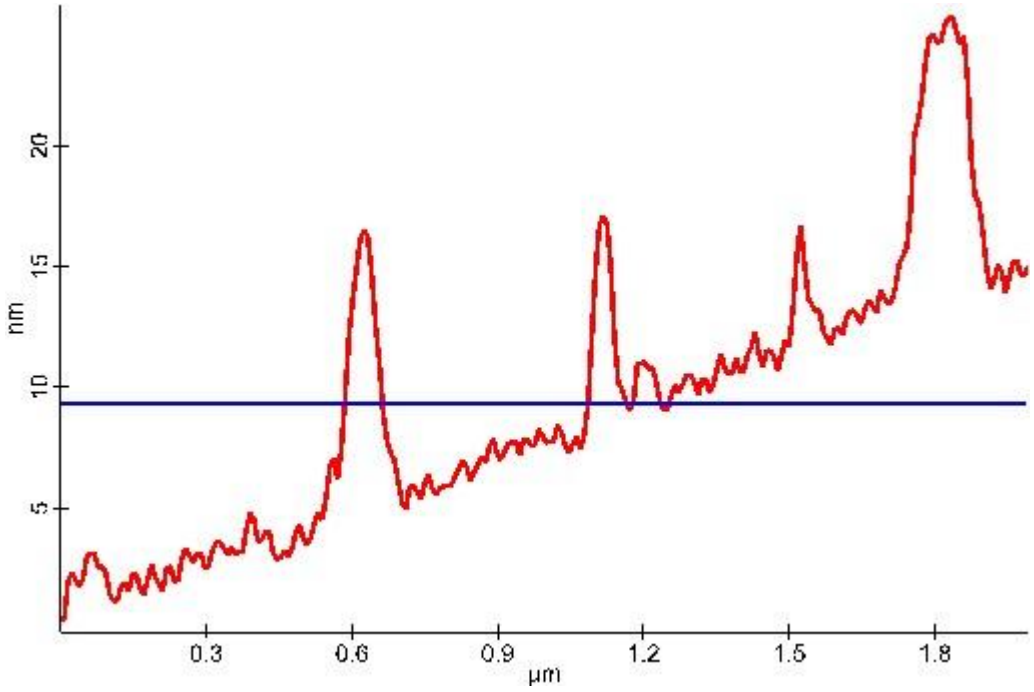
See also

- Image (Def)
- Surface Correction (Math)

Remarks

Average Subtraction

Average subtraction is a special case of the above formula. Here the polynomial is of the order zero. The polynomial is a constant and is equal to the average of the image values.



Average Division

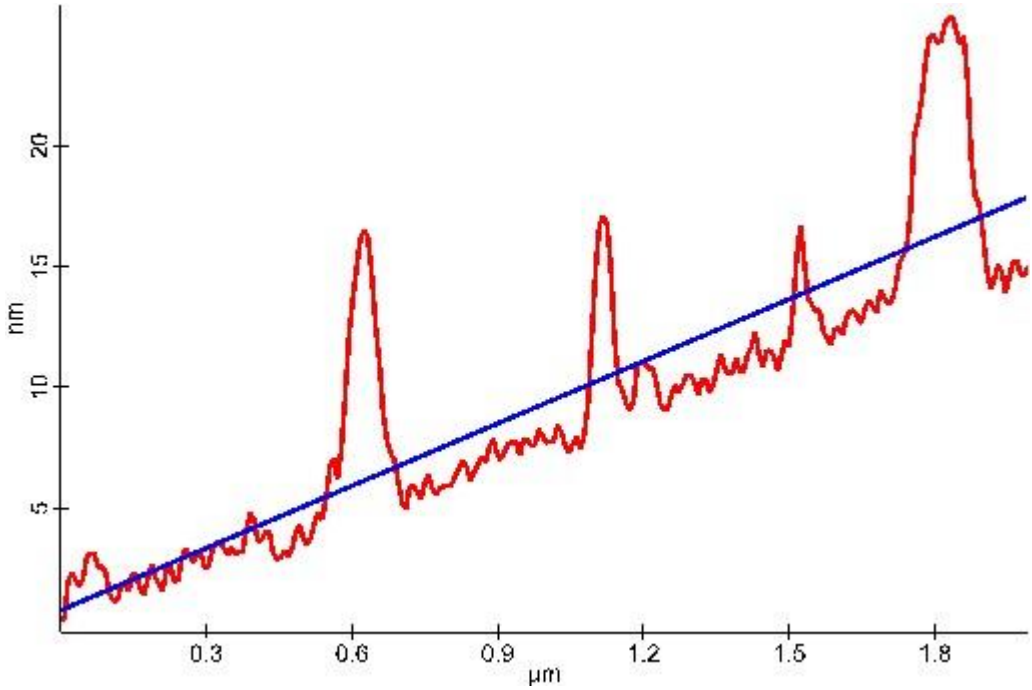
Instead of subtracting the average from the line data, the line data is divided by the average.

$$I_i^{div} = \frac{I_i}{\langle I \rangle}$$

$$\langle I \rangle = \frac{1}{N} \sum_{i=0}^{N-1} I_i$$

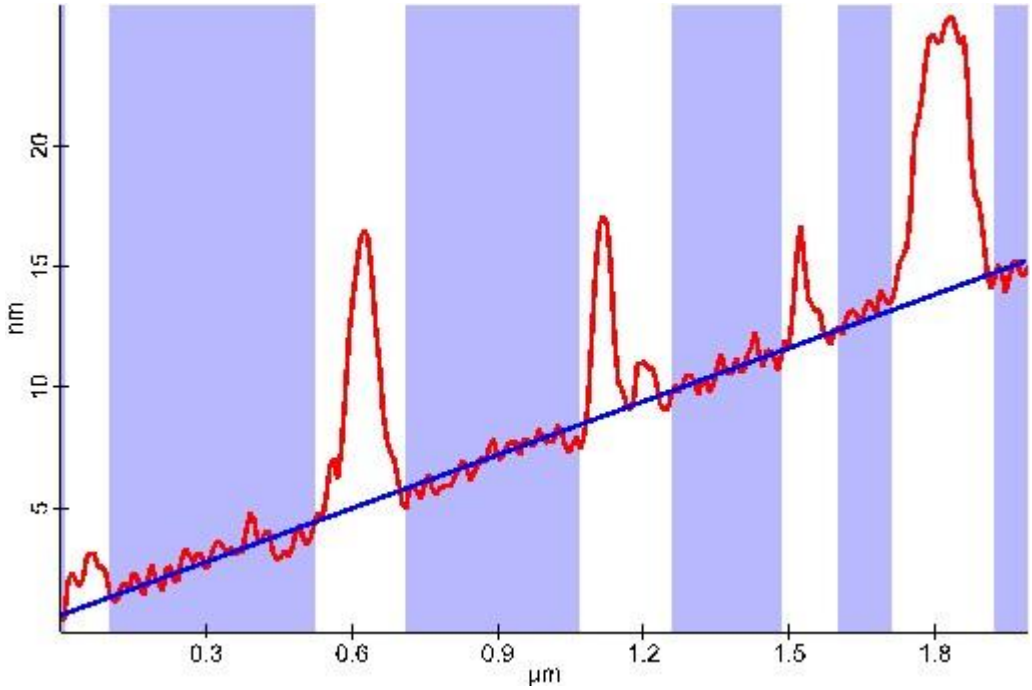
Slope Subtraction

Slope subtraction is a special case of the above formula. Here the polynomial is of the order one (straight line).



Weighting Factors

Using weighting factors ensures that the calculated slope is parallel to some flat surface. Usually the weighting factors are a boolean mask set by the user.



Surface Correction (Math)

To correct the slope of an complete image a 2-dimensional polynomial of k-order can be

subtracted.

$$I_{i,j}^{sub} = I_{i,j} - P_k^{2D}(\vec{a}|i,j)$$

$$P_k^{2D}(\vec{a}|i,j) = \sum_{m=0}^{k}\sum_{n=0}^{m} a_{m,n}(i)^n(j)^{m-n}$$

$$\sum_{j=0}^{M-1}\sum_{i=0}^{N-1} w_{i,j}\left(I_{i,j} - P_k^{2D}(\vec{a}|i,j)\right)^2 = Minimum$$

$I_{i,j}$: *Image value*
$I_{i,j}^{sub}$: *New image value*
$P_k^{2D}(\vec{a}|i,j)$: *2D − Polynomial of order k*
$\vec{a}$: *Fit parameter*
i,j : *Pixel position*
$w_{i,j}$: *Weighting factor*

See also

- Image (Def)
- Line Correction (Math)

Spatial Transformation (Math)

This transformation converts the pixel coordinates into real world space coordinates and vice versa.

$$\vec{x} = \hat{R}\hat{S}\left(\vec{b} - \vec{b}_0\right) + \vec{x}_o$$

$\vec{x} = \begin{pmatrix} x \\ y \\ z \end{pmatrix}$: *Spatial position*
$\vec{x}_o$: *Spatial offset*
$\vec{b} = \begin{pmatrix} i \\ j \\ k \end{pmatrix}$: *Pixel position*
$\vec{b}_0$: *Pixel offset*
$\hat{R}$: *Rotation matrix*
$\hat{S}$: *Scale matrix*

Remarks

Scaling Matrix

$$\hat{S} = \begin{pmatrix} s_x & 0 & 0 \\ 0 & s_y & 0 \\ 0 & 0 & s_z \end{pmatrix}$$

$$s_x, s_y, s_z \quad : Pixel\ size\ (e.g. \mu m/pixel)$$

Rotation Matrix

$$\hat{R} = \hat{R}_\alpha \hat{R}_\beta \hat{R}_\gamma$$

$$\hat{R}_\alpha = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\alpha & -\sin\alpha \\ 0 & \sin\alpha & \cos\alpha \end{pmatrix}$$

$$\hat{R}_\beta = \begin{pmatrix} \cos\beta & 0 & \sin\beta \\ 0 & 1 & 0 \\ -\sin\beta & 0 & \cos\beta \end{pmatrix}$$

$$\hat{R}_\gamma = \begin{pmatrix} \cos\gamma & -\sin\gamma & 0 \\ \sin\gamma & \cos\gamma & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

$$\hat{R} = \begin{pmatrix} \cos\beta\cos\gamma & -\cos\beta\sin\gamma & \sin\beta \\ \sin\alpha\sin\beta\cos\gamma + \cos\alpha\sin\gamma & -\sin\alpha\sin\beta\sin\gamma + \cos\alpha\cos\gamma & -\sin\alpha\cos\beta \\ -\cos\alpha\sin\beta\cos\gamma + \sin\alpha\sin\gamma & \cos\alpha\sin\beta\sin\gamma + \sin\alpha\cos\gamma & \cos\alpha\cos\beta \end{pmatrix}$$

$$\alpha \quad : Rotation\ angle\ of\ x-axis$$
$$\beta \quad : Rotation\ angle\ of\ y-axis$$
$$\gamma \quad : Rotation\ angle\ of\ z-axis$$

Redundancy and Limitations

This form of a 3D linear transformation has some redundant parameters, but it is more convenient. The pixel position offset belongs directly to the spatial position. Also the separation of scaling and rotation makes it easier to analyse the transformation. A shearing matrix is not implemented in order to keep the main spatial directions perpendicular to each other.

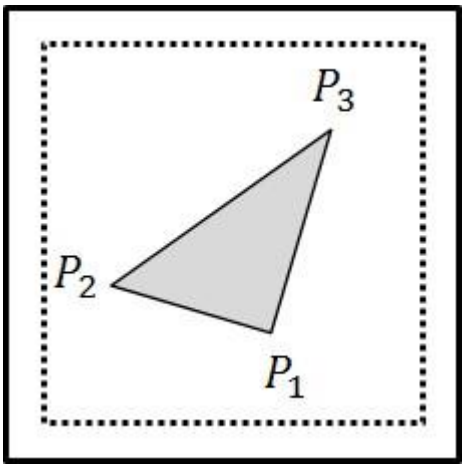
Affine Transformation (Math)

The coordinates of 3 points are transformed into coordinates of 3 new points. The transformation is a linear transformation which includes
translation, scaling, shearing and rotation. The affine transformation transforms a line into a line, a

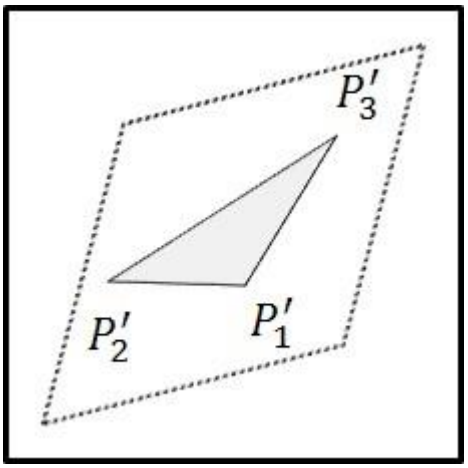
triangle into a triangle and a square into parallelogram.

$$\vec{x}' = \hat{M}\vec{x} + \vec{x}_0$$

$\vec{x}'$: *New point*
$\vec{x}$: *Old point*
$\hat{M}$: *Matrix for scaling, shearing and rotation*
$\vec{x}_0$: *Translation*



Original Image



Transformed Image

See also

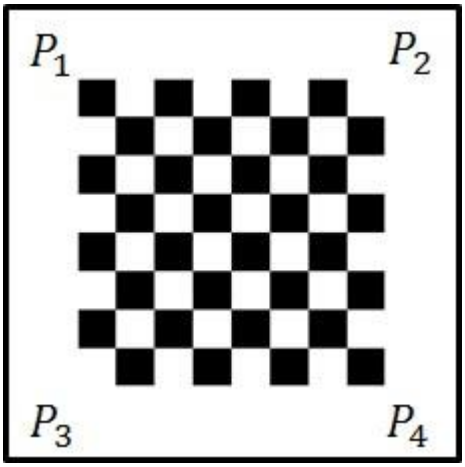
- Bilinear Transformation (Math)
- Spatial Transformation (Math)
- Delaunay-Triangulation (Math)

Bilinear Transformation (Math)

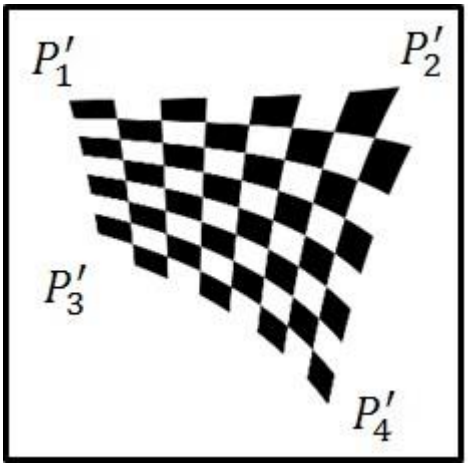
The coordinates of 4 points are transformed into coordinates of 4 new points.

$$x' = a_1 x + a_2 y + a_3 xy + a_4$$
$$y' = b_1 x + b_2 y + b_3 xy + b_4$$

$\vec{x}'$: *New point*
$\vec{x}$: *Old point*
$\vec{a}, \vec{b}$: *Fit parameter*



Original Image



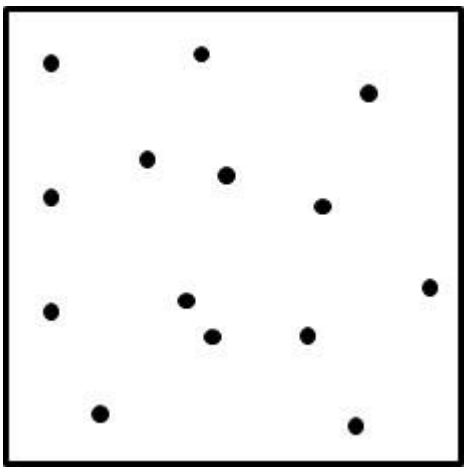
Transformed Image

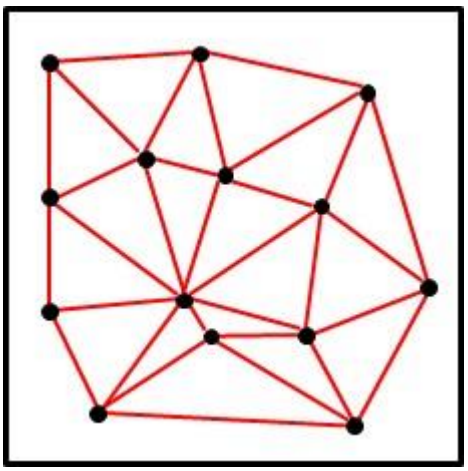
See also

- Affine Transformation (Math)
- Spatial Transformation (Math)
- Delaunay-Triangulation (Math)

Delaunay-Triangulation (Math)

Beginning from a set of points in 2D-space, the Delaunay-Triangulation creates a triangle mesh. This mesh is always a convex area of all points. At each point a additional information is stored. This information together with the coordinates of the points allows to interpolate into the free space between the points.


Set of Points


Delaunay-Triangulation

Remarks

Local Affine Transformations

Together with the Affine Transformation a mesh which describes local deformation can been defined. The old point coordinates are used for triangulation, the new point coordinates as

additional information. This allows to calculate the deformation inside a triangle.

Superposition of Spectra (Geometric View)

Many mathematical descriptions are made easier to understand by using a geometric model of a hyperspectral dataset. This geometric model works for data that follows the superposition principle, particularly Raman spectra which contain a mixture of components.

A complete hyperspectral dataset of two components can be described by three spectra: a constant background spectrum and two component spectra with their corresponding mixing values:

$$\vec{S}_i = h_i^{B_0}\vec{S}_{B_0} + h_i^{B_1}\vec{S}_{B_1} + \vec{S}_{BG}$$

$\vec{S}_{BG}$: *Constant background spectrum*

$\vec{S}_{B_0}, \vec{S}_{B_1}$: *Component Spectra*

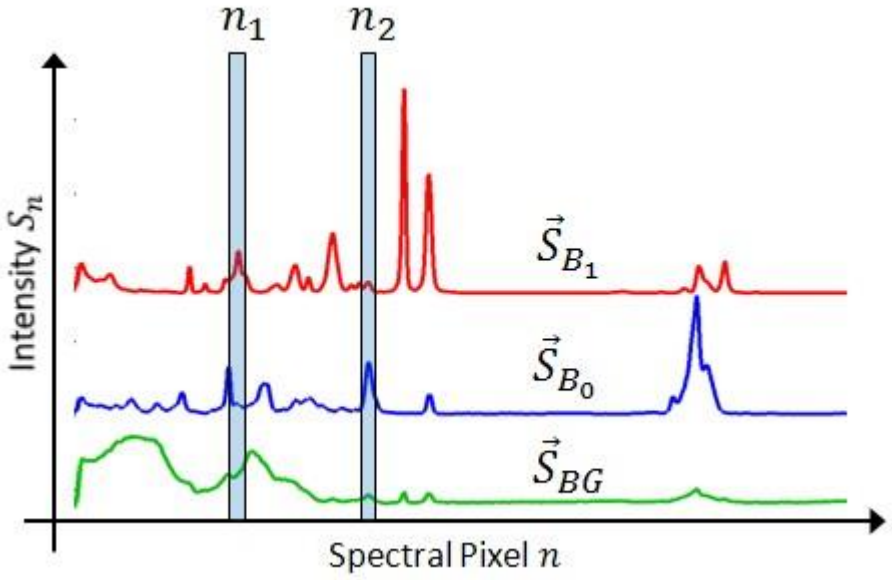
S_{n_1}, S_{n_2} : *Intensity at spectral pixel* n_1, n_2

$\vec{S}_i$: *Spectrum* i *from the hyper spectral dataset*

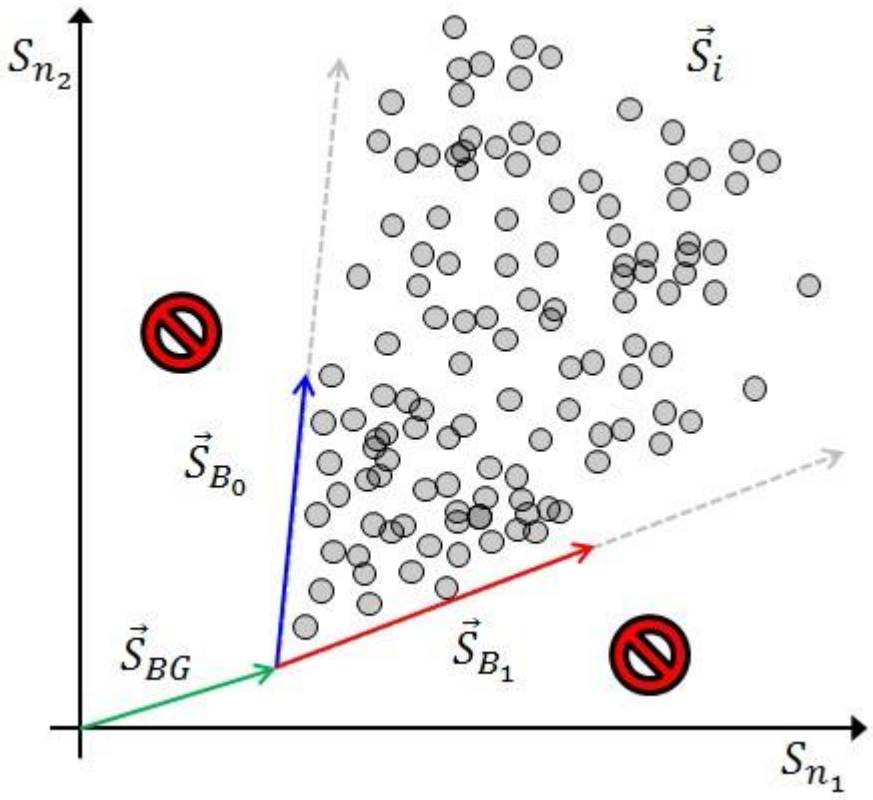
$h_i^{B_0}, h_i^{B_1}$: *Mixing values for spectrum* i

Usually, the component spectra are normalized to one (area normalization). In this case, the mixing values are exactly the amount of signal that belongs to the corresponding component.

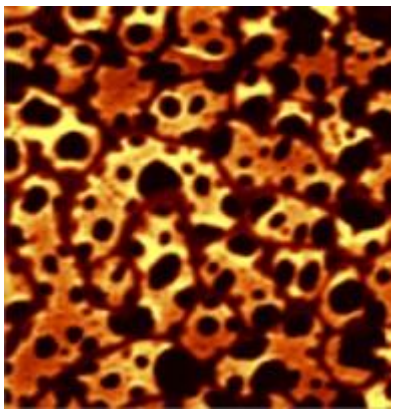
In order to plot the high dimensional vector equation above, a reduction of the dimensions i.e. projection into 2D space is necessary. Two intensity values at spectral pixels n1 and n2 are used as coordinates for the 2-dimensional projection plane:



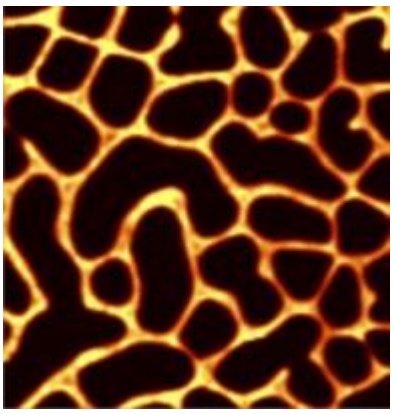
Using these two coordinates, each grey dot in the image below represents a spectrum of the hyperspectral dataset:



If the hyperspectral data is extracted from an image scan, the mixing values can be displayed as images:



$h_i^{B_0}$



$h_i^{B_1}$

Remarks

Limitations
The vector equation is **not** adequate for Raman spectra with Peak-shift, PFM-curves or CARS spectra.

Constant Background Spectrum
The origin of the background spectrum is unimportant as long as it is constant. Examples of constant background spectra are:

- Offset from CCD Camera
- Thermal generated electrons
- Signal from substrate if it is constant (planar scan).

Superposition of Spectra (Math)

One of the models that describe a hyper spectral dataset is based on the principle of superposition. Usually the spectra from the hyper spectral dataset are mixed spectra from different components. Each component points in a different direction of the high dimensional vector space. These spectra or a linear combination of them form a basis which describes only a sub-space of the complete vector space. With this basis all measured spectra can be described as linear combinations.
In addition a constant spectrum, which is equal for all spectra of the dataset, is introduced. Noise from data acquisition and signal which can not be described will be added to the error. Please read Superposition of Spectra (Geometric View) for a further understanding.

$$\hat{S} = \hat{B}\hat{H} + \vec{S}_C\vec{1}^T + \hat{E}$$

$\hat{S}$: *Hyper spectral dataset matrix*
$\hat{B}$: *Matrix of Basis spectra*
$\hat{H}$: *Mixing values as matrix*
$\vec{S}_C$: *Constant spectrum*
$\vec{1}^T$: *Vector filled with 1*
$\hat{E}$: *Error matrix*

Matrix Representation of Hyper Spectral Dataset

$$\hat{S} = (\vec{S}_0 \quad \cdots \quad \vec{S}_m \quad \cdots \quad \vec{S}_{M-1})$$

$\vec{S}_m$: *Spectrum m from the hyper spectral dataset*
M : *Number of spectra in dataset*

See also: Spectrum (Def)

Matrix Representation of Component Spectra (Basis)

$$\hat{B} = (\vec{S}_{B_0} \quad \cdots \quad \vec{S}_{B_n} \quad \cdots \quad \vec{S}_{B_{N-1}})$$

$\vec{S}_{B_n}$: *Basis spectrum n*
N : *Number of basis spectra*

Matrix Representation of Mixing Values

$$\hat{H} = \begin{pmatrix} h_0^{B_0} & \cdots & h_m^{B_0} & \cdots & h_{M-1}^{B_0} \\ \vdots & \ddots & \vdots & & \vdots \\ h_0^{B_n} & & h_m^{B_n} & & h_{M-1}^{B_n} \\ \vdots & & \vdots & \ddots & \vdots \\ h_0^{B_{N-1}} & \cdots & h_m^{B_{N-1}} & \cdots & h_{M-1}^{B_{N-1}} \end{pmatrix}$$

$h_m^{B_n}$: *Mixing value for basis spectrum n and spectrum m*
M : *Number of spectra in dataset*
N : *Number of basis spectra*

Basis Analysis (Math)

The idea of the basis analysis is to describe a measured spectrum or a complete hyper spectral dataset by a linear combination of spectra, which are already known. The mixing values, which are used for the linear combination, is the result of this analysis. In addition to the basis spectra the constant background spectrum must also be known in advance. Usually the latter one is subtracted before using the basis analysis algorithm. This algorithm is based on the basic equation, which is explained in the article Superposition of Spectra (Math):

$$\hat{S} = \hat{B}\hat{H} + \vec{S}_C\vec{1}^T + \hat{E}$$

For basis analysis the equation can be reduced to:

$$\vec{S}_i = \hat{B}\vec{H}_i + \vec{E}_i$$

$\vec{S}_i$: *Spectrum i from the hyper spectral dataset*
$\vec{H}_i$: *Mixing values spectrum i*
$\vec{E}_i$: *Error spectrum*
$\hat{B}$: *Matrix of Basis spectra*

The mixing values are fitted by the method of least squares minimizing the following expression:

$$(\vec{S}_i - \hat{B}\vec{H}_i)^2 = Minimum$$

Percentage Images

Percentage images are calculated using this formula:

$h_m^{B_n}$: *Mixing value for basis spectrum n and spectrum m*
$\tilde{h}_m^{B_n}$: *Normalized mixing value*
i : *Pixel index*
N : *Number of basis spectra*

$$\tilde{h}_i^{B_k} = \frac{h_i^{B_k}}{\sum_{k=0}^{N-1} \left| h_i^{B_k} \right|} \times 100\%$$

See also

- Inverse Basis Analysis (Math)
- Non-Negative Matrix Factorization (Math)

Remarks

Choosing the Correct Basis

In order to analyse Raman spectra it is important first to use a basis matrix including all present components and second that the basis spectra are pure spectra. This assures that the result makes physical sense. If the basis spectra are taken from an average process of a hyperspectral dataset, a decomposing process might be necessary (see Decomposing of Basis Spectra (Math)) before using the basis analysis.
In most cases the best way to get the basis spectra is to use the same hyperspectral dataset instead taking the spectra from a database or an old measurement. This guarantees that the basis spectra are recorded using the same experimental conditions and the fit does not include any systematic error (same spectrograph, grating, spectral center position, calibration, objective, laser excitation, fiber, etc...).

Constrained Minimization (Fitting)

When using the basis analysis often only positive mixing values are reasonable: In the case of Raman analysis, negative values belong to negative matter, which physically makes no sense. Therefore it is possible to fit the above equation constrained by allowing only positive mixing values:

$$h_m^{B_n} \geq 0$$

Constrained fitting can be used to find out whether the basis components are mixed.

Inverse Basis Analysis (Math)

The idea behind inverse basis analysis is to calculate the basis spectra and a constant spectra from a hyper spectral dataset if only images (distribution maps) from the belonging components are

known.
Inverse basis analysis is base on the basic equation, which is explained in the article Superposition of Spectra (Math).

$$\hat{S} = \hat{B}\hat{H} + \vec{S}_C\vec{1}^T + \hat{E}$$

The following equation has to be solved separately for each spectral position.

$$\sum_{j=0}^{N-1}\left|S_i^j - \left(\sum_{k=0}^{M-1} B_i^k H_k^j + S_i^C\right)\right|^2 = Minimum$$

$$\begin{aligned}
i &: i-th\ spectral\ position\\
N &: Number\ of\ spectra\ in\ hyper\ spectral\ dataset\\
M &: Number\ of\ basis\ spectra\\
S_i^j &: j-th\ spectrum\ in\ hyper\ spectral\ dataset\\
B_i^k &: k-th\ basis\ spectrum\\
H_k^j &: k-th\ mixing\ value\\
S_i^C &: Constant\ spectrum\ at\ i-th\ spectral\ position
\end{aligned}$$

See also

- Basis Analysis (Math)
- Non Negative Matrix Factorization (Math)

Remarks

Choosing the right Images

Similar to the basis analysis a complete set of images is necessary which describe all components inside the hyper spectral dataset. In addition each image must contain the background free signal from only one component in order to get physical meaningful spectra.

Constant Spectrum

If the constant background spectrum is flat and there is no need for fitting, the user can set its offset value.

Basis Spectra Reconstruction

In some cases it is possible to get nice images only from a very small part of the spectra. Especially when using non negative matrix factorization. In this case inverse basis analysis can be used to reconstruct the basis spectra over the whole spectral range.

Non Negative Matrix Factorization (Math)

Non-Negative Matrix Factorization (NMF) is a method used to evaluate distribution maps, demixed basis spectra and constant background spectra from a hyperspectral dataset. This multivariate

analysis requires the constraint of only positive values inside the distribution maps, the basis spectra and the constant background spectrum. It is based on the basic equation explained in the article Superposition of Spectra (Math):

$$\hat{S} = \hat{B}\hat{H} + \vec{S}_c\vec{1}^T + \hat{E}$$

Constraints:

$$B_i^k > 0$$

$$H_k^j > 0$$

$$S_i^c > 0$$

$$\begin{aligned} i &: i-th\ spectral\ position \\ k &: k-th\ basis\ spectrum \\ j &: j-th\ spectrum\ of\ hyper\ spectral\ dataset \end{aligned}$$

The NMF algorithm is iterative and converges to a solution with minimal error by:

1. Initializing basis spectra, distribution maps and the constant background spectrum.
2. Optimizing the basis spectra
3. Normalizing the basis spectra
4. Optimizing the distribution maps
5. Optimizing the constant spectrum
6. If it is not the final iteration:
   To pull the constant spectrum and basis spectrum into the data of the hyperspectral dataset
7. Repeat steps 2 to 6 until the error is small enough or final iteration is reached

See also

- Basis Analysis (Math)
- Inverse Basis Analysis (Math)

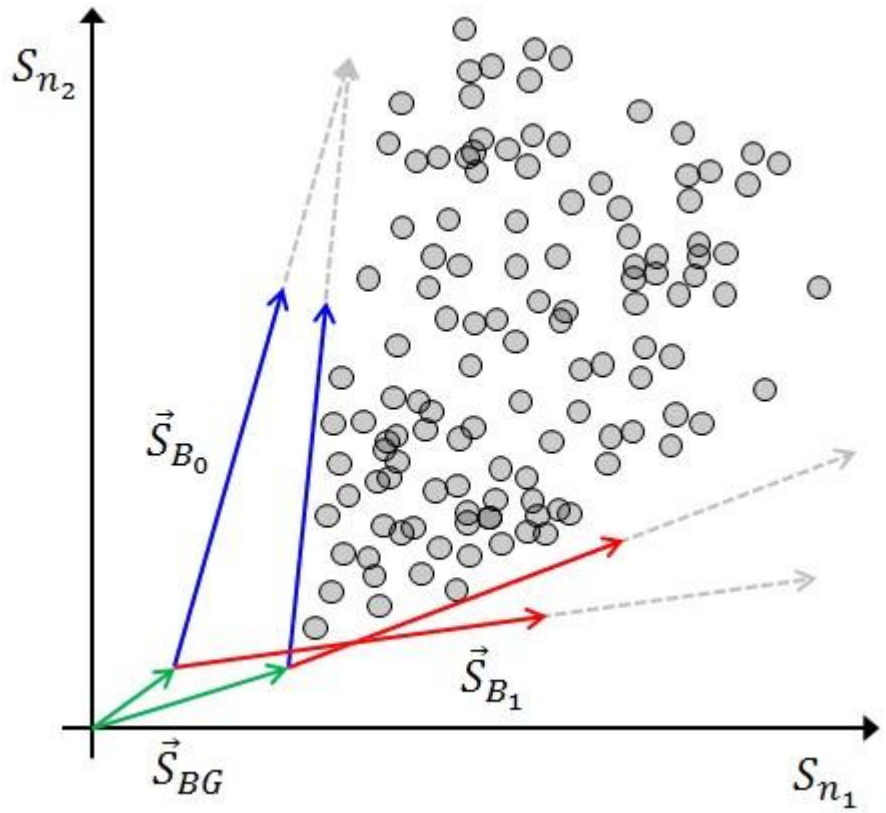
Remarks

Constant Spectrum

If the constant spectrum is flat, a spectral independent offset can be optimized in step 5.

Uniqueness of solution

In general there is no unique solution because an infinite number of solutions exist fulfilling the constraints above.



By pulling the constant spectrum and basis spectra into the cloud of the hyperspectral dataset, a unique solution which is close to pure spectra can be determined. In addition this guarantees that the solution describes the hyperspectral dataset symmetrically to the noise (e.g. readout noise). The strength of the pulling must be adjusted by the user.

Spectrum Normalization (Math)

It is sometimes necessary to normalize a spectrum. If a spectrum is interpreted as a vector there are usually many ways this can be done. For spectral analysis the so-called L1-Norm and L2-Norm are often used, the more general form is the Lp-Norm.

$$\left\|\vec{S}\right\|_p = \sqrt[p]{\sum_{i=0}^{N-1} |S_i|^p}$$

$$\vec{S}_{Norm} = \frac{1}{\left\|\vec{S}\right\|_p}\vec{S}$$

$\left\|\vec{S}\right\|_p$: $L^p - Norm$

$\vec{S}_{Norm}$: $Normalized\ spectrum$

$\vec{S}$: $Spectrum$

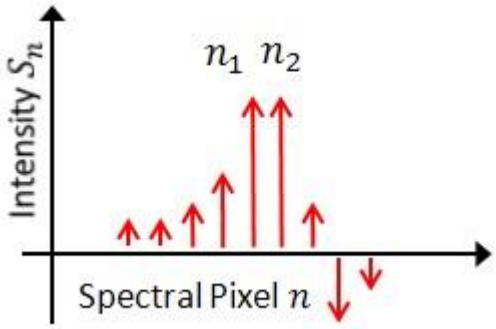
p : $Normalization\ parameter$

See also: Spectrum (Def)

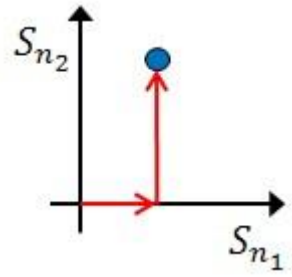
Remarks

Manhattan Norm

For p equals 1, the norm is sometimes called the Manhattan norm. In this case it is equal to the area below the spectrum and therefore proportional to the signal. This type of normalization should be used if proportionality to the signal is important.



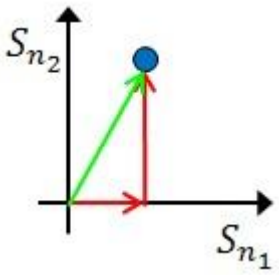
Spectrum



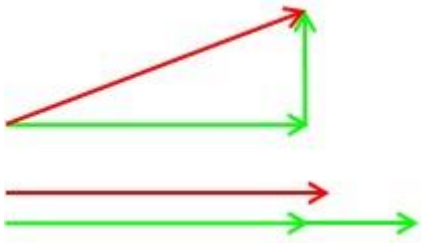
Projection of spectrum into 2D space showing the Manhattan norm

Euclidean Norm

If p equals 2 the norm is called the Euclidean norm. In this case it is equal to the length of the vector, i.e. the influence of a small value inside a vector is smaller compared to a large value. This type of Normalization is appropriate if the influence of small values should be suppressed.



Projection of spectrum into 2D space showing the Euclidean norm



Comparison between Manhattan and Euclidean norm

Decomposing of Basis Spectra (Math)

If the basis spectra are not pure component spectra, a demixing process might be necessary before using them for algorithms, database searching or publication. It does not matter why the spectra are mixed unless the basis is complete and acquired under the same experimental conditions. In this case a decomposition is possible through the following equations:

$$\hat{B}_1 = \left(\hat{B}_0 - \vec{1}\vec{C}_O^T\right)\hat{D}_0$$

$$\hat{B}_k = \hat{B}_{k-1}\hat{D}_{k-1} = \left(\hat{B}_0 - \vec{1}\vec{C}_O^T\right)\hat{D}_0\hat{D}_1 \dots \hat{D}_{k-1}$$

$$\hat{D}_k = \begin{pmatrix} 1 & \cdots & d_{n,0}^k & \cdots & d_{N-1,0}^k \\ \vdots & \ddots & \vdots & & \vdots \\ d_{0,m}^k & & 1 & & d_{N-1,m}^k \\ \vdots & & \vdots & \ddots & \vdots \\ d_{0,N-1}^k & \cdots & d_{n,N-1}^k & \cdots & 1 \end{pmatrix}$$

$$\hat{B}_k = \left(\vec{S}_{B_k^0} \quad \cdots \quad \vec{S}_{B_k^n} \quad \cdots \quad \vec{S}_{B_k^{N-1}}\right)$$

$\hat{B}_0$: *Initial set of spectra*
$\hat{B}_k$: *Final set of spectra after* $k-th$ *iteration*
$\vec{S}_{B_k^n}$: $n-th$ *basis spectrum of* $k-th$ *iteration*
$\vec{C}_O^T$: *Constant Offset*
$\vec{1}$: *Spectrum filled with* 1
$\hat{D}_k$: *Decomposition matrix*
$d_{n,m}^k$: *Elements of decomposition matrix*
N : *Number of basis spectra*

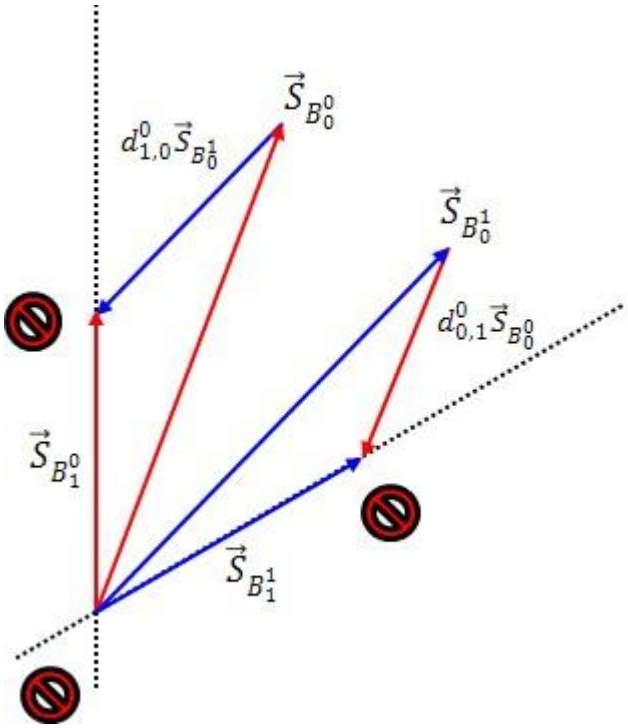
See also: Basis Analysis (Math)

Remarks

Justification

The initial set of basis spectra is only a sub-space of the completely possible vector space. By subtracting or adding the spectra it is not possible to exit this sub-space, i.e. this procedure can not create new components.

The following plot shows how this works for a set of two basis spectra. It is not possible to leave the plane defined by the initial set of basis spectra.



Principal Component Analysis (Math)

Principal component analysis (PCA) is a multivariate analysis. This analysis calculates a new orthogonal basis and transforms the original data into this basis. Similar to other technics the basis equation Superposition of Spectra (Math) can be used as a starting point.

$$\hat{S} = \hat{B}\hat{H} + \vec{S}_c\vec{1}^T + \hat{E}$$

The only difference is that the symbol of right side have a different meaning. The constant spectrum is calculated by the average of the hyper spectral dataset (see Average Spectrum (Math)). The matrix of basis spectra is filled by the eigenvectors with the highest eigenvalues of the covariance matrix. The mixing values are just calculated by the new coordinate transformation.

The eigenvectors and eigenvalues are calculated by the following equation.

$$\hat{C} = \begin{pmatrix} cov(S_0,S_0) & \cdots & cov(S_0,S_m) & \cdots & cov(S_0,S_{M-1}) \\ \vdots & \ddots & \vdots & & \vdots \\ cov(S_n,S_0) & & cov(S_n,S_m) & & cov(S_n,S_{M-1}) \\ \vdots & & \vdots & \ddots & \vdots \\ cov(S_{M-1},S_0) & \cdots & cov(S_{M-1},S_m) & \cdots & cov(S_{M-1},S_{M-1}) \end{pmatrix}$$

$$cov(S_n,S_m) = cov(S_m,S_n) = \frac{1}{N-1}\sum_{k=0}^{N-1}(S_n^k - S_n^{Ave})(S_m^k - S_m^{Ave})$$

$$\hat{C}\vec{X}_k = \lambda_k\vec{X}_k$$

$$\hat{B} = \begin{pmatrix} \vec{X}_0 & \cdots & \vec{X}_m & \cdots & \vec{X}_{M-1} \end{pmatrix}$$

$\hat{S}$: *Hyper spectral dataset matrix*
$\hat{C}$: *Covarianz matrix*
$\vec{X}_k$: *Eigenvector of* $\hat{C}$
λ_k : *Eigenvalues of* $\vec{X}_k$ *and* $\hat{C}$ $(\lambda_k > \lambda_{k+1})$
$\hat{B}$: *Complete matrix of eigenvectors*
N : *Number of spectra in hyper spectral dataset*
M : *Dimension of spectra*
$\vec{S}^{Ave}$: *Average spectrum of hyper spectral dataset*

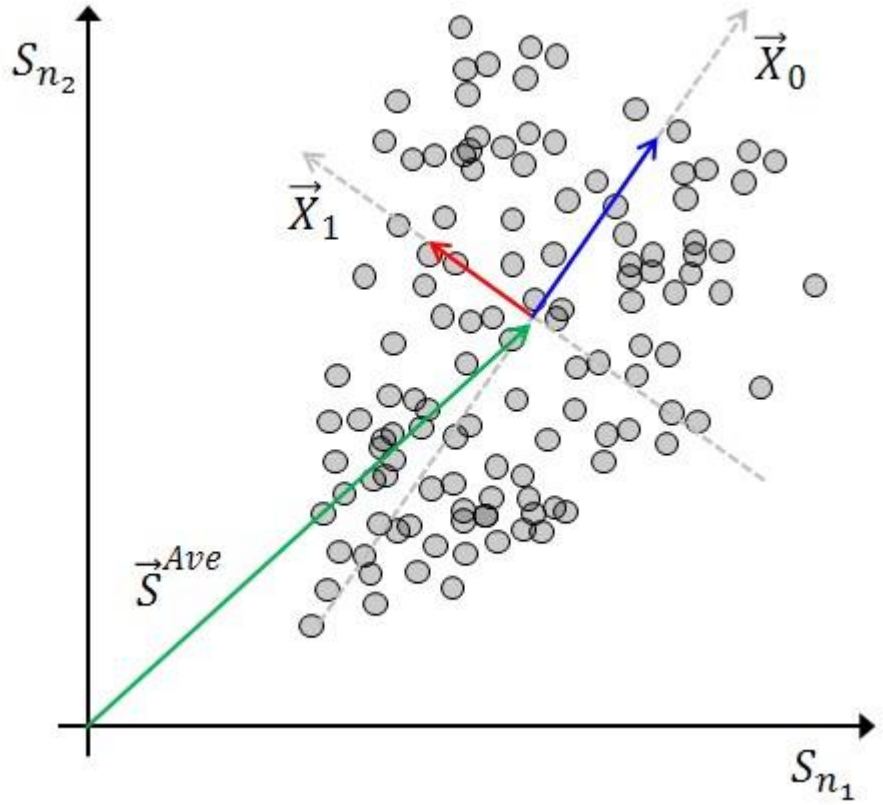
See also

- Non Negative Matrix Factorization (Math)

Remarks

Basis of Eigenvectors

Due to the properties of the covariance matrix the eigenvectors are orthogonal to each other. PCA can be seen as a shift and rotation of the old coordinate system.



If all eigenvector are taking as a new basis, there is no loss of information. In general only the eigenvector with the highest eigenvalues are chosen. In this case not the complete information is preserve. The goal is to chose as many eigenvectors necessary to preserve the measured signal, but take as less to remove the noise.
Principle component Analysis can be used to reduce dimensionality for further analysis or just as a noise filter.

Eigenvalues and Eigenvectors

The eigenvalue can be seen as the variance of the data into the direction of the eigenvector. In this context the meaning of variance is not error but content information.

Uniqueness

For a given hyper spectral dataset a principal component analysis will always give the same result, but a similar dataset with a different e.g. concentration distribution will lead to a different basis. There is no intrinsic physical meaning of the eigenvectors. In addition the elements of the eigenvectors and the mixing values can have negative values.

Cluster Analysis K-Means (Math)

The k-means cluster algorithm splits a hyper spectral dataset into a user defined number of sets (cluster). The sets are chosen by minimizing the following equation.

$$\sum_{k=1}^{N} \sum_{\vec{S}_i \in \Gamma_k} \left| \vec{S}_i - \vec{S}_{Ave}^{k} \right|^2 = Minimum$$

N : *Number of clusters*

$\vec{S}_i$: *Spectrum i from the hyper spectral dataset*

Γ_k : *Set of all spectra inside cluster k*

$\vec{S}_{Ave}^{k}$: *Average spectrum of cluster k (cluster center)*

For a large number of spectra it is not possible to solve the above equation in a reasonable amount of time. Therefore a iterative algorithm is used to find the minimum. This minimum is not necessarily equal with global minimum. The algorithm performs the following steps.

1. Initialize all cluster center.
2. Compare each spectrum of the complete hyper spectral dataset with all cluster centers. Put the spectrum into the most similar set.
3. Calculate new cluster center.
4. Repeat step 2-3 until the sets keep unchanged.

See also

- Average Spectrum (Math)
- Cluster Analysis K-Means (Geometric View)

Remarks

Similarity of two Spectra

In order to calculate the similarity of two spectra the length of the difference spectrum can be used. the inverse of the length is a measure for the similarity. Usually the Euclidean norm is used for k-means clustering, but sometimes the Manhattan norm gives better results (see Spectrum Normalization (Math)).

Pre-Transformations

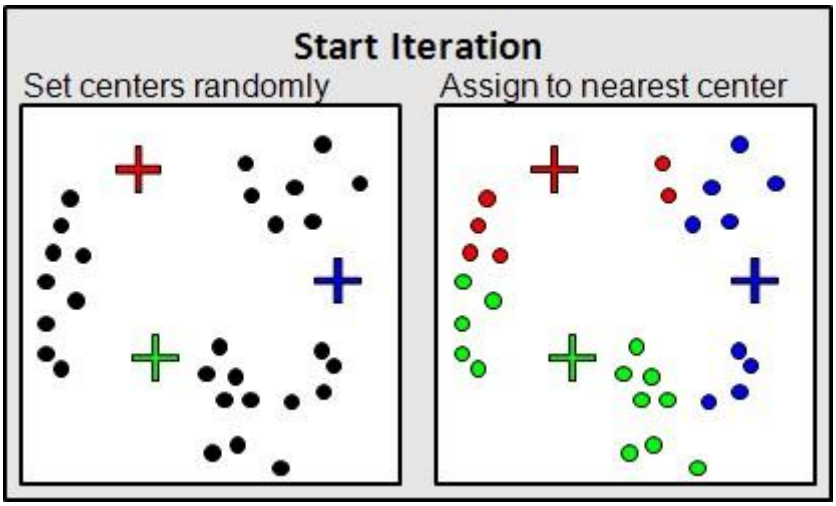
Instead of using the original spectra for clustering a applied pre-transformation to all spectra inside the hyper spectral dataset can make sense.

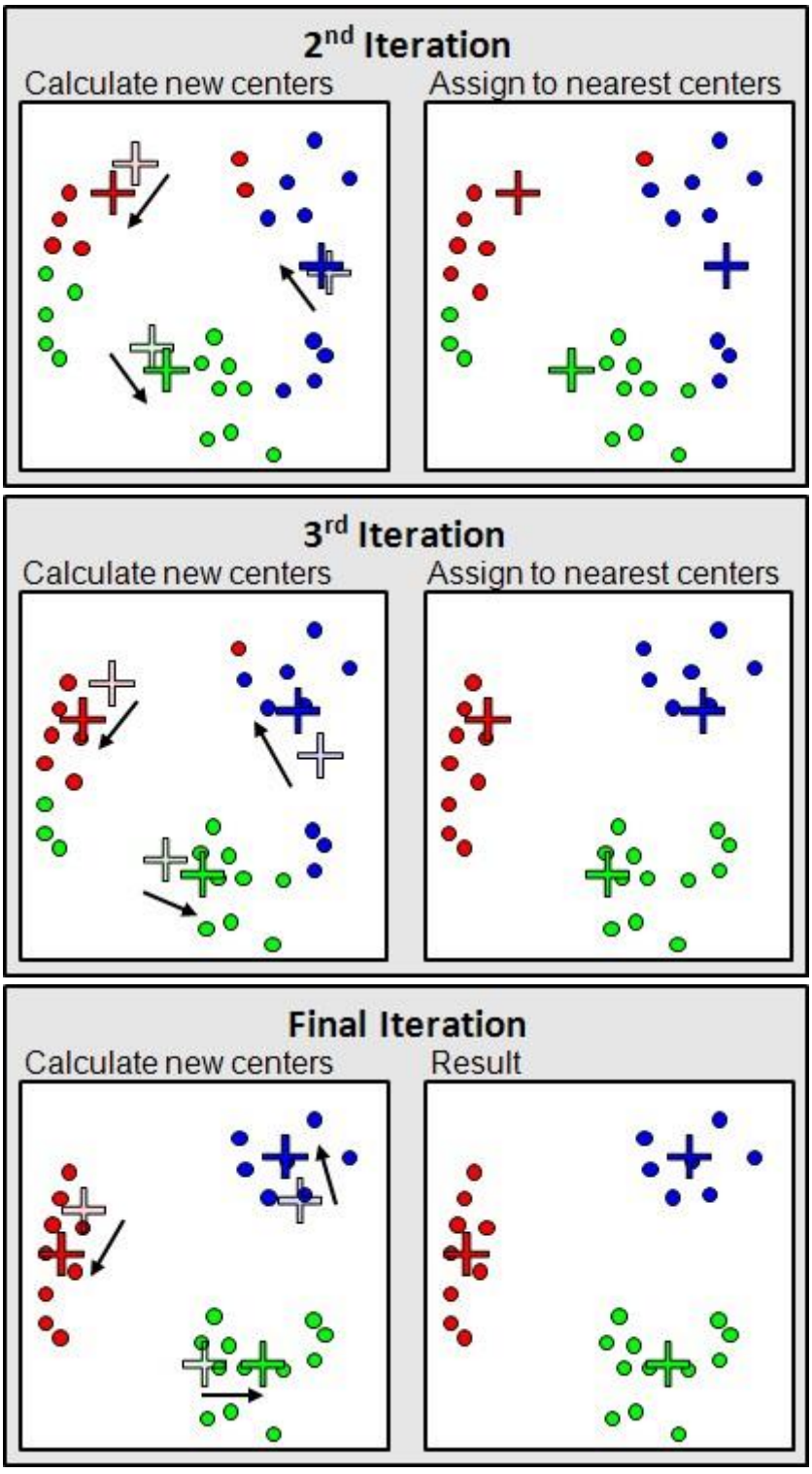
- Data Reduction (speed up and more distinct clusters because of less noise)
- Normalization (only sensitive to spectra shape, insensitive to intensity, integration time and material density)
- Derivative (only sensitive to sharp Raman Lines, insensitive to fluorescence)

These pre-transformed spectra are only used for the clustering process. In order to get a nice averaged spectrum from the original spectra the set information (mask) can be used.

Cluster Analysis K-Means (Geometric View)

In order to solve the k-means clustering condition (see Cluster Analysis K-Means (Math)) a iterative algorithm is used. Below a visualization of the algorithm is shown for three clusters. The crosses show the cluster centers. Each dot is a individual spectrum of the hyper spectral dataset. Please read Superposition of Spectra (Geometric View) for further understanding.





Spectrum (Def)

Many mathematical algorithms for spectral analysis can be described with vector and matrix algebra. The N pixels of a spectrum determine the coordinates of the N-dimensional vector:

$$\vec{S} = \begin{pmatrix} S_0 \\ \vdots \\ S_{N-1} \end{pmatrix}$$

N : *Number of spectral pixels*
S_i : *Intensity of* $i-th$ *pixel*
$\vec{S}$: *Spectrum*

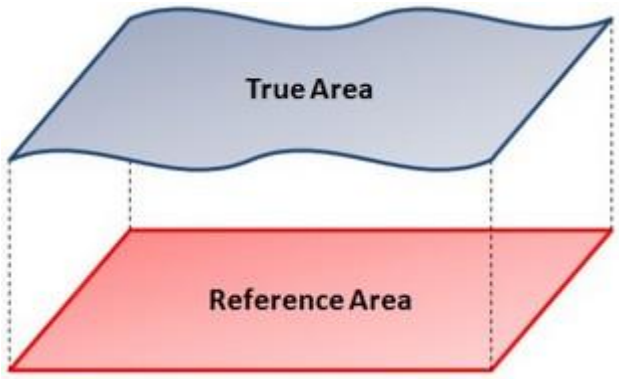
Image Statistics (Math)

Histogram | Statistics | Roughness | Draw Field Mask

Image Roughness Parameters

Number of Pixels:	262144
True Area [µm²]:	3125.68
Reference Area [µm²]:	2490.24
SDR [%]:	27.5646
SDQ [µm]:	1.24282
SSC [1/µm]:	0.693558
Average [µm]:	1.26278
SA [µm]:	1.02088
SQ [µm]:	1.17234
SSK:	0.0125672
SKU:	1.85145
Peak-Peak [µm]:	7.3184
# Local Maxima:	1583

True Area / Reference Area:



The roughness average, SA, is defined as:

$$\mathrm{SA} = \frac{1}{< MN >} \sum_{j=1}^{N} \sum_{i=1}^{M} \epsilon_{i,j} |z(x_i, y_j) - \bar{z}|$$

with $\bar{z}$ representing the mean hight.

$$\bar{z} = \frac{1}{< MN >} \sum_{j=1}^{N} \sum_{i=1}^{M} \epsilon_{i,j} z(x_i, y_j)$$

The root mean square SQ is defined as:

$$\mathrm{SQ} = \sqrt{\frac{1}{< MN >} \sum_{j=1}^{N} \sum_{i=1}^{M} \epsilon_{i,j} [z(x_i, y_j) - \bar{z}]^2}$$

The surface skewness SKK, describes the asymmetry of the height distribution histogram and is defined as:

$$\mathrm{SSK} = \frac{1}{< MN > \mathrm{SQ}^3} \sum_{j=1}^{N} \sum_{i=1}^{M} \epsilon_{i,j} [z(x_i, y_j) - \bar{z}]^3$$

If $\mathrm{SSK} = 0$, a symmetric height distribution is indicated, e.g. Gaussian.

If $\mathrm{SSK} < 0$, it can be a bearing surface with holes and if $\mathrm{SSK} > 0$, it can be a flat surface with peaks. Values of SSK numerically greater than 1.0 may indicate extreme holes or peaks on the surface.

The surface kurtosis SKU, describes the peaked-ness on the surface topography and is defined as:

$$\mathrm{SKU} = \frac{1}{< MN > \mathrm{SQ}^4} \sum_{j=1}^{N} \sum_{i=1}^{M} \epsilon_{i,j} [z(x_i, y_j) - \bar{z}]^4$$

For Gaussian height distributions SKU approaches 3.0 when increasing the number of pixels. Smaller values indicate broader height distributions.

The extreme values are defined by:

$$\mathrm{Max} = \max\{z(x_i, y_j) : 1 \leqq i \leqq M, 1 \leqq j \leqq N, \epsilon_{i,j} = 1\}$$
$$\mathrm{Min} = \min\{z(x_i, y_j) : 1 \leqq i \leqq M, 1 \leqq j \leqq N, \epsilon_{i,j} = 1\}$$

The peak-peak parameter is defined as:

$$\text{Peak-Peak} = |\mathrm{Max} - \mathrm{Min}|$$

Pearson Correlation Coefficient

The following formula is used to calculate the Pearson Correlation Coefficient:

$$r = \frac{\sum_{i=0}^{N-1}(S_i - \overline{S})(S_i^{Ref} - \overline{S}^{Ref})}{\sqrt{\sum_{i=0}^{N-1}(S_i - \overline{S})^2}\sqrt{\sum_{i=0}^{N-1}(S_i^{Ref} - \overline{S}^{Ref})^2}}$$

$$\overline{S} = \frac{1}{N}\sum_{i=0}^{N-1} S_i \qquad \overline{S}^{Ref} = \frac{1}{N}\sum_{i=0}^{N-1} S_i^{Ref}$$

$\vec{S}^{Ref}$: *Reference Spectrum*

$\vec{S}$: *Spectrum*

Examples / How To's

Creating Raman Overlay

Raman Overlay on Video Image

1. Open your video image in an image viewer.
2. Drag and drop your Raman image on the image viewer and select "Use as Overlay".
3. Adjust the overlay in the Image Transform and Overlay Dialog. Press "Extract Bitmap".

Video Image on Topography

1. Open your topography image in an image viewer.
2. Drag and drop your video image on the topography and select "Use as Overlay".
3. Adjust to full overlay in the Image Transform and Overlay Dialog. Press "Extract Bitmap".
4. Drag and drop the new bitmap on the image viewer showing the topography and select "Use as Color Image".

Using Graph Intensity Correction

To execute a proper intensity correction, the following steps have to be done.

Note: For all measurements you have to use the same laser, the same spectral position and the same CCD camera settings !

Preparation

- Measure a series of **reference spectra** (≈ 500 to 2000, time series), e.g. fluorescence spectra There must be **no Raman signal** on the fluorescence!

- The correction only works if the **background** can be subtracted from the reference.
  If the background can't be determined using the reference spectrum itself (from an area without any signal, e.g. near Rayleigh) or if the background has a strong variation coming from the CCD, you have to measure a series of **dark spectra** (≈ 500 to 2000, time series). The average of those dark spectra can be used to subtract the background.

Measurement and Correction

1. Execute your Raman measurement under the same conditions as the reference spectra.

2. If necessary, remove cosmic rays from your Raman measurement.
   Don't do any background subtraction. Only use 0-order polynomial / horizontal line subtraction in case you see that the offset had drifted during the measurement.

3. Drop your Raman measurement on the Graph Intensity Correction Dialog Drop Action

4. Drop your reference series on the "Drop Reference" Button

5. Subtract the background from the reference:
   - Set the blue mask in the reference preview graph viewer to an area without any signal
     and press "Get Mask Average" in the Reference Offset Group Box.
     or
   - Drop the **dark spectra** series on the "Drop Spectrum" button in the Reference Offset

Group Box.

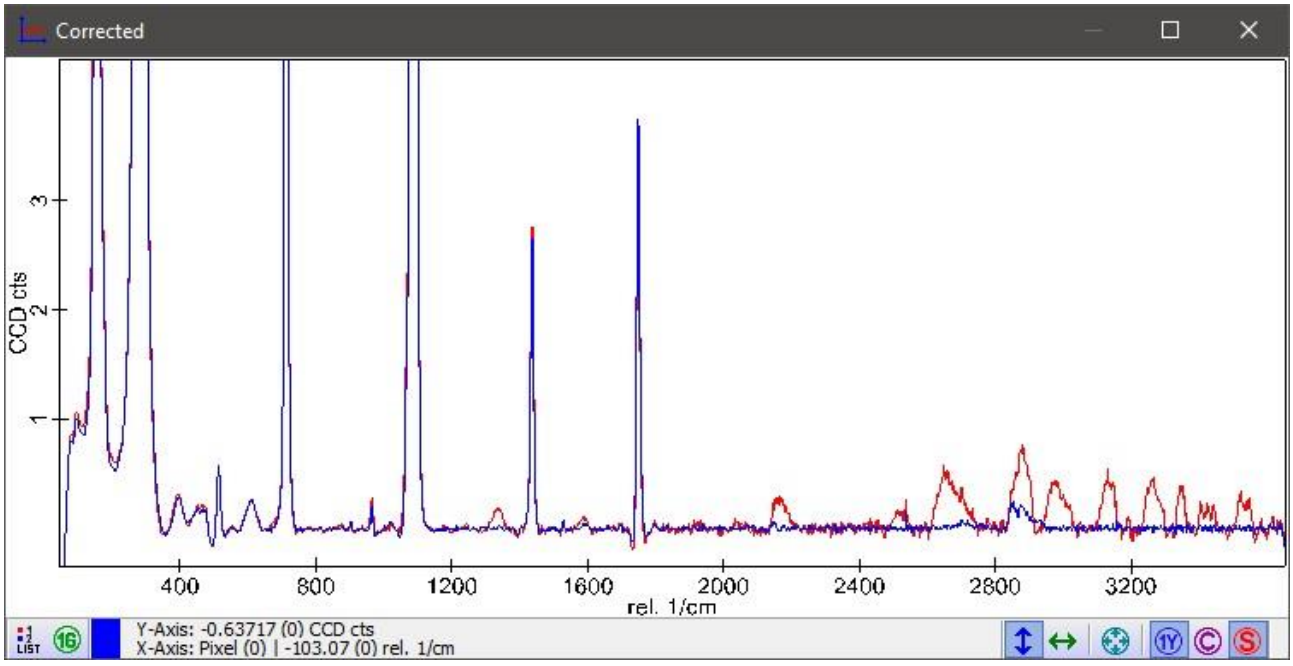
6. Subtract the background from your Raman measurement:
   - Set the blue mask in the result preview graph viewer to an area without any signal and press "Get Mask Average" in the Measurement Offset Group Box.
     or
   - Drop the **dark spectra** series on the "Drop Spectrum" button in the Measurement Offset Group Box.

7. If your fluorescence spectrum has a region without any signal, remove it from the green mask

8. Press Extract.

How does the result look like?

In the graph viewer below you can see the result of an intensity correction.
You won't see the difference in a single noisy spectrum, but in a spectrum with high intensity or in averaged spectra.
Both spectra show an average Raman spectrum having a fluorescence offset which is subtracted using the shape background subtraction.
The red one shows the result without the intensity correction, the blue one with the correction:



Creating Image Masks

Creating Image Masks - Overview

Image Masks are needed to select which pixel (or spectra of a hyper-spectral data) set should be considered for a calculation, e.g. for Averaging Spectra, Cluster Analysis, Image Statistics and so on.

You can create Image masks using several software features:

Features	Examples
Image Viewer Draw Tools (manual + thresholding)	Creating Image Masks using the Draw Tools
Calculator Dialog (boolean formulas)	Creating Image Masks using the Calculator
Advanced Graph Average Dialog (advanced thresholding)	

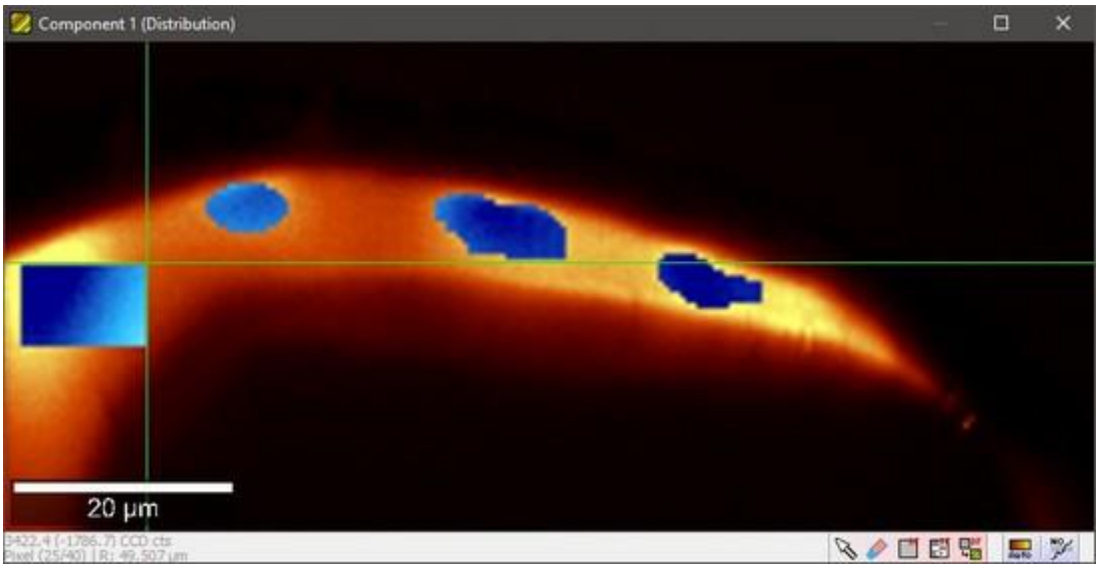
Creating Image Masks using the Draw Tools

You can create Masks using the Draw Tools in any Image Viewer.

Steps

1. Open a new Image Viewer or select an existing Image Viewer
2. Open the draw tools via the circle menu and select a desired drawing tool

3. Draw on the image using the left mouse button or select a threashold in the threshold tool



Hint: If the mask is used for averaging multiple spectra, make sure you only mask pixels that contain only one component.

4. Extract the mask using the tool button in the Image Viewer status bar or via the draw tools circle menu > Extract button

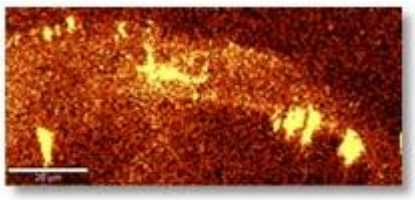
5. Switch back to the Mouse Move mode by pressing the tool button with the mouse icon or by using Circle Menu Up.

Creating Image Masks using the Calculator

You can create masks using a boolean formula in the Calculator Dialog.
The formula gives you the chance to combine any kind of logic in order to create a mask from different images also.

For example we could have a sum image showing a Raman component with flluorescence and a sum image showing fluorescence only:

Sum Image Layer 1

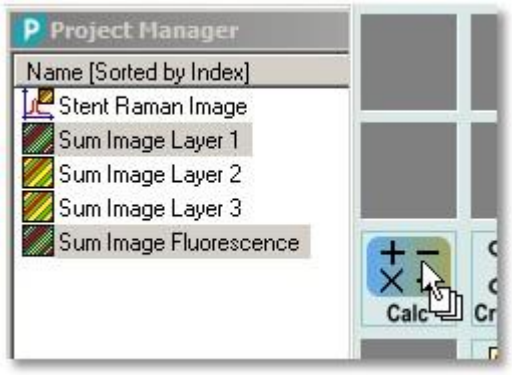


Sum Image Fluorescence

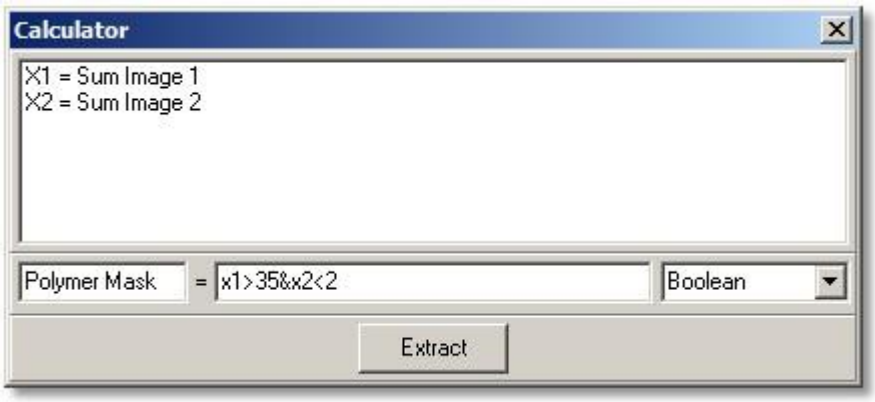
The calculator could be used to create a mask where only the Raman component of the first image is masked.

Steps

1. Drag and drop both images on the Calculator Drop Action.



2. Now you can type a boolean formula (leading to a mask result with values of 0 and 1). This can be achieved by using the larger (>) and smaller (<) operator as well as the logical "and" (&) and "or" (|) operators:



3. Use the formula "x1 > 150":
   This will set all pixels that have a value larger than 150 in the Sum Image Layer 1 (Polymer), thus selecting only bright pixels.
   The threshold (in this case 150) depends on the sum image. Move the mouse cursor over the original sum image to get an idea which value could be a good threshold for thresholding bright pixels and change the value to see what happens.

4. Use the formula "x2 < 30":
   This will clear all pixels that are larger or equal than 30 in the Sum Image Fluorescence, thus removing fluorescent pixels.

5. Use the "logical and" operator (&) to combine the two masks (both conditions have to be true to set the mask pixel), thus removing the fluorescent pixel from the Polymer mask.

6. Optional: Use the unit "Boolean" and rename the image to recognize the mask later on

7. You can calculate the masks also for the Polymer Layer 2 (Threshold 120) and Polymer Layer 3 (Threshold 350).

8. Compare your results with the predefined mask data objects Mask Layer 1/2/3.

Results

Calculator Result with Formula "**x1 > 150**"

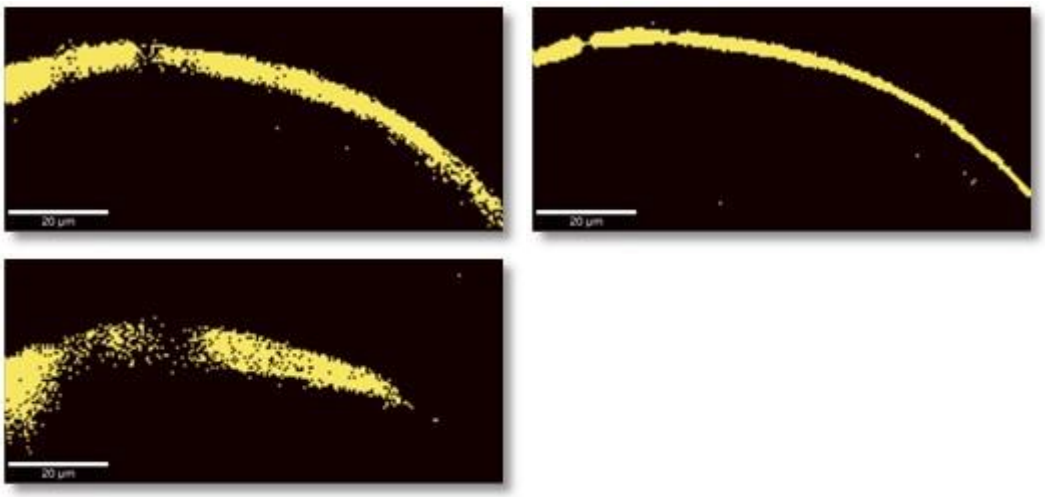
Calculator Result with Formula "**x2 < 30**"

Calculator Result with Formula "**x1 > 150 & x2 < 30**"

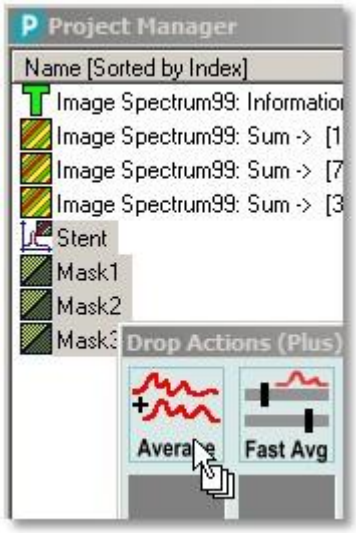
Creating Average Spectra using Masks

If you would like to create a nice, noise-free spectrum for each component, averaging multiple spectra from the same component is a suitable way.
To achieve that, you have to do the following steps:

1. Create Image Masks for each component.

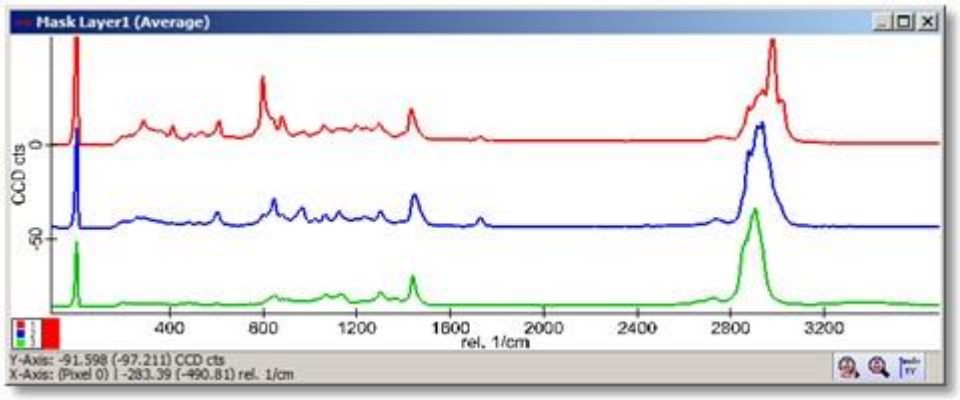


2. Having those masks, you can select the masks together with the spectral Raman image data set and drag and drop it onto the Average Spectrum Drop Action:



Result

The result is one nice average spectrum for each dropped mask:



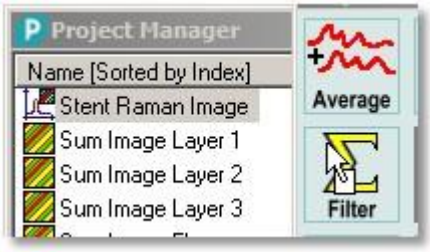
Creating Images using the Filter Viewer

During or after a Raman Image Scan measurement, you can create images with the Filter Viewer.
Hint: In WITec Control, a Filter Viewer might be automatically created upon starting an Image Scan or Large Area Scan.

The Filter Viewer helps you finding the interesting Raman bands and creates images which show structures of your components.

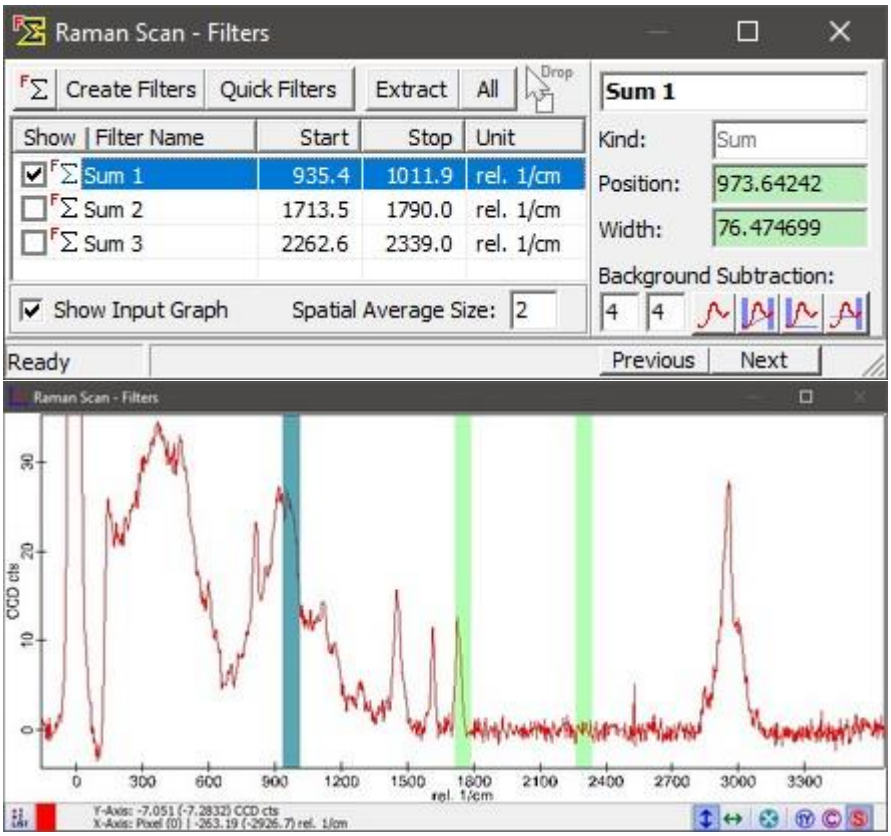
Steps

1. Drag and drop the Raman Image Data Object onto the Filter Viewer Drop Action



2. Add new sum filters

3. Select a sum filter to change the filter parameters (position, width, background subtraction, ...)



4. You can extract the currently selected filter image or all filters using the buttons "Extract" and "All".

3D Data Analysis

This article describes how to prepare 3D stack data in WITec Project for the export. An example of the data analysis procedure is given in the following. The best way to analyze a set of data will depend on the specific data and your requirements, so the following procedure might need to be modified and adjusted.

Our goal is to apply the same analysis steps like background subtraction and TrueComponent Analysis (TCA) to all layers with the same parameters. However, TCA or other analysis tools need the whole dataset in order to find all components. The solution is to stitch the layers together in one image, and set the analysis parameters with this single stitched object. Then, we apply the analysis to all individual layers by drag and drop them to the Extract button.

If the number of stack layers is very high, it is better to choose only few representative layers (e.g. 2

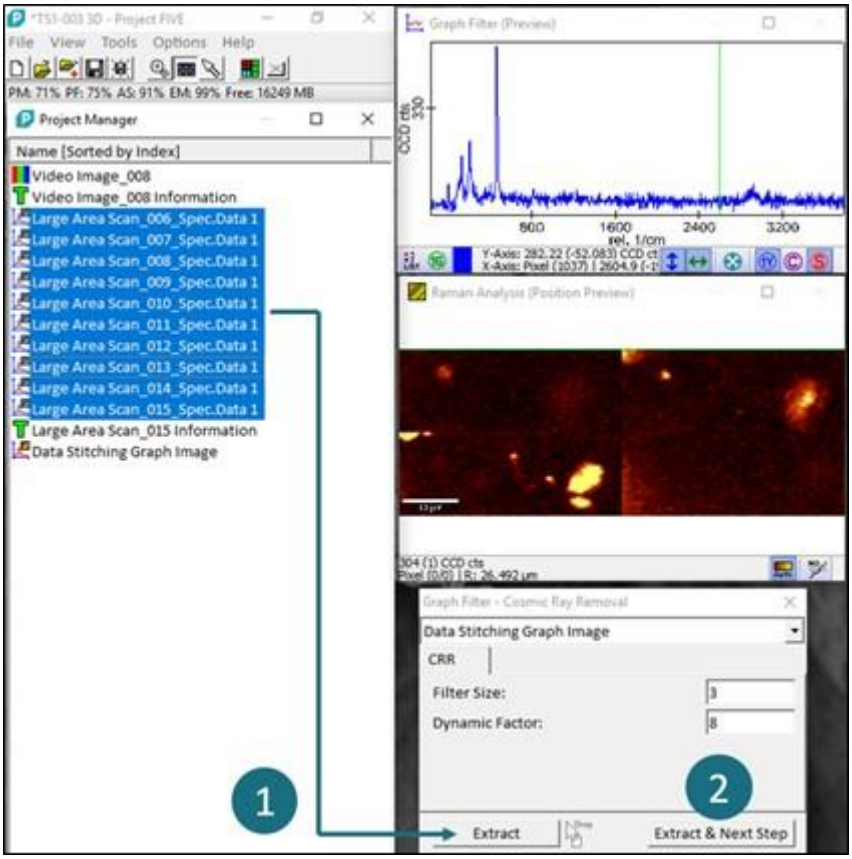
or 4) for stitching, this makes the analysis much faster.

The procedure suggested here uses the TrueComponent analysis; however, other tools like the fitting tool can also be used.

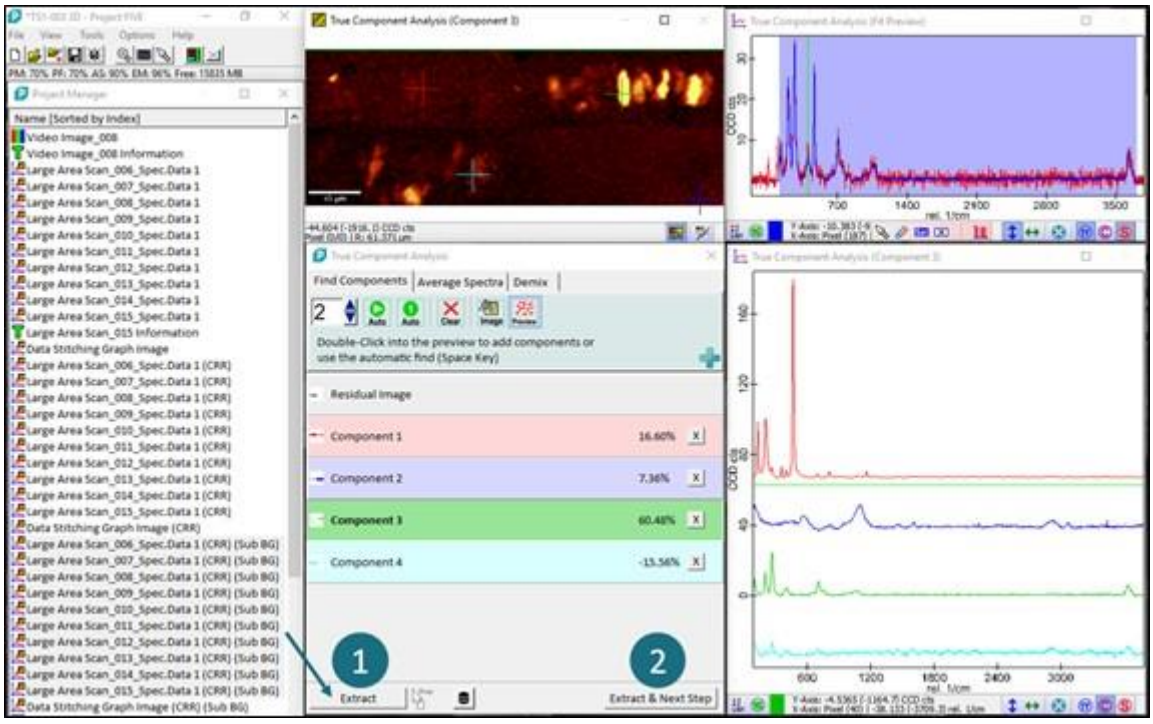
The data in the figures show some inclusions (green, yellow) in a garnet sample (red) mounted on a glass slide (blue).

Data analysis

- Stitch the images to one object using the Stitching tool from the Drop Action Menu. In the example here, I choose two layers.
- Drag and drop the stitched image to the Raman Wizard, it will open a preview image showing your representative layers.
- The first step is cosmic ray removal (CRR). Set the values as usual.
- Important: Drag and drop all individual layers (not the stitched image) of your stack on the Extract (1) button before pushing the Extract & Next Step (2) button! (See figure below)



- Do the same for baseline correction: Choose the settings with the help of the preview image, then drag and drop all (CRR) corrected layers on the Extract button and finally push the Extract & Next Step button.
- Continue your data evaluation of the stitched data object with the TCA (as in our example):
- Drag and drop all single layers to the Extract button. This will create a (long) list of objects, namely one image for each component in each layer.



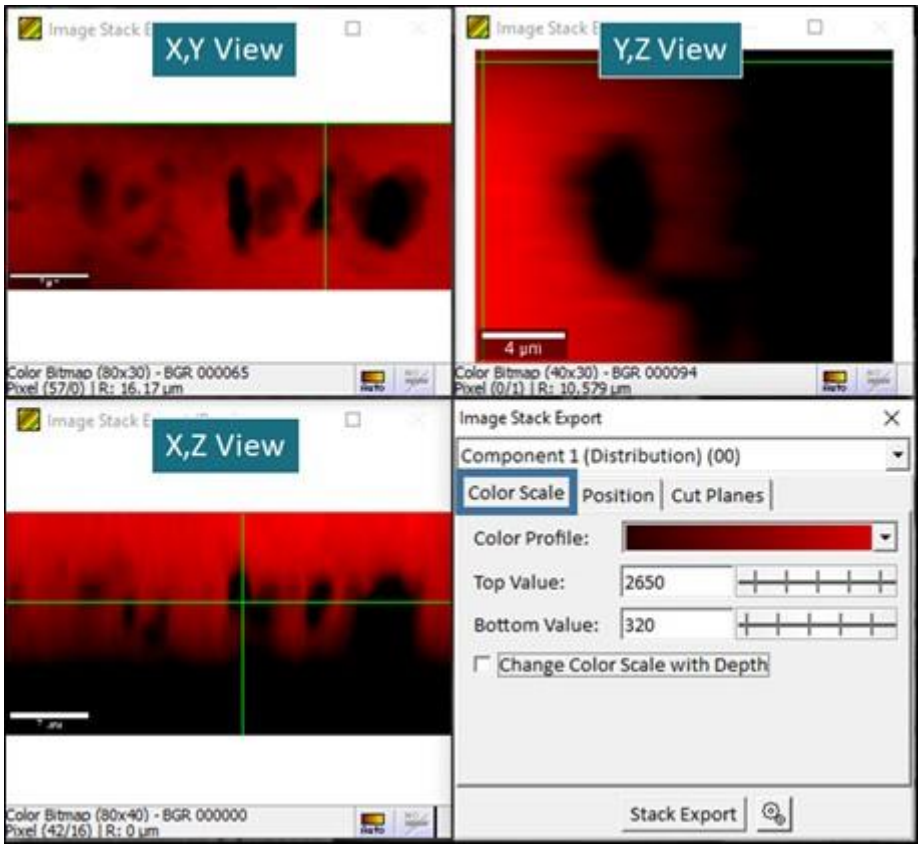
- Optional: Consider image smoothing at this point.

Data Export

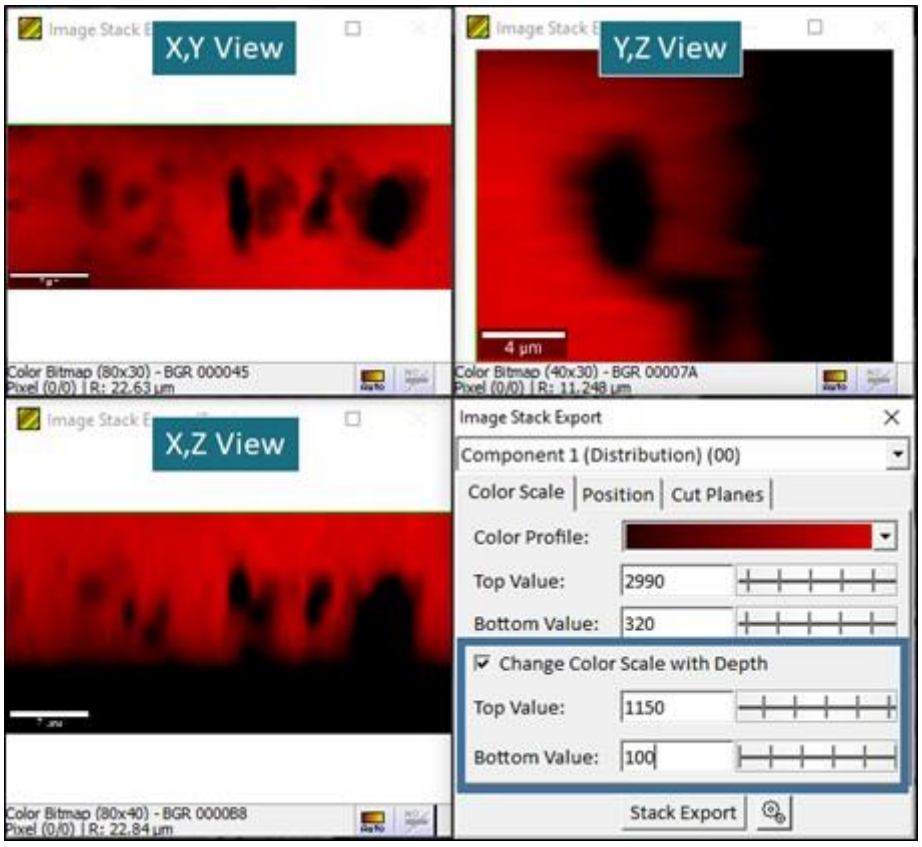
- Extract the images of each component by using the Image Stack Export button in the drop actions menu.

Sort the objects in the project manager by name first.

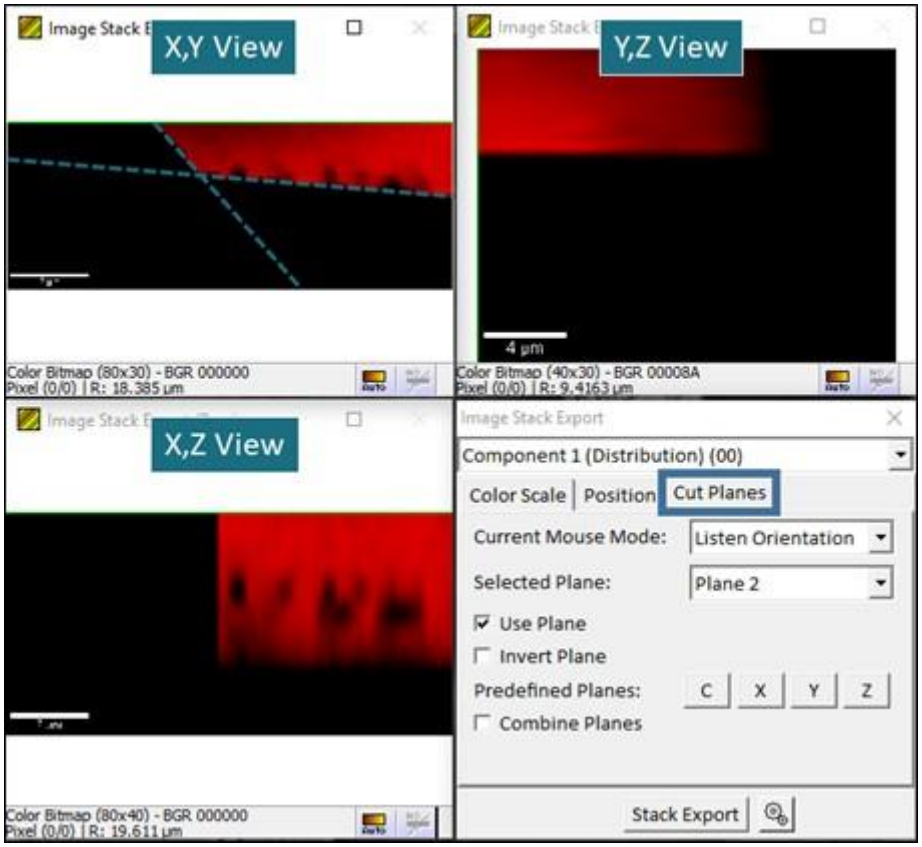
- The Image Stack Export tool provides x,y x,z and y,z views. The color and color scale can be adjusted



- To compensate for the typical intensity decrease when focusing deeper inside a sample, activate "Change Color Scale with Depth".



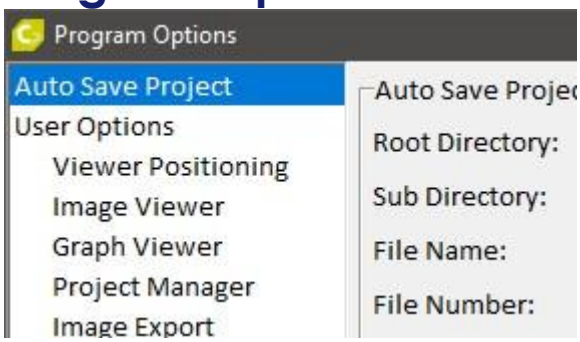
- Use "Cut Planes" to remove parts of single components, e.g. if you want to show only a part of a matrix with inclusions:



- Greyscale is fine – if you choose the color of each component later i.e. in the ImageJ 3D Viewer. (Note: if you choose another 3D Plugin, definition of the color scale might be necessary here.)
- Export as single tif file
- The data can be visualized in any 3D image software i.e. ImageJ

Program Options

Program Options Window



You can open the Program Options window via the main menu **Options > Program Options**.

Notes:

- Options are not saved to the hard drive until the application is closed.
- Some options take effect immediately, some require a program restart.

There are several places in which options will be saved, depending on the option:

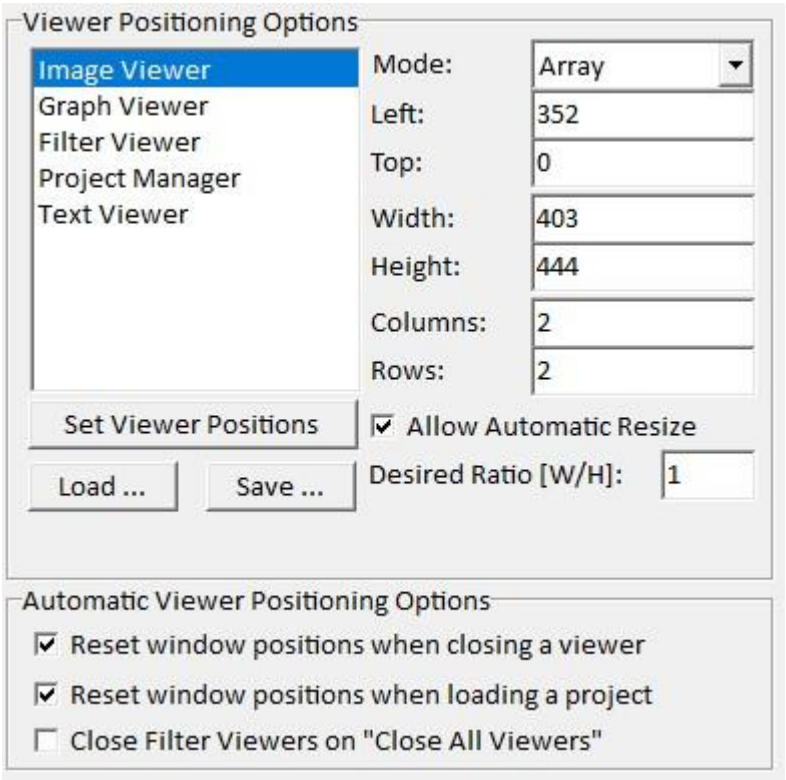
- Most options are saved for the current user only.
- Some options are saved for all users of the current application version.
- Some options are saved system-wide for all WITec applications and users.

The following options are available:

- Viewer Positioning Options
- Image Viewer Options
- Graph Viewer Options
- Project Manager Options
- Image Export Options
- User Interface Options
- OpenGL Options
- Graph ASCII to External Program Options
- Memory Options
- Auto Save Project Options

Viewer Positioning Options

You can change the way of automatic viewer positioning for each viewer category in the **Program Options > Viewer Positioning**.
To change the viewer positioning, first select a viewer category in the list box. Second, change the values in the edits on the right to adjust the positioning.



Viewer Positioning Options

Mode (Combo Box):

- None: No automatic positioning, use operating system default position (cascade).
- Array: Tile the windows side by side with a given number of columns and rows.
- Cascade: Cascade the windows with a given X and Y step size.
- Single Position: Use one static position and size for each viewer.

Left/Top (Integer Edits):
The most left and top position of the first viewer.

Width/Height (Integer Edits):
The width and height of each viewer;
in Array Mode, the maximum area for the viewers is <Width * Columns> x <Height * Rows>.

Columns/Rows (Integer Edits):
number of rows and columns for Array Mode.

Allow Automatic Resize (Check Box):
In Array Mode, window sizes are automatically scaled down if too many windows are open and no more tile is left, this is the case if Number of Viewers > Columns * Rows.

Desired Ratio (Float Edit):
If "Allow Automatic Resize" is turned on, the automatic resize algorithm tries to get a good number of rows and columns that fit best the desired ratio.

Set Viewer Positions
Resets all the viewer positions if one or more viewers were moved by the user.

Load/Save
Here you can save or load your current viewer positioning options.

The file name is important: the software automatically chooses the filename for the current screen/monitor resolution.
If you press "Save", the file name is automatically set to the file name that will be searched for for the current resolution.

Automatic Viewer Positioning Options

Reset window positions for a viewer kind when closing a viewer (Check Box):
If checked, the window positions for a viewer kind are automatically reset if a viewer of this kind is closed.

Reset window positions when loading/appending a project (Check Box):
If checked, the positions of all loaded viewers are reset after loading the project.

Close Filter Viewers when using "Close All Viewers" Button (Check Box):
If checked, all Filter Viewers will be closed when using the "Close All Viewers" feature in the main window.

Image Viewer Options

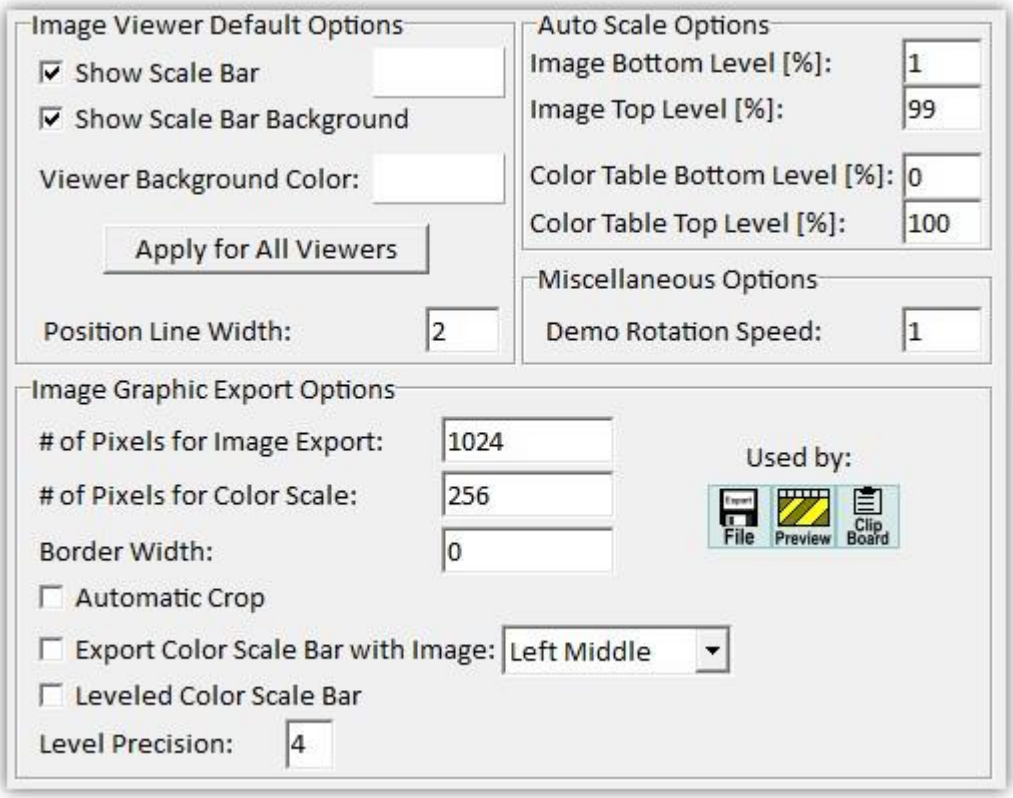


Image Viewer Default Options

Those options are used each time a new image viewer is opened.

Show Scale Bar (Check Box):
Changes the default visibility of the scale bar.

Default Scale Bar Color (Color Panel):
Click on the colored area to change the default scale bar color.

Show Scale Bar with Background (Check Box):
Changes the default visibility of the semi-transparent background of the scale bar (and other labels).

Viewer Background Color (Color Panel):
Changes the default background color for Image Viewers.

Apply for All Viewers (Button)
Applies the current default settings to all currently opened image viewers

Position Line Width (Integer Edit):
Sets the line width of transformation drawings (if you drop another measurement object onto an image viewer to see the measurement position).

Auto Scale Options

Image Bottom/Top Level (Float Edits):
set the bottom and top level for automatic color scale calculation. All values smaller than the bottom level or higher than the top level are ignored.

Color Table Bottom/Top Level (Float Edits):
set the bottom and top color table level for automatic color scale calculation. All values smaller than the bottom level or higher than the top level are ignored.

Miscellaneous Options

Demo Rotation Speed:
Changes the animation speed of the demonstration rotation function.

Image Graphic Export Options

# of Pixels for Image Export (Integer Edit):
Defines the width in pixels of an exported bitmap. The height in pixels is automatically adjusted using the current viewer size ratio.

# of Pixels for Color Scale (Integer Edit)
Defines the width in pixels of an exported color scale bitmap.

Border Width (Integer Edit)
Defines the width in pixels for an additional border around the exported image.

Automatic Crop (Check Box):
If checked, the white border is removed automatically before exporting.

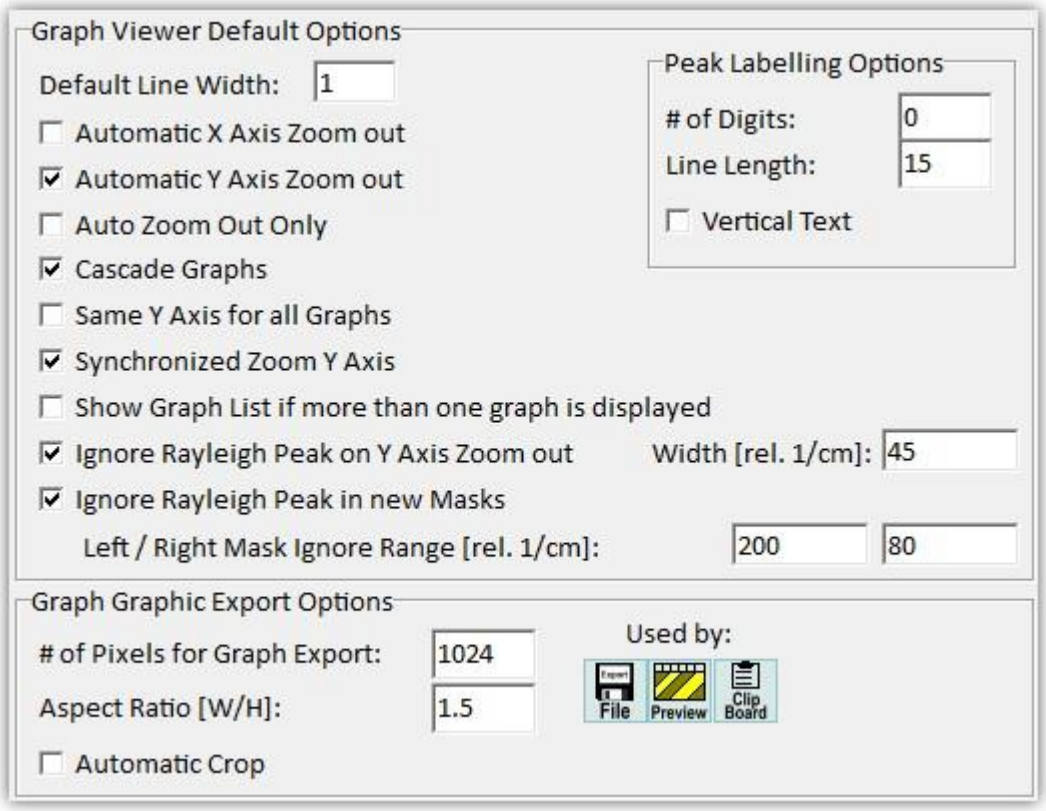
Export Color Scale Bar with Image (Check Box):
If checked, the color scale bar is automatically exported next to the image when exporting an image.

Color Scale Bar Position (Combo Box):
Defines the position of the exported color scale bar, if "**Export Color Scale Bar with Image**" is checked.

Leveled Color Scale bar (Check Box):
If checked, the values of the labels of the exported color scale bar are leveled to an absolute color scale value range (from 0 to <range>).

Level Precision (Float Edit):
Sets the precision of the numbers shown at the level bar.

Graph Viewer Options



Graph Viewer Default Options

These options are used each time a new Graph Viewer is created.

Default Line Width (Integer Edit):
Sets the default line with for drawing graph objects.

Automatic X Axis Zoom out (Check Box):
if checked, the X Axis is automatically zoomed when the displayed graph is changing.

Automatic Y Axis Zoom out (Check Box):
if checked, the Y Axis is automatically zoomed when the displayed graph is changing.

Cascade Graphs (Check Box):
if checked, the Y Axis is cascaded when showing multiple Graph Objects.

Same Y Axis for all Graphs (Check Box):
if checked, all Graph Objects in the same viewer share the same Y Axis scale.

Synchronized Zoom Y Axis (Check Box):

if checked, all Graph Objects are zoomed synchronously when using the mouse wheel for zooming.

Show Graph List if more than one graph is displayed (Check Box):
if checked, a list of displayed Graph Objects will be shown automatically if more than one graph is displayed.

Ignore Rayleigh Peak on Zoom out Y (Check Box and Float Edit):
if checked, the Y Axis zoom on spectral data automatically ignores the Rayleigh Peak.
In the float edit you can define the spectral range that should be ignored (in relative wavenumbers).

Ignore Rayleigh Peak in new Masks (Check Box and Float Edits):
if checked, the Rayleigh peak is automatically removed from masks in analysis dialogs. You can define the range that should be removed by setting the left and right distance from the excitation wavelength.

Peak Labelling Options

# of Digits (Integer Edit):
Sets the default number of digits for peak labels.

Line Length (Integer Edit):
Sets the default line length for peak labels.

Vertical Text (Check Box):
If checked, the peak label text is drawn vertically.

Graph Graphic Export Options

# of Pixels for Graph Export (Integer Edit):
defines the width of an exported bitmap in pixels.

Aspect Ratio (Float Edit):
defines the width/height ratio of an exported bitmap.

Automatic Crop (Check Box):
if checked, the white border is removed automatically before exporting.

Image Export Options

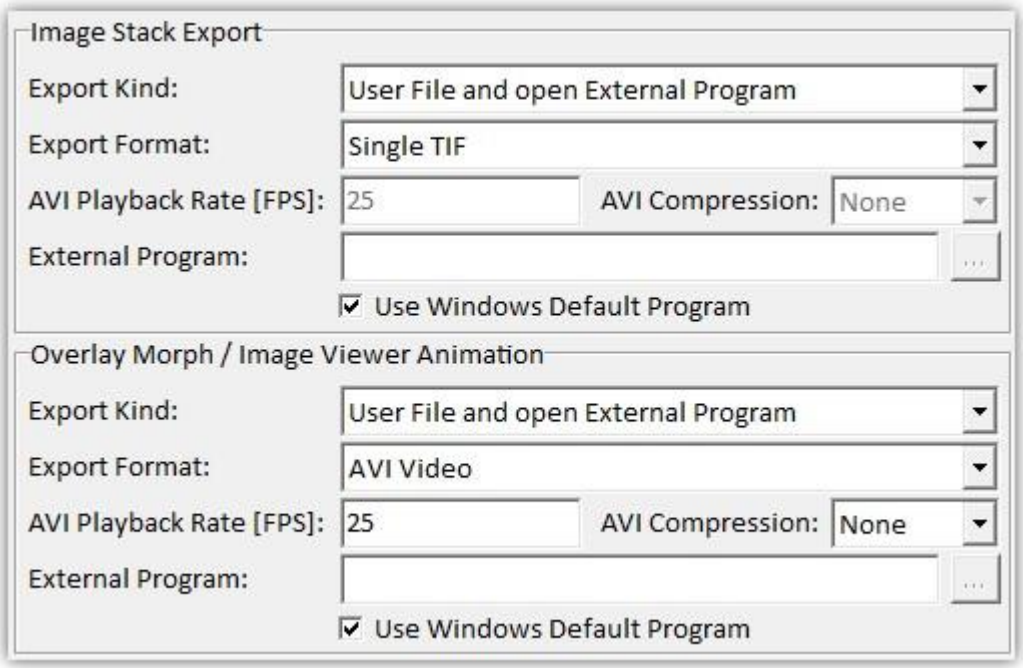


Image Stack Export

Used for the Image Stack Export Dialog.

Export Kind:
Here you can choose the following options:

- User File: just saves the file(s) to a user defined location
- User File and open External Program: saves the file(s) to a user defined location and opens the file using the program defined in "External Program"
- Temporary File and open External Program: saves the file(s) to a temporary folder and opens the file using the program defined in "External Program"

Export Format:
Defines the file format for the image export:

- Single TIF: Stores all images into a multi-frame TIF file
- Multiple Bitmaps: Stores all images into separate bitmap files, a number is automatically added to the file names (0001, 0002, ...)
- AVI Video: Stores all images to an AVI video file.

AVI Playback Rate:
Default playback rate for AVI videos.

AVI Compression:
Defines the compression for AVI videos:

- None: no compression
- MSVC: Simple Microsoft Video Compression - might not work with several video players.

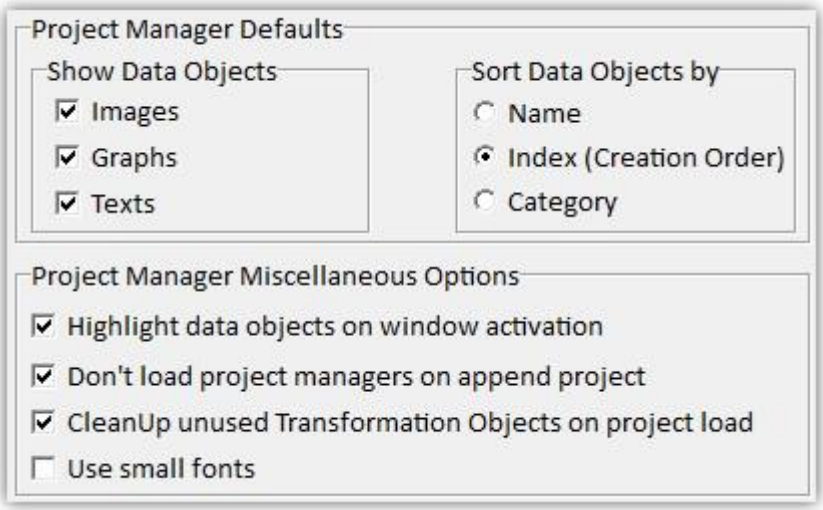
External Program:
Here you can define an external program file name that will be used for opening the stored image file.

Use Windows Default Program:
If checked, the windows default program is used instead of a user defined external program.

Overlay Morph / Image Viewer Animation

Parameters see above.
Used for the Image Viewer Animation Export and the Overlay Morph in Image Transform and Overlay Dialog.

Project Manager Options



Project Manager Defaults

Those options are used each time a new project manager is created.

Show Data Objects

Images:
If checked, Image Data Objects are shown

Graphs:
If checked, Graph Data Objects are shown.

Texts:
If checked, Text Data Objects are shown.

Sort Data Objects by

Caption:
If checked, Data Objects are sorted by their name / caption.

ID (Creation Order / Index):
If checked, Data Objects are sorted by their creation order. The newest object is at the end of the list.

Category:
If checked, Data Objects are sorted by their category.

Project Manager Miscellaneous Options

Highlight Data Objects in project managers on window activation.
If checked, each Project Manager will highlight data objects that are used by another software part upon clicking on a viewer or a data analysis dialog.

Don't load project managers when appending a project:
If checked, Project Manager windows of an appended project are not loaded.

CleanUp unused Transformation Objects on project load:
If checked, transformation objects (that are hidden by default) are deleted if not used by any image or graph object.
Can be turned off e.g. for special import actions.

Use small fonts:
If checked, Project Manager uses the old smaller font style. Create a new Project Manager to see the effect.

Image Export Options

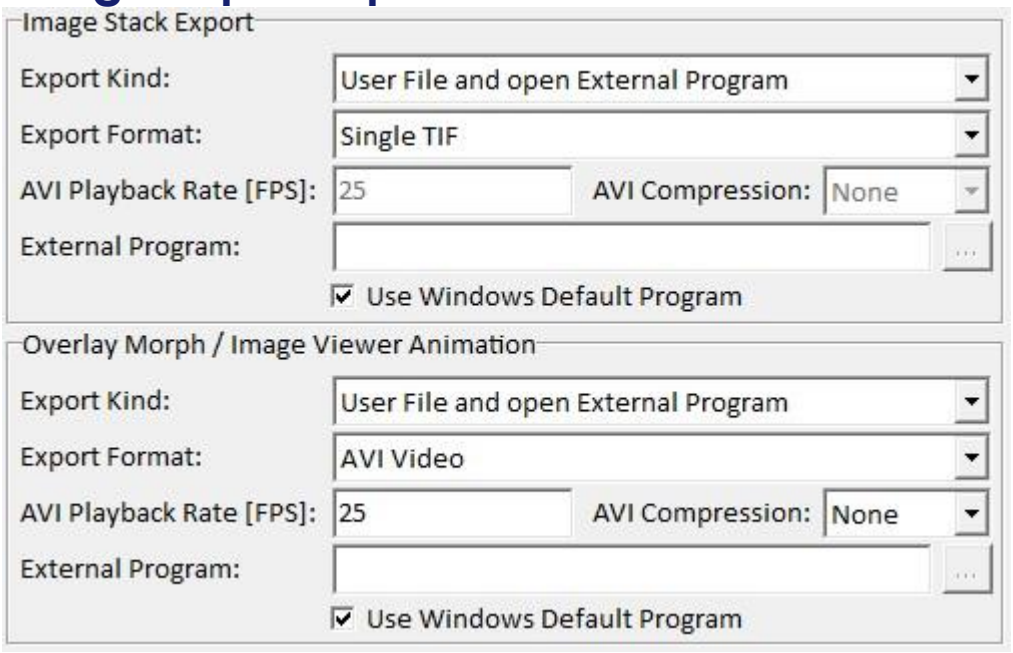


Image Stack Export

These settings are used for the Image Stack Export Drop Action.

Overlay Morph / Image Viewer Animation

These settings are used for

- The Image Transform and Overlay Drop Action (Export Overlay Morph)
- The Image Viewer Animation Editor

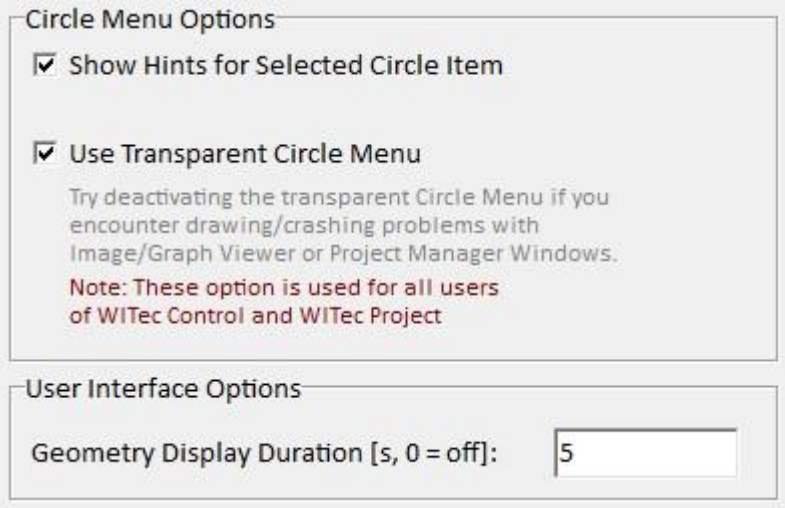
Export Kind
User File: Just saves a file using a save file dialog.
User File and open External Program: Saves the file using a save file dialog, then opens the configured external program.
Temporary File and open External Program: Saves the file in the Temp-Directory, then opens the configured external program.

Export Format
Single TIF: Saves one or multiple images into a single (multi-frame-)TIF image file.
Multiple Bitmaps: Saves one or multiple images as one or multiple bitmap files.

AVI Video: Creates an AVI Video File for movie playback, using the AVI Playback Rate and AVI Compression options.

External Program:
Enter the full path of an external executable, e.g. ImageJ or another program of your choice. Will be automatically opened depending on the Export Kind.

User Interface Options



Show Hints for Selected Circle Item
If checked, the Circle Menu shows a text when moving over an option.

Use Transparent Circle Menu
Can be turned off if there are problems displaying the circle menu (e.g. remote sessions).

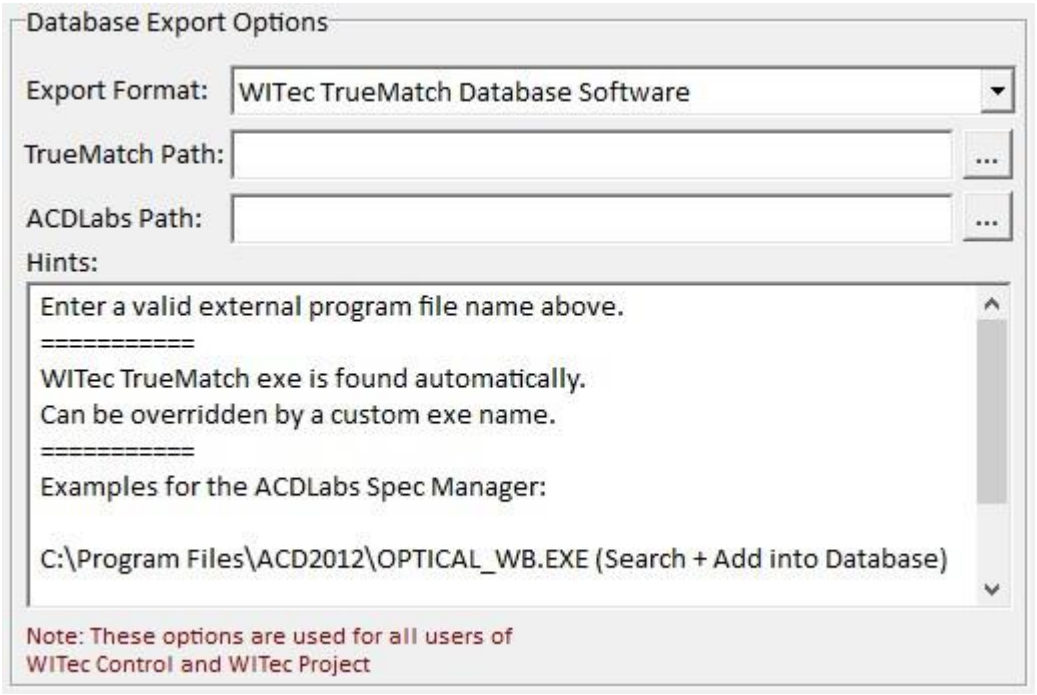
Geometry Display Duration
In image and graph viewers, you can press the "G" Button to send the current position to all other windows. This will define how long the position is shown in the other windows.

OpenGL Options

Use OpenGL Graphic Hardware Acceleration:
If checked, the software will use the graphic hardware acceleration to improve graphic performance (especially for large 3D presentations in Image Viewers).
This setting is used for **all users** and for both WITec Project and WITec Control from the same Suite Version.

Database Export Options

In the Database Export Options you can define which database software should used.
These options are shared for **all users** of the compter in WITec Control and WITec Project.
See Database Search in WITec Project.



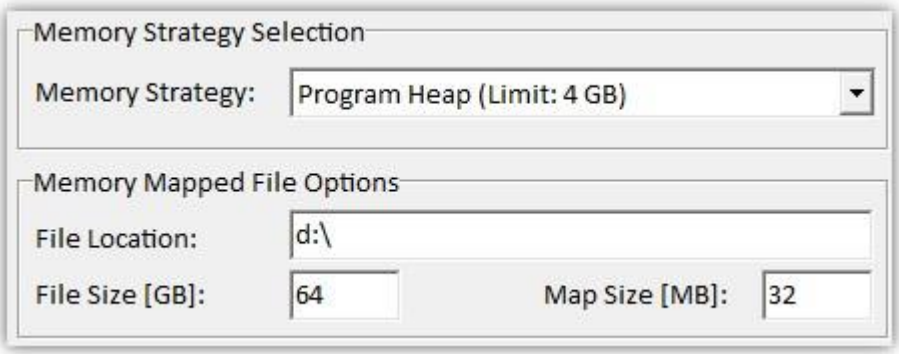
Export Format
Here you can choose between the WITec TrueMatch Database Software and ACDLabs.

TrueMatch Path
Should be empty. The TrueMatch executable will be found automatically for the WITec TrueMatch Database Software.

ACDLabs Path
Enter the path / executable file name for the ACDLabs Database Software.

The file name should be something like
C:\Program Files\ACD2012\OPTICAL_WB.EXE (Search + Add into Database)
C:\Program Files\ACD2012\SPECPROC.EXE (Search in Database Only)
C:\Program Files\ACD12\UVIRMAN.EXE (Search + Add into Database)

Memory Options



Memory Strategy

The Memory Strategy affects the way Data Objects are held in memory while the software is running and working with the data.
The main difference between the strategies is the amount of data that can be handled by the software as well as the performance while loading/saving/calculating.

Please take a look at the Data Object Memory Consumption article to get a feeling about the size of your measurement data.

These options are shared for **all users** of the computer.

Memory Strategy "Program Heap"

This is the standard "Memory Strategy"; if there are no memory problems this strategy should be used.
The software uses up to 4 GB of fast physical memory for data objects (the real maximum is < 4 GB).

Use this Memory Strategy if:

- you are working with small graph Data Objects (< 500 MB)
- you don't have a fast Solid State Disk (SSD) Hard Drive
- you have a 32-bit Operating System

Memory Strategy "Memory Mapped File"

This strategy enables the software to work with project data larger than 4 GB.
For this purpose the software uses a big file on a fast hard drive for Data Objects. This file is cached automatically by the operating system using the fast physical memory.

Use this Memory Strategy if:

- You have a 64-bit operating system
- The amount of installed physical memory is larger than your project size (e.g. > 8 GB)

Before using this Memory Strategy pay attention to the following notes:

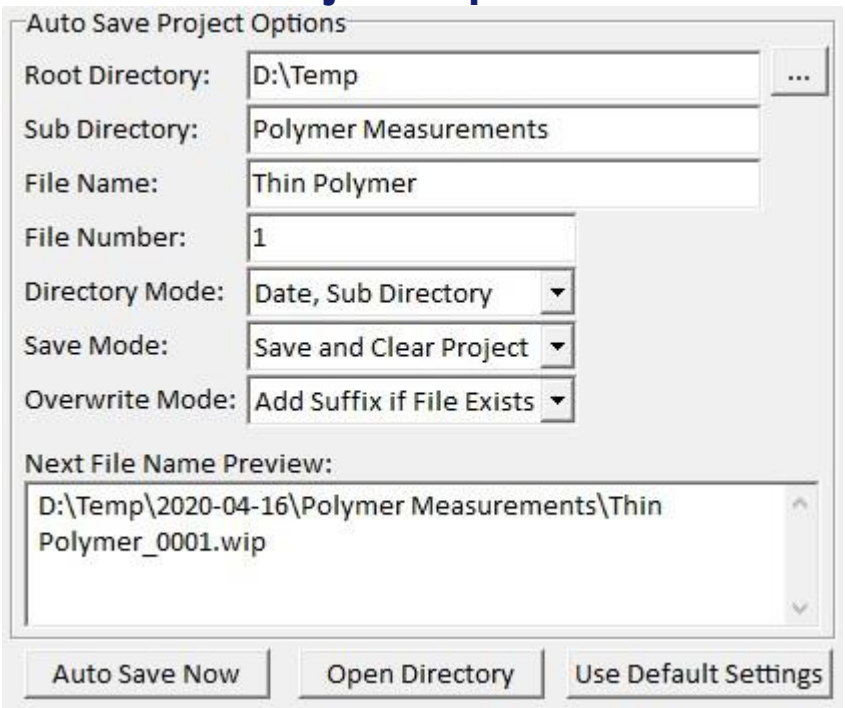
- For performance reasons, the "File Location" should be on a fast Solid State Disk (SSD) Hard Drive.
- The file size should be adjusted to the SSD size and to your project size needs.
- Each software instance will create a memory mapped file with the given size!

File Location:
The location of the memory mapped file; should be on a fast Solid State Disk (e.g. "E:\" if the drive letter of the SSD is E.)

File Size:
The size of the memory mapped file, i.e. the amount of space each instance of WITec Project/Control will need on the SSD.

Map Size:
The amount of physical memory used to map data between the memory mapped file and the application.
We recommend a value of 32 MB.

Auto Save Project Options



These options are used if you press the Auto Save Project Button:

Every time you perform the "Auto Save Project" action, the file number will be increased and a new file name will be generated.

Licensing

Licensing Overview

The WITec Control as well as the WITec Project software components need valid license keys before they can be started.

WITec Project

WITec Project is the basic post-processing software for analyzing WITec microscope data. The WITec Project (basic version) License Key is delivered with a microscope system and can be used by the whole user group of this microscope.

This Key is not bound to a computer and can be installed on a certain number of computers according to the license product. To install an existing license key onto a new computer, use the License Manager.

WITec Project Plus

WITec Project Plus is an add-on to the basic WITec Project software containing sophisticated data evaluation algorithms (see WITec Project Plus Features). All Plus features are available with limitations (demonstration mode) if you don't have a license.

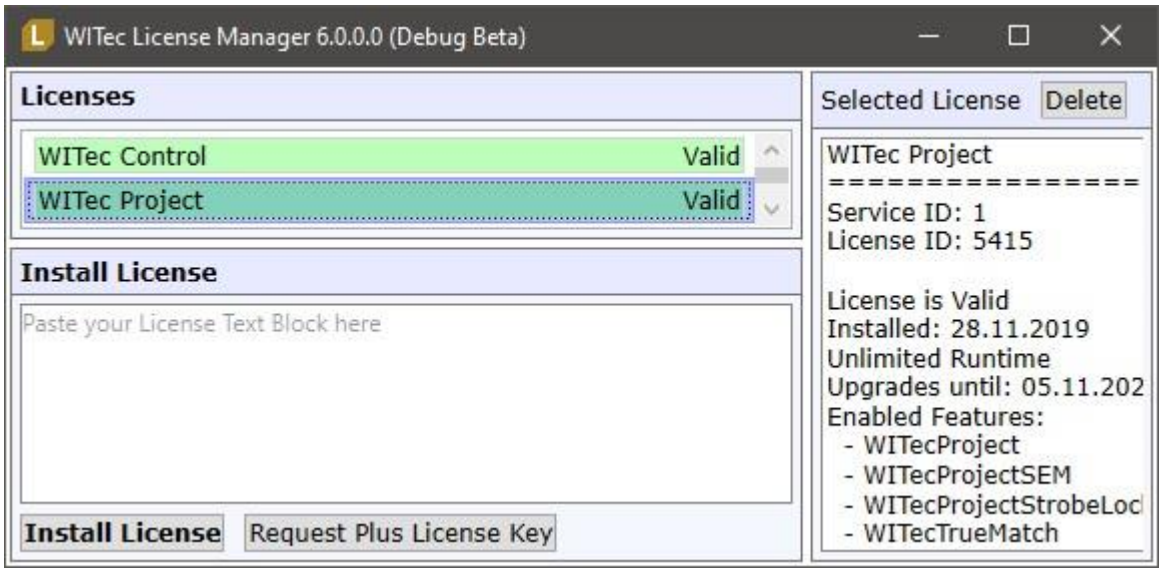
To use the plus features without limitations, you have to buy a computer-bound license, see WITec Project Plus Activation. This Key is bound to exactly one computer and cannot be installed on a different one.

WITec Control

WITec Control is only installed on the microscope system computer that comes from WITec and should already have a valid license installed. In case of (remote) software upgrades the WITec support team will install this license.

License Manager

The License Manager shows the currently installed licenses and allows you to install or remove licenses:



License List
Click on a License to see the License details.

Delete
Removes the currently selected License.

Please only delete a license if it is no longer valid or you really know what you are doing!

Install License
Copy your license text from the windows clipboard into the white area beside the black key symbol (using the Ctrl-V shortcut or via right-click Context Menu -> Paste). Then press the "Install License" Button.

Request WITec Project or Plus License Key:
This will open a request window which allows you to send a request to WITec.

Before sending a WITec Project Key request, check whether your company/department already has a License Key. In general, a WITec Project license is shipped with the WITec System.

Before sending a WITec Project Plus Key request, make sure WITec already received a license order. The Request is needed to create a computer-bound license key for WITec Project Plus.

Please at least enter your full name and your company/university, wherever applicable also the

other fields.

Send Request eMail:
If you have an eMail client installed and configured properly, you can press this button in order to automatically create a new eMail containing the request text.
If you don't have an eMail client on the certain computer, save the request to a file and take this file to a computer with an eMail client in order to send its contents to key@witec.de

Save Request To File:
Saves the request eMail Text to a file on hard drive.

WITec Project Plus Activation

Request Price Quotation

If you are interested in our Advanced Data Evaluation Software **WITec Project Plus**, feel free to ask for a price quotation: info@WITec.de.

Activation Process

Before you can use your WITec Project Plus License, you have to activate the license:

Send Request:

- Start the WITec Software on the computer on which the plus license should be activated.
- Open the License Manager via "Main Menu > Help > License Manager".
- Press the "Request WITec Project Plus Key" Button.
- Enter your contact information.
- If you bought multiple licenses to activate the software on more than one computer, enter the computer name in the reason field to be able to distinguish the keys later on.
- Press the "Send Request eMail" Button to send the Request to WITec if you have an eMail Client installed on the same computer; otherwise:
- Press the "Save Request To File" Button to store the request in a Text File and copy it to a computer with an eMail Client, then copy the content of the Text File into a new eMail addressed to key@witec.de.

Receive and Install License:

- WITec will send you a License Key via eMail (the Key is sent as an encrypted text block).
- Open the License Manager of WITec Project (or WITec Control) on the computer where the request was created.
- Copy the encrypted text block into the white empty field next to the key symbol and press "Install New License".
- Restart WITec Project. The splash screen should now display that WITec Project Plus is active ("Plus Version"). You can check whether the Plus Version is active at any time by opening the About and Support Panel in the Help Menu of WITec Project.

Note that the WITec Project PLUS license only works on the computer from which you created the license request.

If you have problems activating your WITec Project Plus Software, please contact key@witec.de.

WITec Project Plus Features

List of all WITec Project Plus Features

The following Drop Actions are features of the WITec Project Plus Software.
Some standard Drop Actions contain parts that only work with the Plus license.

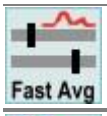
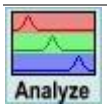
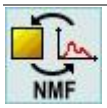
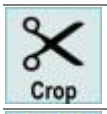
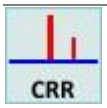
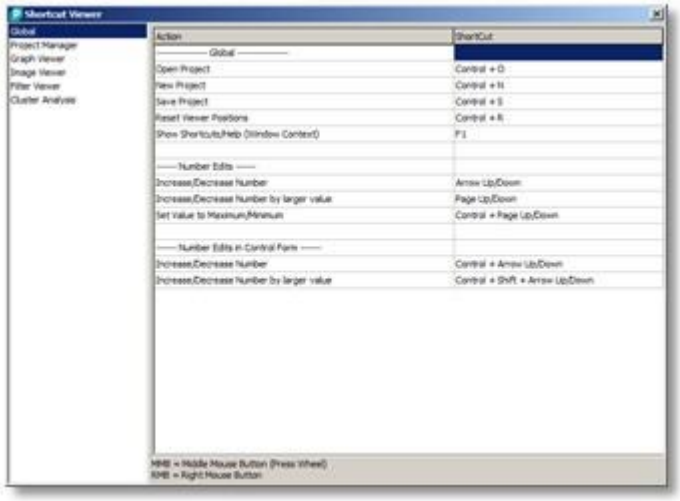
	**Advanced Graph Average** fast threshold mask creation from images and "real-time" average spectra
	**Graph Background Subtraction (only parts)** shape subtraction, automatic mask in polynomial subtraction, weighted constant spectrum subtraction
	**TrueComponent Analysis** find components, demix spectra, create intensity images
	**Inverse Basis Analysis** create component spectra using intensity images and the original image spectra
	**Advanced Fitting Tool (only parts)** fit all spectra in a multi spectral data object in one step
	**Cluster Analysis** automatically find similar spectra in a multi spectral data object
	**Non-Negative Matrix Factorization** automatic creation of component spectra and distribution images
	**Principal Component Analysis** PCA Transformation of spectral image data objects
	**Graph Repair** replace spectral pixels by a simple interpolation
	**Data Cropping and Reduction** change the size of images, bitmaps, spectral data objects
	**Graph and Image Stitching** stitch multiple images or image spectral objects side by side into one larger image or spectral object
	**Image Transform and Overlay** overlay 2 images, adjust Image position and scale including advanced transformations
	**Image Correlation** calculate correlation graphs to plot a correlation point cloud of two or more images
	**Image Filter (only parts)** Anisotropic, Sobel, Kirsch, Variance, Laplace, Sharpen and Custom Image Filters
	**Image Fourier Filter** create Fourier Amplitude Images or remove noise frequencies in fourier space with back transform

	**Image Repair** replace bad image pixels using user values with noise, interpolation or texture analysis algorithms
	**Cosmic Ray Removal (only parts)** advanced cosmic ray removal / possibility to browse and manually deselect found cosmics

Shortcuts

There are a lot of shortcuts in WITec Project.
An overview is available in the Shortcut Viewer: **Main Menu > Help > Show Shortcuts.**



Here you can find:

- Global shortcuts
- Project Manager shortcuts
- Graph Viewer shortcuts
- Image Viewer shortcuts
- Text Viewer shortcuts
- Filter Viewer shortcuts
- Cluster Analysis shortcuts.

Some shortcuts are also shown in the hint of an UI element if the mouse is just moved over the element.

WITec TrueMatch

TrueMatch Overview

Welcome to the WITec True Match Database Search Software Help.

Installation	Infos about the installation
User Interface	Help for menu, spectrum selector, spectrum viewer, sample properties
Search	Help for all search features
Database Management	Handling custom databases and ST Japan integration

Press the **F1 key** anywhere in the software to open the
context help or browse the Help Menu to open the help contents

Installation

Installation

The WITec TrueMatch Software is delivered in a combined setup with WITec Project (or available as a separate .msi installer).
It contains the following software components:

- WITec TrueMatch Executable and all depending DLLs
- WITec ParticleScout and WITec TrueMatch are integrated in the same application

Program Start

TrueMatch is started automatically when exporting spectra from WITec Control or WITec Project to TrueMatch.
You can also start TrueMatch using the start menu of windows or the "WITec ParticleScout" shortcut on the desktop.

Licensing

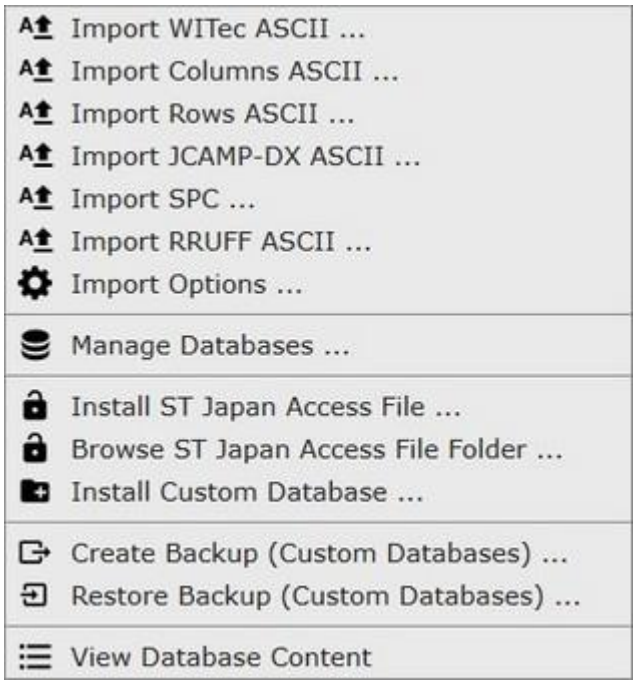
A special TrueMatch license is needed to use TrueMatch without any limitations.
Once you have ordered the TrueMatch license, it is included in the WITec Project license and can be used on any number of computers.

Computer Requirements

- 4 GB RAM minimum, 8 GB recommended
- 1 GB Hard Drive Space
- Direct3D compatible display adapter
- Windows 10 64-bit
- .NET Runtime 4.8 or newer
  (Direct Download Link: https://go.microsoft.com/fwlink/?linkid=2088631)

User Interface

TrueMatch Menu



Import

Imports spectra that can be used for searching or for adding into your own database.

Import WITec ASCII
Imports spectra from one or multiple WITec ASCII files.

Import Columns ASCII
Imports spectra from one or multiple files containing simple data columns.
Decimal Separator must be a point ".".
Value Separator can be <Tabulator> or <Semicolon> or <Comma>

```
XData1 YData1 YData1 [...]
XData2 YData2 YData2 [...]
[...]
```

Import Rows ASCII
Imports spectra from one or multiple files containing simple data rows.
Decimal Separator must be a point ".".
Value Separator can be <Tabulator> or <Semicolon> or <Comma>

With Captions:

```
<separator>XData1 XData2 [...]
Caption1 YData1 YData [...]
Caption2 YData1 YData [...]
[...]
```

Without Captions:

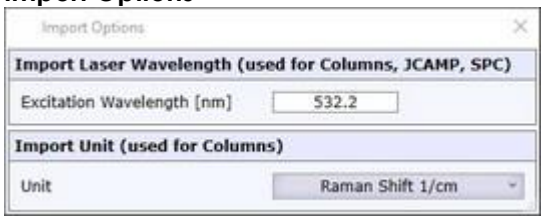
```
XData1 XData2 [...]
YData1 YData2 [...]
YData1 YData2 [...]
[...]
```

Import JCAMP-DX ASCII
Imports spectra from one or multiple JCAMP-DX ASCII files.
See https://en.wikipedia.org/wiki/JCAMP-DX

Import SPC
Imports spectra from one or multiple SPC binary files.
See https://en.wikipedia.org/wiki/SPC_file_format

Import RRUFF ASCII
Imports spectra from one or multiple RRUFF ASCII files.
See https://rruff.info/

Import Options



Excitation Wavelength
Defines the laser wavelength for imported data from "Columns", "Rows", "JCAMP" and "SPC" files.

Unit
Defines the unit of X-Values of imported data from "Columns" and "Rows".

Manage Databases
This opens the database manager. Here you can create or delete your own databases and show or hide the ST Japan and WITec Demo Databases.

Install ST Japan Access File
This will install the ST Japan Access File which is needed for running the ST Japan database. This file defines which ST Japan Sub-Databases are licensed. Also, a hardware USB Dongle must be used.
See ST Japan Database.

Browse ST Japan Access File Folder
Opens a explorer window and browses to the ST Japan Database folder.
See ST Japan Database.

Install Custom Database
Copies a .h5 database file into the TrueMatch custom databases folder.

Create Backup
This will put all your custom databases into a compressed ZIP file.

Restore Backup
This will restore custom databases from a compressed ZIP file. You will be asked if a database already exists.

View Database Content

This will open a database viewer which allows you to browse through all databases.

Spectrum Selector

Spectrum Selector

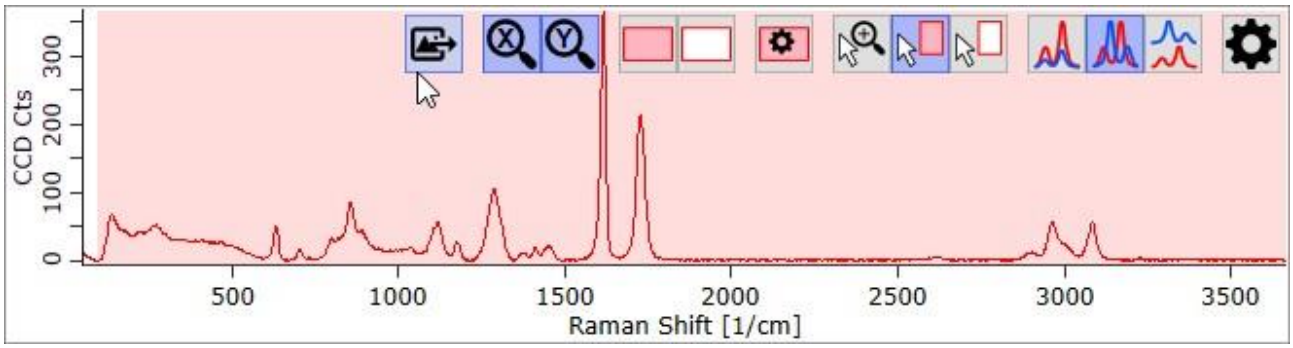
If multiple spectra are sent from WITec Project or were imported, you can select the current spectrum with the Spectrum Selector.
This will change the red preview graph to the selected spectrum and show the selected result.

If you want to add a single spectrum to a custom database, the currently selected spectrum will be added.

You can remove a spectrum from the list using the remove button on the right.

Spectrum Viewer

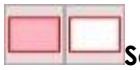
Spectrum Viewer



Buttons


Export
This allows you to export the current view into a image file or to a preview where you can define the line width, font size etc.
See Spectrum Viewer Export.


Zoom X / Y
Auto Zooms the X/Y-Axis.
Right-Click to turn on/off the automatic X/Y-Axis Zoom on graph change.


Set / Clear Mask
Sets the mask to a default mask or clears the whole mask.
Only visible if the Mouse Set/Clear Mask Mode is enabled.


Load/Save Mask
Enables to save or load masks.

This is saved per user.

 **Mouse Zoom Mode**
If selected, you can simply drag a region with the left mouse button to zoom that region.
Double-Click to automatically zoom the X- and Y-Axis.

 **Mouse Set Mask Mode**
If selected, you can drag a region (drawn as red background) to set mask pixels.
This way you define which spectrum pixels should be used for the search or to add into the custom database.
Double-Click into the viewer to reset the region to the default region defined in the Search Options.

 **Mouse Clear Mask Mode**
If selected, you can drag a region (drawn as red background) to clear mask pixels.

 **Same Y Axis**
If selected, uses the same Y-Axis for all spectra.

 **Overlay Y Axes**
If selected, each spectrum has its own Y-Axis and will be automatically zoomed to fit the window height.

 **Stacked Y Axes**
If selected, each spectrum has its own Y-Axis and will be stacked above each other.

 **Options**

Show Legend
Shows or hides the legend.

Show X/Y Axis
Show or hide the X/Y Axis drawing.

Hide Rayleigh Peak
If checked, the Rayleigh Peak will be hidden upon automatically zooming the X-Axis. You can zoom in and out the X-Axis any time using the mouse wheel (and holding the control key on the keyboard).

Zooming with the Mouse

Zoom Y Axis
You can simple turn the mouse wheel for zooming the Y-Axis.

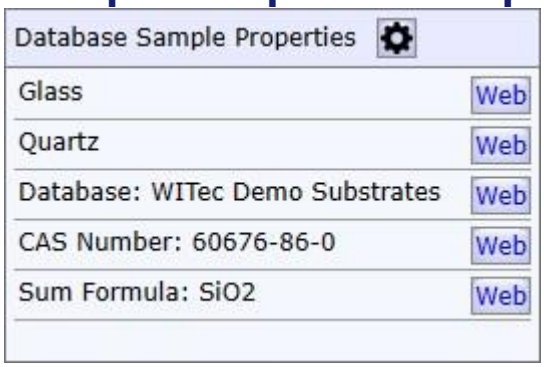
Zoom X Axis

Holding down the control key will zoom the X-Axis.

In the Mouse Zoom Mode, you can also drag a rectangle to zoom that region.
A double click into the viewer will zoom out automatically.

You can move the mouse to a position and press the <Space> key on the keyboard.
This will zoom into the region at the mouse cursor.

Sample Properties Display

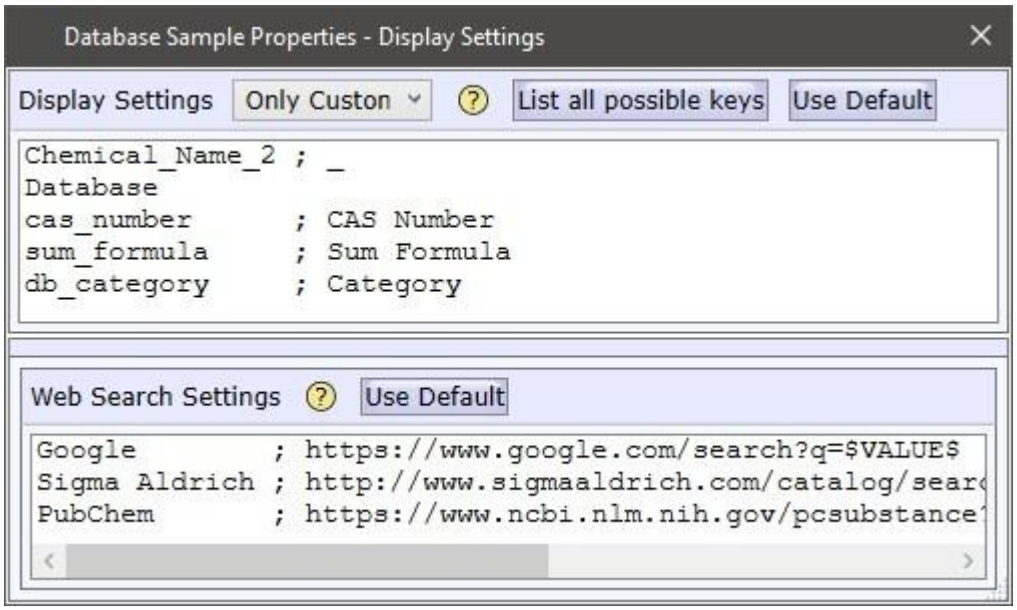


The Sample Properties box shows certain additional information about a database spectrum or search result.
For editing Sample Properties of your own custom database, see Database Editor and Sample Property Editor.

Web Search
You can click on the Web button in order to search for a certain property (uses default search).

With a *right-click*, you can select between a customizable list of searches:


Open Settings
You can define which properties should be displayed and also define a web link for each kind of information:



Display Settings

Show All (ComboBox)
You can select between the following options:

- Don't show: shows no properties
- Show All: shows all sample properties, no matter which are defined in the text. Though your definitions will be used.
- Only Custom: shows only the defined sample properties

List all possible keys
Enumerates all info keys from all databases and shows them.

Use Default
Uses a default set of sample properties.

Text Box
Here you can define, which properties should be shown.
Syntax:
<Property Name> ; <Optional Display Name>

Web Search Settings

Here you can define any number of web search URLs that can be used to search all sample properties in the web.
Each line in the text box defines a search.

The first line is the **default search** which is performed on a left click.

Syntax:
<Search Name> ; <Search URL>

The string $VALUE$ in the Search URL will be replaced by the particular sample information.

User Spectrum Properties

The small button on the far right will show the properties of the user spectrum, if any set. Here you can also define which properties should be shown.

Search

Search Overview

Search and Options

Simple Search
This will start a simple database search: each unknown spectrum will be compared with each database spectrum.
Only spectra from databases and categories of the Main Search Set defined in the Database Selection are used.

2-Component-Search
This will start a two component search: two database spectra are mixed to fit the unknown spectra.
Only spectra from databases and categories of the "Set for Component 2" defined in the Database Selection are used.

3-Component-Search
This will start a three component search: three database spectra are mixed to fit the unknown spectra.
Only spectra from databases and categories of the "Set for Component 3" defined in the Database Selection are used.

Demix-Search
This will start the demix search: all unknown spectra are demixed to fit the database spectrum.

Self-Search
Starts the self search: only all unknown spectra are compared with each other.
Can be used to see if there are similarities between your unknown spectra.

Search Options
See Search Options

 **Result Display Settings**
See Result Display Settings

 **Database Selection**
See Database Selection

 **Export Results**
See Result Export

 **Report**
See Generate PDF Report

Selected Result

Shows information about the selected result, such as the full name and the overlap (the overlapping region of the unknown search spectrum and the selected database spectrum)

Show Original Search Spectrum (Visible on Demix Search Result)
If not checked: the fitted / demixed spectrum is shown
If checked: the original search spectrum is shown

Arrow Buttons (Visible on Simple Search Result, if sorted by material)

Jumps to the result list of the previous / next material.

Result Lists

Shows the current search results.
For each unknown spectrum, one result list is displayed showing the best results on top.

You can click any result with the mouse or move through the results using the arrow keys left/right/up/down.
You can select multiple results for exporting or for the report by clicking on the little flag or by pressing the space bar.

This button is shown, if one of the following settings lead to a result hiding:

- Minimum HQI
- Minimum Overlap

- Search/Filter Name
- Result deleted using the Delete-Key

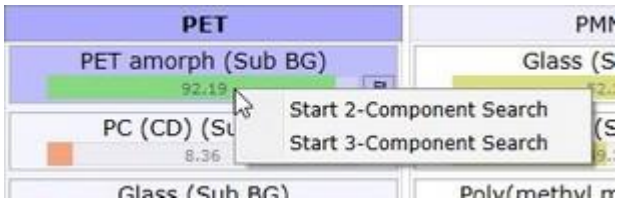
If this button is clicked, all results are shown again and deleted results are recovered.

This label is shown if the Minimum Overlap in the Search Options leads to less results.

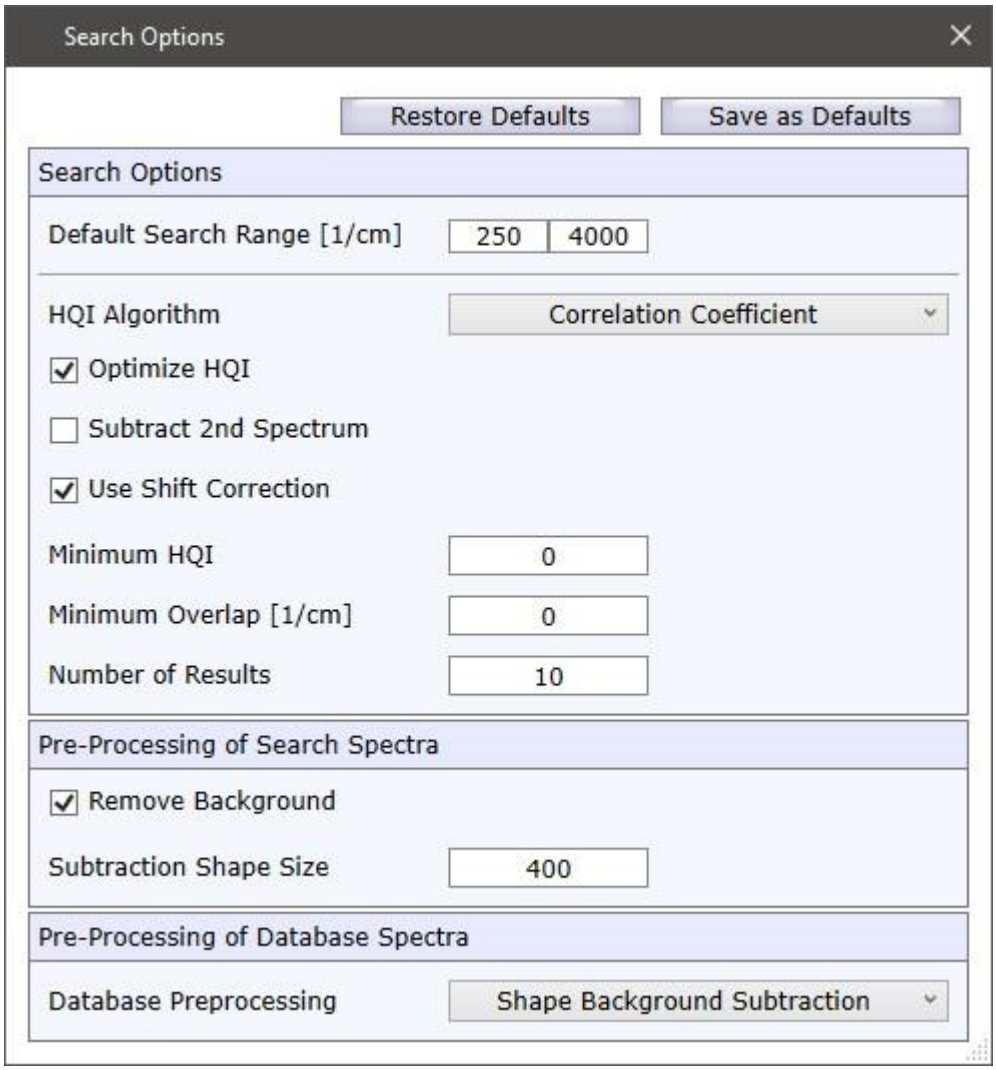
Use Result Database Spectrum for Multi-Component-Search

Press the 2 / 3 magnify icon in the "Selected Result" area in order to start a 2- or 3-Component Search using the result database spectrum as one (or two) of the components. A new tab will open and show the result.

You can also Right-Click on a search result to start the search:



Search Options



Search Options

Default Search Range
Defines the default mask region for the search.
You can manipulate the mask in the graph viewer in order to define which part of the spectrum should be used for the database comparison.

HQI Algorithm
Defines the way how spectra are compared:

- Correlation Coefficient
- Absolute Difference
- Least Squares
- Euclidean Distance

See Detection Algorithms

Optimize HQI
Improves the HQI on noisy spectra.

Subtract 2nd Spectrum
Subtracts the 2nd Spectrum from the first one when using 2- or 3-Component Search.
E.g. subtract the substrate from the interesting material, thus reducing the influence of the substrate

on the HQI.

Use Shift Correction
If checked, the search spectrum is shifted in a range of ±10 [1/cm] to get the best database match.
After searching, the shift with the best match is shown in the Selected Result area of the search tab.

Minimum HQI
Filters the result (only results with a minimum HQI are shown).

Minimum Overlap
Filters the result (only results with a minimum overlap are calculated and shown).

Number of Results
Defines the number of displayed results.

Pre-Processing of Search Spectra

Defines the preprocessing for the unknown spectra.

Remove Background
If checked, the background subtraction is done before searching. The WITec Project shape algorithm is used for the subtraction.

Subtraction Shape Size
Defines the shape size for the background subtraction. A lower value will subtract more details from peaks.

Pre-Processing of Database Spectra

Database Preprocessing
Defines the way how database spectra are preprocessed before comparing to the unknown spectra:

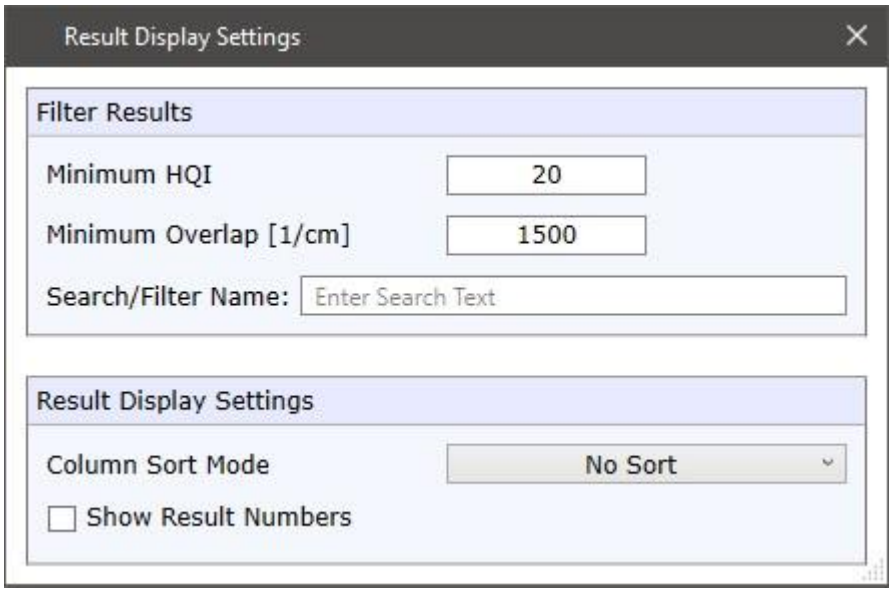
- No Preprocessing
- Use Derivative
- Shape Background Subtraction

Defaults

Restore Defaults
Uses the defaults and overwrites all current search options.

Save as Defaults
Saves the current settings as your defaults for future searches.

Result Display Settings



All display settings can be changed before AND after doing a search.

Minimum HQI
Filters the result (only results with a minimum HQI are shown).

Minimum Overlap
Filters the result (only results with a minimum overlap are shown).

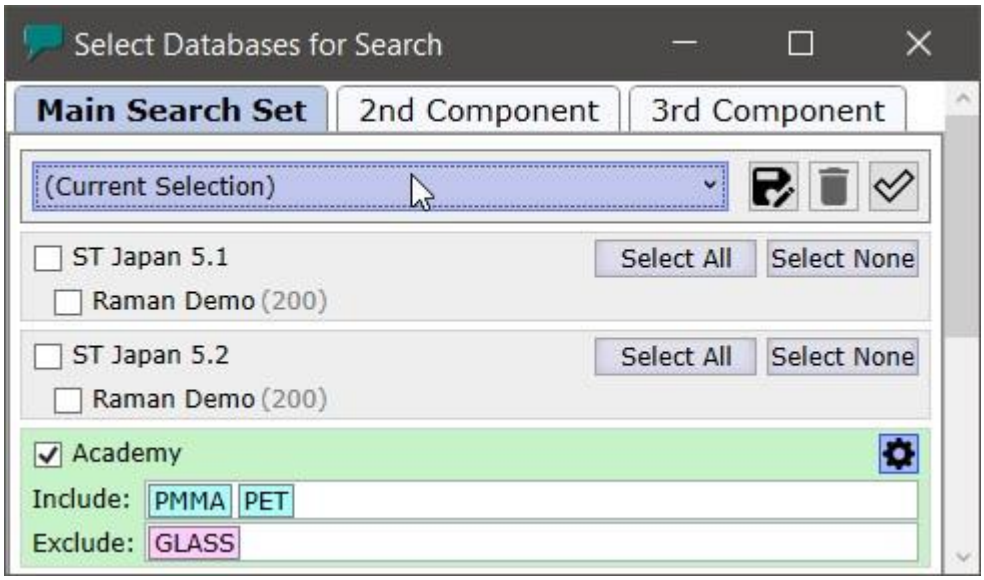
Search/Filter Name
Can be used to filter the result lists by database sample name.

Column Sort Mode

- No Sort - Uses the order of search spectra (imported or from WITec Project/Control)
- Best HQI - Sorts result columns by the HQI of the best result
- Material Name - Sorts result columns by the material name of the best result
- Best HQI & Material Name - Sorts result columns by the HQI of the best result and groups by the material name

Show Result Numbers
If checked, shows a result number / index on each result

Database Selection



Databases used for Search

Here you can select which databases should be used for the search.
The ST Japan contains multiple categories that can be checked or unchecked.
If no ST Japan Dongle and Access File is present, only the Raman Demo category containing 200 random Spectra is available for testing.

Main Search Set
This selection is used:
- for the simple search
- as the first component for a 2- or 3-component search
- and for the demix search

2nd component
This set is used as a second component for the 2- or 3-component search

3rd component
This set is used as a third component for the 3-component search

Current Selection (ComboBox)
When selecting a search set using the combo box, all selections are changed according to the saved selections.

Save current selection as new "Search Set"
Creates a new set from the current selection. You have to enter a name that will be used in the set selection combo boxes.

Delete selected
Deletes the selected main search set from your search set list.

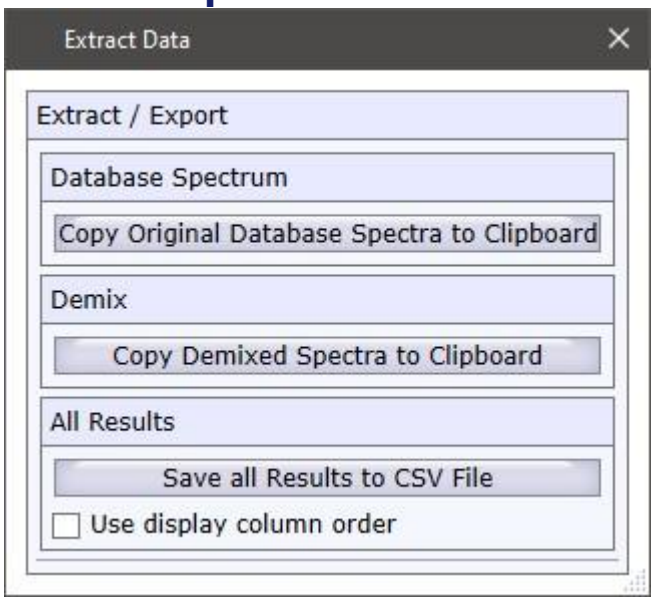
Select All / None
Toggle select all or none of the databases.

Include / Exclude
Type a sample name you want to include or exclude in your search. Hit the Enter or Return Key to accept the sample name.

If names are included, only samples with the given names are used for the search.
If names are excluded, samples whose name matches the typed names will be excluded for the search.

Note: All sets and selections are stored on the hard drive.

Result Export



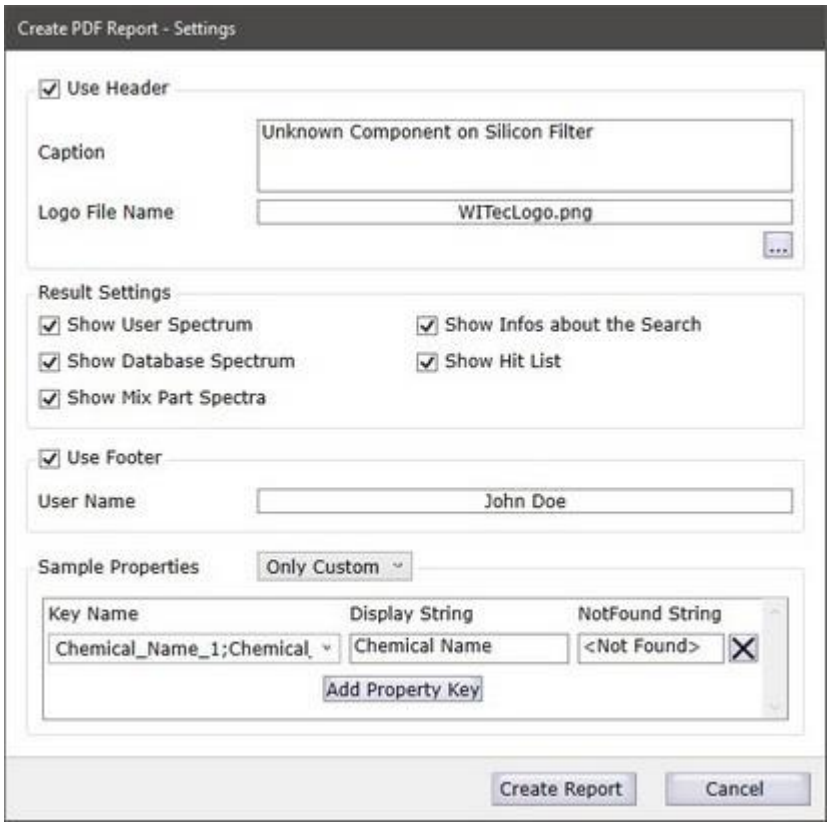
Copy Original Database Spectra to Clipboard
Copies the original database spectrum to the clipboard. Only works with custom databases. When adding spectra to a custom database, the original spectrum is always stored additionally with the preprocessed search spectrum.
You can paste the data into any WITec Project instance.

Copy Demixed Spectra to Clipboard
Copies the demixed result spectrum to the clipboard.
You can paste the data into any WITec Project instance.

Save all Results to CSV File
Saves all displayed results into a semicolon-separated ASCII file.

Use display column order
If checked, the results of the csv file will have the same order like the currently displayed result lists (e.g. sorted by HQI).
Otherwise uses the original order of search spectra coming from WITec Project or the import file.

PDF Report



Use Header
Defines if a header is used in all pages.

Caption
Defines the caption in the header

Logo File Name
Defines the logo file name which is displayed in the right edge of the header

Show User Spectrum
You can define whether the unknown user spectrum is shown in as a graph.

Show Database Spectrum
You can define whether the found database spectrum is shown in as a graph.

Show Mix Part Spectra
When doing a 2- or 3-component search, all mix parts can be shown as single graphs.

Show Infos about the Search
If checked, a simple information about the used search is added at the beginning.

Show Hit List
If checked, shows a list of all results for each component.

Use Footer
Defines if a footer is used in all pages, showing a page number and e.g. a user name.

User Name
Here you can enter your name that will be displayed in the middle of the footer.

Sample Properties

Here you can define which sample properties should be shown for each result.
In the field <Key Name> you can enter multiple keys separated with semicolon.
All found properties for the given key names are displayed using the <Display String>.
If the property is not found, you can define a "not found string". Leave it empty to not display anything if the property is not found.

Detection Algorithms

Common Notations

$$S = \frac{1}{N}\sum_{i=0}^{N-1} S_i \qquad R = \frac{1}{N}\sum_{i=0}^{N-1} R_i$$

$\vec{R}$: *Reference Spectrum or Database Spectrum*
$\vec{S}$: *Spectrum*
R : *Average of Spectrum* $\vec{R}$
S : *Average of Spectrum* $\vec{S}$
N : *Number of Pixels in Spectrum*

Correlation Coefficient

$$HQI_{Cor} = \frac{\sum_{i=0}^{N-1}(S_i - S)(R_i - R)\left|\sum_{i=0}^{N-1}(S_i - S)(R_i - R)\right|}{\sum_{i=0}^{N-1}(S_i - S)^2 \sum_{i=0}^{N-1}(R_i - R)^2}$$

Absolute Difference

$$HQI_{Abs} = \left(1 - \frac{V}{D}\right) \cdot 100$$

$$V = \sum_{i=0}^{N-1} |S_i - R_i|$$

$$D = \sum_{i=0}^{N-1} |S_i|$$

Least Squares

$$HQI_{LS} = \left(1 - \frac{V}{D}\right) \cdot 100$$

$$V = \sum_{i=0}^{N-1} (S_i - R_i)^2$$

$$D = \sum_{i=0}^{N-1} (S_i)^2$$

Euclidean Distance

$$HQI_{ED} = \left(1 - \frac{V}{D}\right) \cdot 100$$

$$V = \sqrt{\sum_{i=0}^{N-1} (S_i - R_i)^2}$$

$$D = \sqrt{\sum_{i=0}^{N-1} (S_i)^2}$$

ST Japan Database

How to buy ST Japan Databases

Because of technical reasons, it is not possible to buy the ST Japan Database directly from ST Japan.
Instead, please contact WITec or a local representative in order to buy a license and dongle from WITec (there is no price difference).

Installation of ST Japan Database

The following installation files are available:

1. ST Japan (All Spectra), Version 51
2. ST Japan (All Spectra), Version 52 (newer with more spectra)
3. ST Japan (All Spectra), Version 61 (newer with more spectra)
4. ST Japan Psychoactive, Version 52 (only psychoactive and drug substances)
5. ST Japan Psychoactive, Version 61 (only psychoactive and drug substances)

The setup for 1, 2 and 3 contains a demo license for 200 demo spectra.

Note that only WITec ST Japan Installation Files will work with TrueMatch / ParticleScout and you have to install the version which fits to your ST Japan License.

Installation of ST Japan License

After buying the ST Japan license, you will get an USB hardware dongle and a so called "Access File".
The Access File has the suffix ".stx".
To install the Access File, you can open WITec TrueMatch and then use the menu "TrueMatch -> Install ST Japan Access File". Browse your Access File and click OK.
The software will ask you, whether you want to install the USB Dongle driver. If the dongle driver was not installed before, click yes.

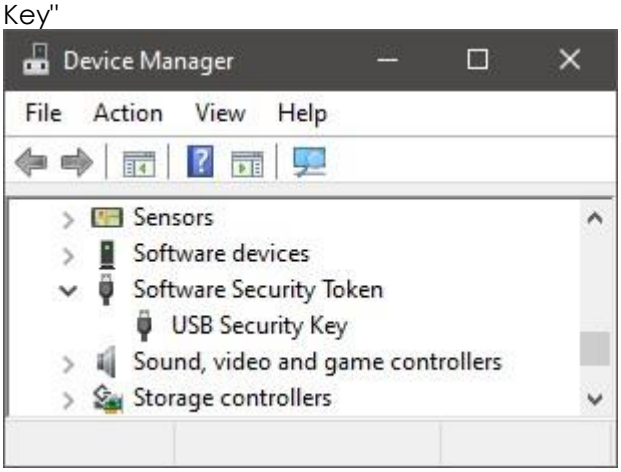
Make sure ST Japan is activated in the "Database -> Manage Databases .." Menu: "Show ST Japan". Now the ST Japan license should work.

Before searching, you can define which databases and categories should be used for the search. Just click on this icon:

Troubleshooting

If the ST Japan is not working correctly, please consider the following solutions:

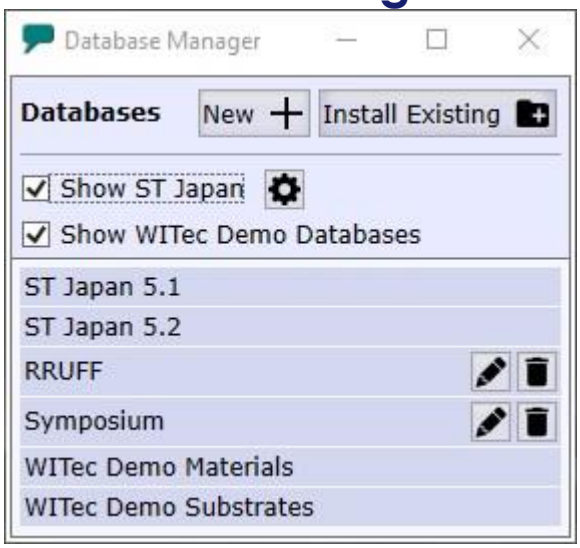
- Make sure the ST Japan USB Dongle is plugged in correctly.
- Ensure that the USB Hardware Slot is generally working.
- Check whether the USB Dongle is recognized by windows.
  For this, Open the device manager and look out for a "Software Security Token / USB Security Key"



  If the device is not listed, ensure that the USB Dongle Driver is installed.
  This normally happens automatically upon installing an Access file using the TrueMatch Menu.
- Make sure, the correct Version of the WITec ST Japan Database Setup is installed (51, 52, ...)
- Ensure that the correct .stx Access File is installed.
  In rare cases that you have installed multiple .stx Files (e.g. an older one which activates less spectra and a newer one which activates more spectra), you might have to remove the old/wrong .stx file using "Browse ST Japan Access File Folder" in the TrueMatch Menu.
- Make sure that the CheckBox "Show ST Japan" is checked in the Database Manager.
- Check whether the ST Japan Database or any of the available Categories is selected for searching.

Database Management

Database Manager



The database list shows all databases that can be used for the search.

New **Create New Database**
Creates a new customer database. The database file is automatically stored in a dedicated WITec databases directory.
You can backup and restore your databases using the TrueMatch Menu.

Install Existing **Install Existing**
Here you can select a custom database file that will be installed in the dedicated WITec databases directory.

Show ST Japan
If checked, the ST Japan Database can be used for searching. If you don't have a dongle with access-file, you can use the demo category of the ST Japan.
Uncheck if you didn't buy the ST Japan database.

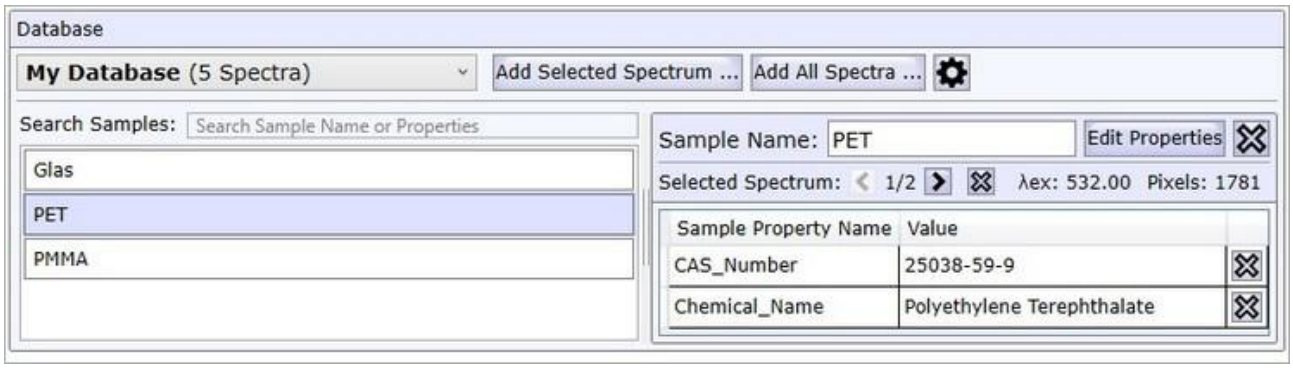
ST Japan Options
For WITec support team only.

Show WITec Demo Databases
Not yet supported: If checked, the WITec Demo Databases can be used for searching. Uncheck if you don't need those databases.

Rename Selected Database
Renames the selected database. The file name on the hard drive will be changed.

Delete Selected Database(s)
Deletes the selected customer databases from disk. The ST Japan and the WITec Demo Databases can not be deleted.

Database Editor



The Database Editor / Database Viewer can be used to browse all database spectra of all configured databases.
You can also add or remove samples/spectra to your own database.

"My Database" Database Selection (ComboBox)
Here you can select the desired database that you want to browse or to add spectra to.

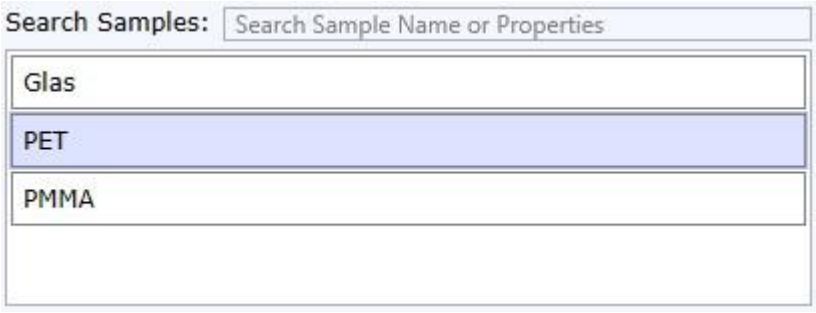
Add Selected Spectrum
Allows you to add the currently selected user spectrum to the selected database.
If a sample is selected, you can add the spectrum to this existing sample or you can create a new one (the software will ask you).

Add All Spectra
Adds all user spectra to the database and uses the names of each user spectrum as new sample names.
In WITec Project you can rename your single spectrum data objects, so the name is used here.

Add Options
See Add Options

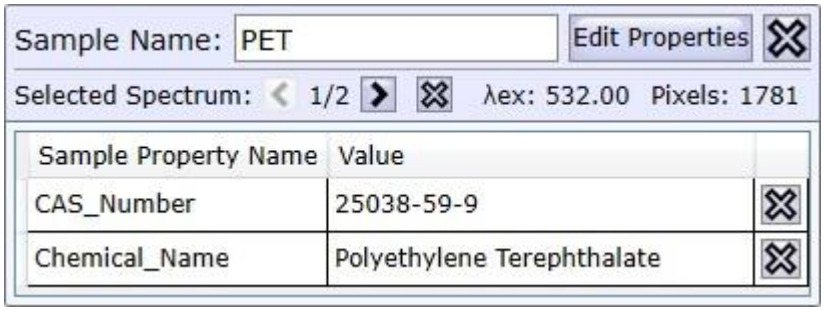
Current HQI

Shows the HQI of the selected user spectrum with the selected database spectrum.
Here you can also change the HQI Algorithm and create a report with those two spectra.



Search Samples
You can search in the sample names as well as in all sample properties.

Samples List View
Here you can select one of the samples. Clicking on a sample will show the first spectrum of the sample (there can be multiple spectra for each sample).
On the right side you can see details about the selected sample.



Sample Name
Here you can change the sample name, if a custom database is selected.

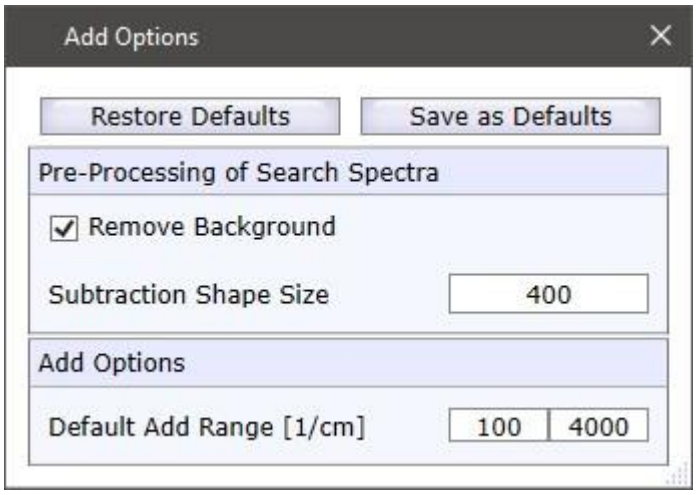
Edit Properties
You can edit the sample properties, see Sample Property Editor.

Selected Spectrum
If multiple spectra are stored for the same sample, you can browse through those spectra. Some information about the selected spectrum are displayed: the excitation wavelength and number of pixels.

Sample Property List
Just shows all sample properties. You can delete properties here or by unselecting the "Use" Checkbox in the Sample Property Editor.

Add options

Here you can define which part of the spectrum should be added and also if the Rayleigh peak should be removed and a background subtraction should be done.



Remove Background
If checked, the background subtraction is done before searching. The WITec Project shape algorithm is used for the subtraction.

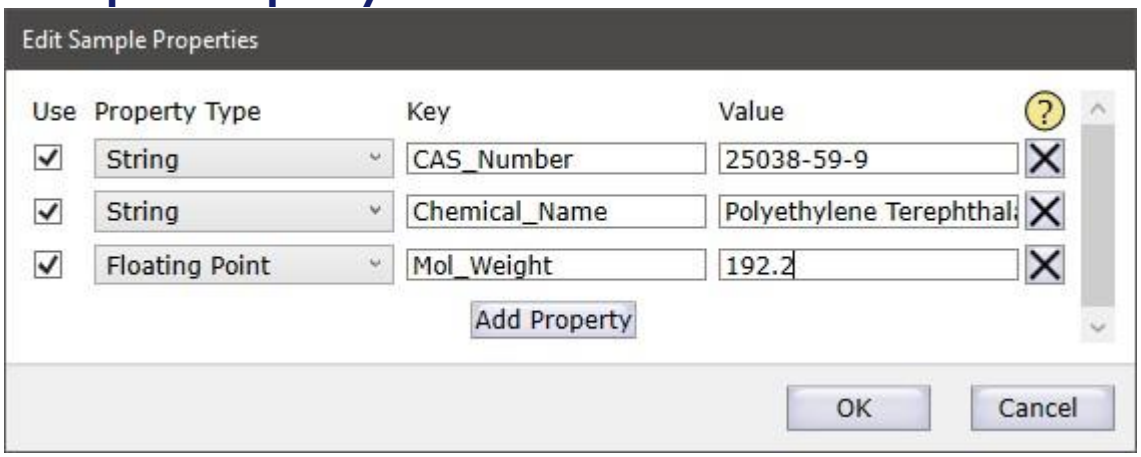
Subtraction Shape Size
Defines the shape size for the background subtraction. A lower value will subtract more details from peaks.

Default Add Range
Defines the default range that will be added into the database. You can manipulate the mask in the graph viewer in order to define which range of the spectrum should be saved as a database search spectrum.
Note: The original spectrum without preprocessing and region will be stored always in the database so you can reimport it to WITec Project after searching.

Save as Defaults
Saves the current Add Options as defaults.

Restore Defaults
Loads the defaults and overwrites the current settings.

Sample Property Editor



In the Sample Property Editor you can define your own property keys which are stored on the hard drive so you can reuse it for different samples/spectra that you add into your custom database. The Property Type can be String, Floating Point or Integer.

To add properties to your sample, simply change the value of a property and the "Use"-Checkbox will automatically be checked (upon changing the keyboard focus to another edit or if you press enter).

WITec ASCII Import

You can import one ore multiple spectra using the following WITec ASCII format in order to execute a search or to add the spectra to a custom database.

The file must start with the [WITEC_TRUEMATCH_ASCII_HEADER].

Each spectrum must begin with the [SpectrumHeader] containing basic information about the spectrum.

Optionally, a [SampleMetaData] section can be defined in order to add some sample properties when adding the spectrum to a custom database.
The format is <key> = <value>, if "double" or "int" is preceding, you can define a floating point or an integer number as value.

At last, the [SpectrumData] data section must follow using one spectrum value per line, beginning with the xdata / spectral position (in nm or 1/cm), followed by a <tabulator> character, followed by the ydata / CCD counts of this pixel.
The floating point number format must use a "." as decimal separator.

Example:

```
[WITEC_TRUEMATCH_ASCII_HEADER]

[SpectrumHeader]
Title = Component 1
ExcitationWavelength = 532.235
SpectrumSize = 1024
XDataKind = nm (or 1/cm)

[SampleMetaData]
CAS_NUMBER = 123-54578
double mol_weight = 63.123
int NumComponents = 2

[SpectrumData]
YourXData1 <tabulator> YourYData1
YourXData2 <tabulator> YourYData2
....
YourXData1024 <tabulator> YourYData1024

[SpectrumHeader]
Title = Component 2
ExcitationWavelength = 785.122
SpectrumSize = 1600
XDataKind = 1/cm

[SampleMetaData]
CAS_NUMBER = 123-11222
double mol_weight = 11.55

[SpectrumData]
YourXData1 <tabulator> YourYData1
YourXData2 <tabulator> YourYData2
....
YourXData1600 <tabulator> YourYData1600

...
...
...
```

Shared XData

For huge amount of spectra that share the same X-Axis values / XData, it is also possible to define the XData only once before the first [SpectrumHeader] is defined. This way, the ASCII save/load time can be reduced. In this case each spectrum only has to define the YData. The number of XData values must be the SpectrumSize and all spectra must have the same size.

Example:

```
[WITEC_TRUEMATCH_ASCII_HEADER]

[XData]
YourXData1
YourXData2
...
YourXData1600

[SpectrumHeader]
...
SpectrumSize = 1600
...
...
```

WITec ParticleScout

ParticleScout Overview

Welcome to the Particle Scout Help.

Installation	Installation Infos
Menu	The ParticleScout Menu
Find Particles	Find and filter particles on (Stitching) Video Images
Particle Manager	Browse and manage all particles
Raman Measurement	Perform Raman measurements on particles
Material Search	Use TrueMatch to perform a material search for each particle spectrum
Report	Create a report and perform statistical analysis

Press the **F1 key** anywhere in the software to open the
context help or browse the Help Menu to open the help contents

Installation

Installation

WITec ParticleScout and WITec TrueMatch are both included in the same executable and distributed with the same setup file.

Program Start

ParticleScout is started automatically when

- measuring a Particle Stitching image from the WITec Control Video Measurement Dialog
- when exporting images from the Project Manager Context Menu of WITecProject.

You can also double-click a .witscout (WITec ParticleScout) file or start the ParticleScout software using the start menu of windows or the desktop shortcut and load a particle project.

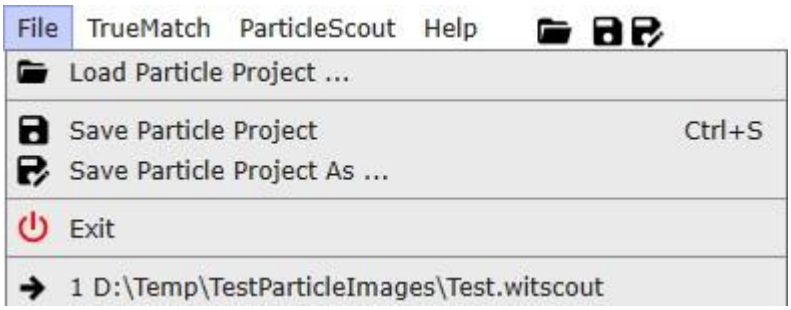
Please note that only one instance of WITec TrueMatch / ParticleScout is allowed.

Licensing

A special ParticleScout license is needed to use ParticleScout without any limitations.
Once you have ordered the ParticleScout license, it is included in the WITecProject license and can be used on any number of computers.

Menu

File Menu



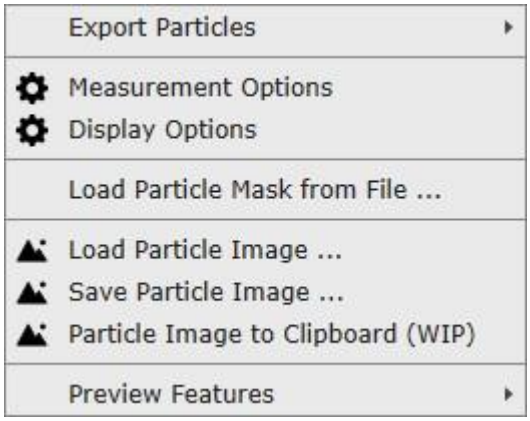
Load Particle Project
Loads a previously saved particle project.

Save Particle Project (As)
Saves the current particle project including all particles with their properties, thumbnail images, spectra and database search results.

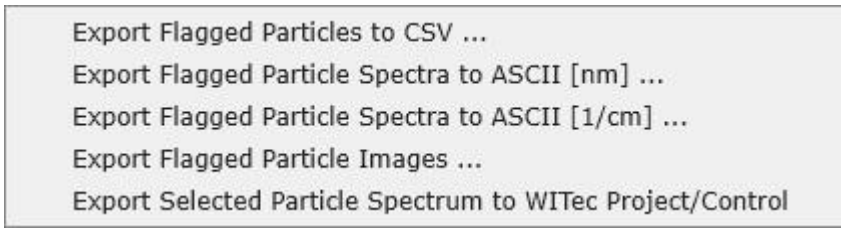
Exit
Exits the application.

Recent Files
Recent particle scout files are shown at the bottom. Just click it to load the particle project.

ParticleScout Menu



Export Particles



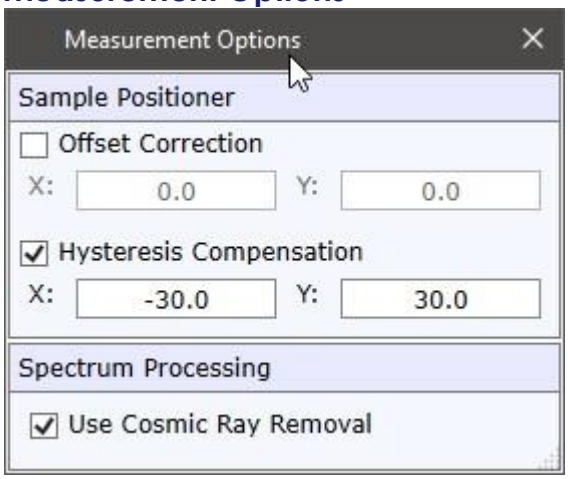
Export Flagged Particles to CSV
Exports all flagged particles with properties into a semicolon separated file.

Export Flagged Particle Spectra to ASCII [nm] / [1/cm]
Exports the measured spectra of all flagged particles into an ASCII file,
the format is the WITec TrueMatch ASCII Import format.
Choose a menu item for the desired X axis unit.

Export Flagged Particle Images
Exports the particle images of the flagged particles into a desired folder.

Export Selected Particle Spectrum to WITec Project/Control
Exports the measured spectrum of the currently selected particle to the clipboard.
Can be pasted as new single spectrum object into WITec Project/Control project manager.
Works only if a single particle is selected.

Measurement Options

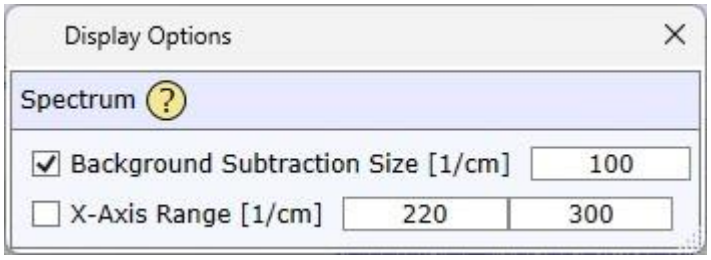


Offset Correction
If set, ParticleScout will move the sample positioner with an offset for each particle or if the move to particle button is used.

Hysteresis Compensation
If set, a hysteresis compensation is performed before moving to a particle.

Use Cosmic Ray Removal
If checked, cosmic rays in measured Raman spectra will automatically be removed using a cosmic ray detection algorithm.
Only works if at least 2 accumulations are used.

Display Options



Background Subtraction Size
If checked, a shape background subtraction is performed in all views that show spectra.

X-Axis Range
If checked, an exactly defined spectral range is shown in all views that show spectra.

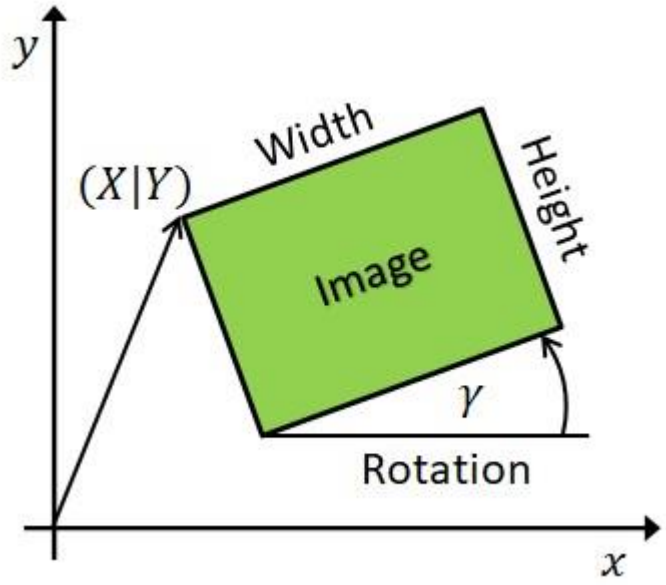
Load Particle Mask from File ...
Loads a bitmap and uses all white pixels as mask pixels for adding new particles.
If there are already particles in the current project, you can decide whether to create a new project or add the particles to the current project.

Load Particle Image ...
Loads one or multiple particle image files (e.g. *.bmp or *.png).
If there is already a particle project opened, a message box will open and the user can choose

- to use these images for adding more particles
- to use the image as a main image (if only one image is loaded)
- to create a new project (the first image will be used as the main particle image)

You can load the image with a coordinate information that contains the size, offset and the rotation.
It can be defined by saving a text file (.txt) with the same name as the image file name in the same directory.
Use "." as decimal separator.
Width/Height unit is microns.
X/Y/Z coordinates is the upper left corner of the image, in microns.
Rotation unit is radian.
Pixels must be quadratic.

Example:
Width: 558.24
Height: 558.24
X: 0
Y: 0
Z: 0
Rotation: 0



Save Particle Image ...
Saves the current particle image into a windows bitmap file.
Note that the software allows to save bitmaps with a maximum size of ≈700.000.000 Pixels, which might not be readable in every software.

Particle Image to Clipboard (WIP)
Copies the particle image to the clipboard in WIP format, so it can be pasted into the Project Manager of a running WITecProject or WITecControl instance.
The image will be downsized to 8000x8000 pixels, if larger.

Find Particles

Find Particles

Bright / Dark Particles (Automatically set)
Bright Particles: finds particles that are brighter than the background (e.g. dark field image)
Dark Particles: finds particles that are darker than the background (e.g. bright field image)

Mask by Threshold

Defines which brightness level should be used to detect the particles.

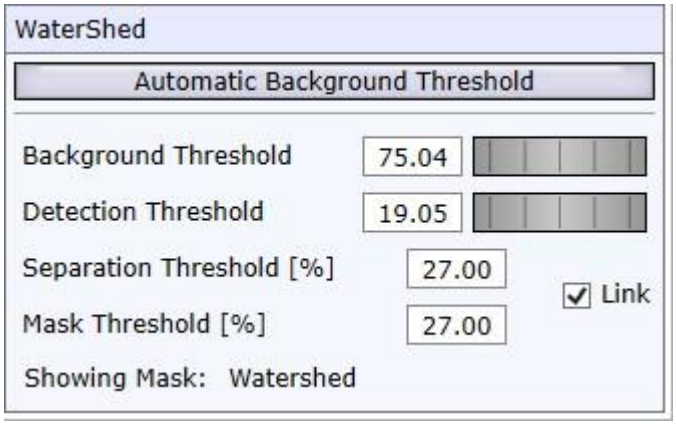
Auto
Calculates an automatic threshold (also used on startup).

For the automatic threshold, "Otsu's method" is used: Wikipedia Link

Mask by Watershed

This algorithm can be used to solve two problems in particle detection.

- Bright and less bright particles are both masked with an individual threshold depending on the particle brightness.
- Particles that are very near to each other can be separated.



To get the best results, please define the parameters step by step in the following order:

Background Threshold
Define a threshold for the background.
When changing this parameter, the preview will temporarily show the background mask.

Detection Threshold
Only areas that are above this threshold (background threshold + detection threshold) will be used to find the particles.
When changing this parameter, the preview will temporarily show the detection mask.

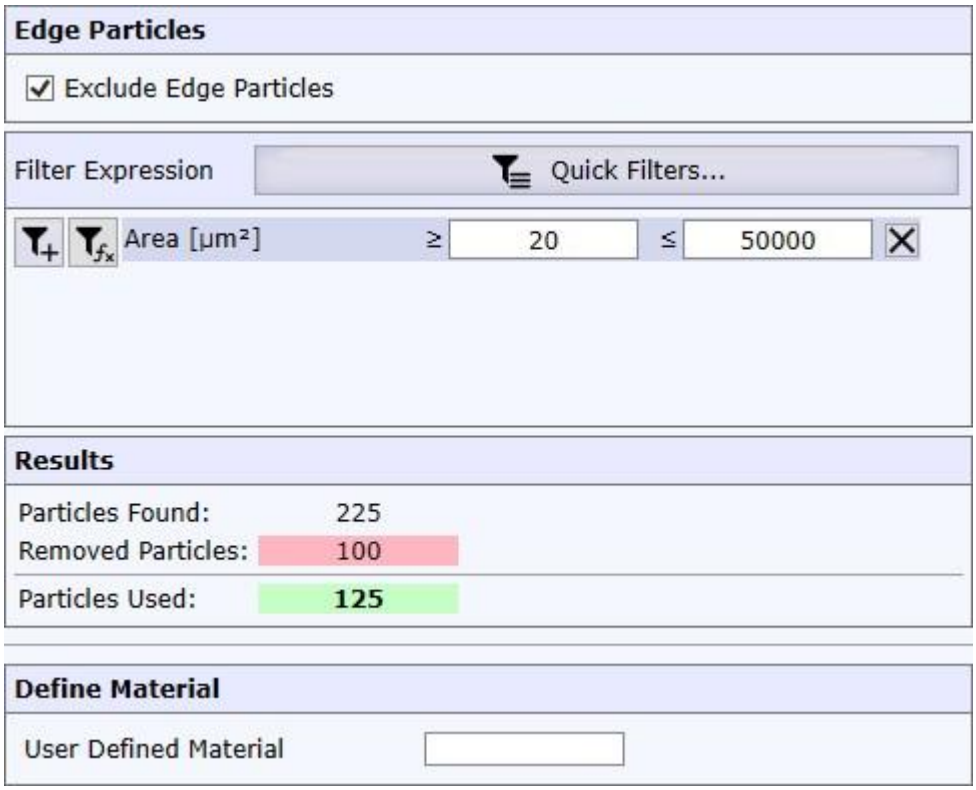
Link
If the link check box is checked, the separation and mask thresholds are set equal. This is useful if you are only interested in having individual thresholds for each particle.

Separation Threshold
During the calculation the particles are expanded around local intensity maxima. If the intensity drops below the separation threshold the region becomes a particle by its own. It will not be joined with other particles, which are "falling" below their separation threshold.

Mask Threshold
This parameter defines the mask threshold relative to the local maxima.

See Watershed Examples

Filter Particles



Exclude Edge Particles
If set, all particles whose contours are not completely within the image are skipped.

Filter Expression / Quick filters
You can filter out particles that do not match certain conditions or a custom filter expression.
E.g. you can define that only particles with an area larger than 50 µm should be detected.
See Filter Expression Editor.

User Defined Material
It's possible to export particle images from WITec Project with known material. In this case you can just enter the material name which is then assigned to the particle objects in the resulting particle list.

Actions

 **Use Results**
This will accept the current mask and proceed with the particle filter.

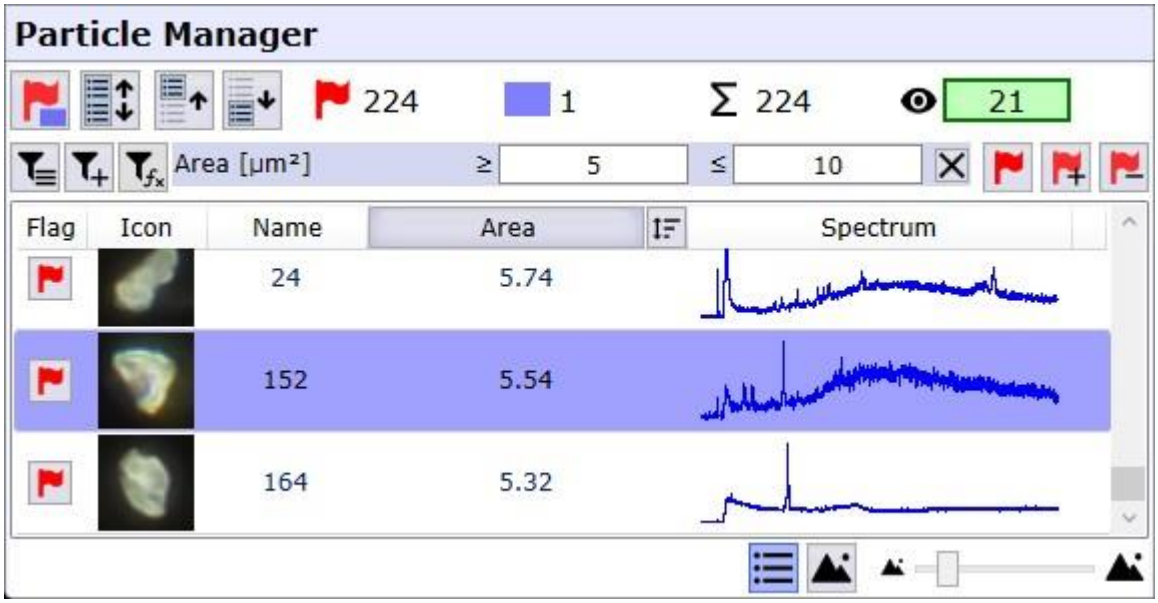
Preview

The preview image shows the original particle image and the current mask as an overlay.
In the upper right corner you can open options in order to change the brightness / contrast and change the opacity and color of the mask.

Particle Manager

The Particle Manager shows all particles of the current particle project.

It can be used to select particles and use certain particles for Raman measurement, database search or statistical analysis.



Selection for Analysis

Flags
Only flagged particles are used for

- Raman Measurement
- TrueMatch Database Search
- Statistical Analysis
- Export

Selection
The selection (blue rows) can be used to

- Flag/unflag particles
- Delete particles, particle spectra or particle material
- Calculate spectrum properties
- Define custom material

 Toggles the flag state of all selected particles.

 Selects all particles in the list.

 Selects all particles in the list that are above the currently selected particle.

 Selects all particles in the list that are below the currently selected particle.

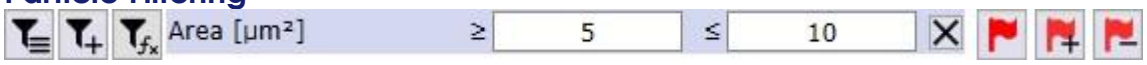
Select via List
You can click on any row to select a single particle. The selected particle will be shown in the Particle Detail View.
Use the Shift or Control keys on the keyboard in order to select multiple items.

Toggle Flag in List
You can click the box on the very left side of a particle row entry to flag or unflag this particle. Use the shortcut space to toggle the flags of all selected particles.

Number of Particles

54: Number of flagged particles.
1: Number of selected particles.
55: Total number of particles in the current project.
48: Number of visible particles in the list view.

Particle Filtering



Here you can filter particles matching a certain condition.

 Opens the quick filter menu to save and reload predefined filters

See Filter Expression Editor

 Flag only currently visible particles

 Add currently visible particles to existing flagged items

 Remove currently visible particles from existing flagged items

Sorting

Click on the button in the column header area in order to define which particle property should be shown in the column.
The selected property is also used for sorting the items.

 **Reverse**
If checked, the order of the current list of particles will be reversed.

If particles are sorted by material or any Boolean property, you can quickly jump to the next material / different value using the arrow buttons:

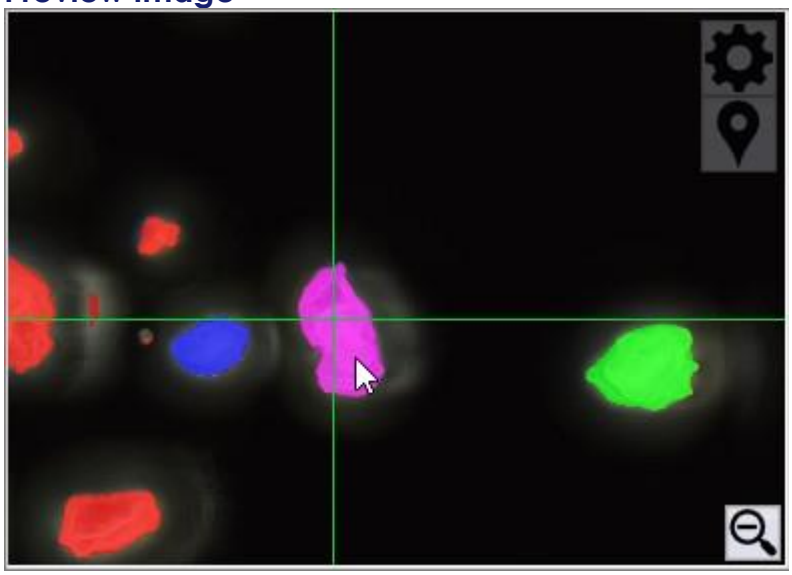
List View Settings

Here you can switch between the list view and the thumbnail view.
Both views have a custom thumbnail size.

Particle Details

See ParticleScout Particle Details.

Preview Image



The preview image shows the particle image.
All particles, that are visible in the list view are shown as a green overlay.
Red particles are flagged. Blue particles are selected, Pink particles are selected and flagged.

Click to Select
Click on any particle in order to select it in the list. Hold down the left mouse button to change the selection while moving the mouse.

Move Sample to Mouse Position
When enabled, click somewhere in the image in order to move the sample positioner to the desired position.

Actions

Find Particles

Returns to the Find Particles view so you can e.g. change the mask and create a new particle list.

Actions on Flagged Particles

Measurement
Opens the Measurement View. Here you can acquire a Raman spectrum at each particle and assign it to the particles.
Only available if WITec Control is running.

Material Search with TrueMatch
Opens the TrueMatch Search View. Here you can search all particle spectra in a spectral database and assign a material name to each particle.
Only available if particles with spectra are flagged.

Particle Report
Opens the Particle Report View. Here you create and customize a report of all flagged particles.

Calculate Spectrum Properties
This will calculate an estimation of the amount of Raman or fluorescence signal and a yes-no-info about the over-saturation for all selected particles.
See Spectrum Properties.

Actions on Selected Particles

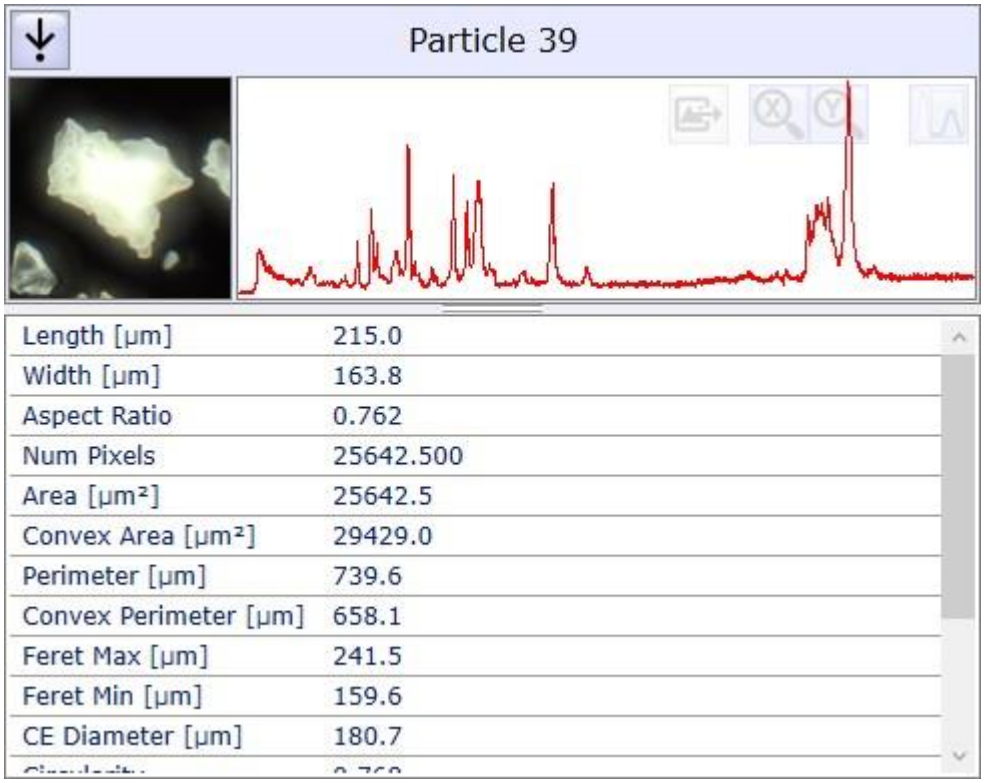
Define custom material
Here you can enter a material name and assign it to the selected particles.

Delete Spectrum
This will delete the spectrum and its information (material, spectrum properties) from the selected particles.
Only available if particles with spectra are selected.

Delete Material
This will delete the material name from the selected particles.
Only available if particles with spectra and assigned material are selected.

Delete Particle
Deletes all selected particles.

Particle Details



This view shows the details of a selected or measured particle.

You can select multiple particles in order to see some statistics of the properties of all selected particles:
Min, Max, Average, Standard Deviation and Median.

 **Move to Particle**
With this button you can move the sample positioner to the currently selected particle.

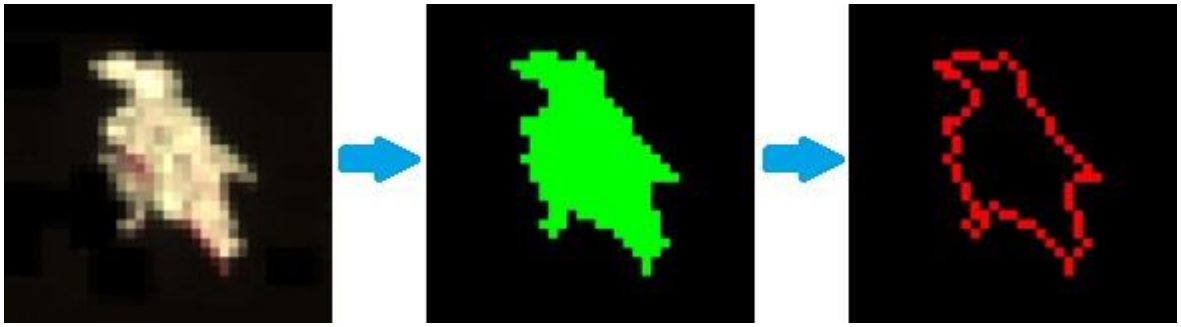
Spectrum Viewer
Shows the measured Raman spectrum. See Spectrum Viewer.

Particle Properties
Shows all particle properties.
For a detailed description for each property, see Particle Properties.

Particle Properties

In order to calculate descriptive particle properties from an image the following steps are done:

- The image is converted to a mask
- A list of particles is created (all connected mask pixels belong to one particle)
- For each particle a contour line is calculated
- The pixel positions and the contour line is used to calculate the descriptive particle properties



Definition of Particle Pixels

$$\mathbb{N} = \left\{ \vec{n}_i = \begin{pmatrix} n_x^i \\ n_y^i \end{pmatrix} : 0 \leq i < M_n \right\}$$

$\mathbb{N}$: *Set of all Mask Particles*
M_n : *Number of Mask Particles*
$\vec{n}_i$: *Pixel Coordinates*

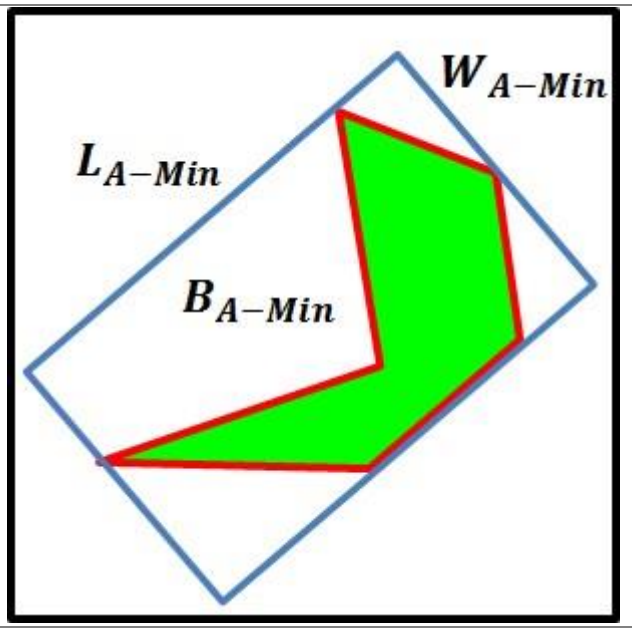
Definition of Particle Contour

$$\mathbb{C} = \left\{ \vec{c}_i = \begin{pmatrix} c_x^i \\ c_y^i \end{pmatrix} : 0 \leq i \leq M_c;\ \vec{c}_0 = \vec{c}_{M_c} \right\}$$

$\mathbb{C}$: *Set of all Contour Supporting Po*
M_c : *Number of Supporting Points*
$\vec{c}_i$: *Pixel Coordinates of Supporting*

Length and Width

In order to calculate the Length and the Width of the Particle the bounding box with the smallest area is fitted to the contour of the particle.
The larger side is defined as the Length.



L_{A-Min} : *Length of Bounding Box with smallest Area*
W_{A-Min} : *Width of Bounding Box with smallest Area*
$L_{A-Min} > W_{A-Min}$

Aspect Ratio

$$Aspect\ Ratio = \frac{W_{A-Min}}{L_{A-Min}}$$

The Aspect Ratio range is between 0 to 1.
A quadratic bounding box has an Aspect Ratio of 1.

Number of Pixels

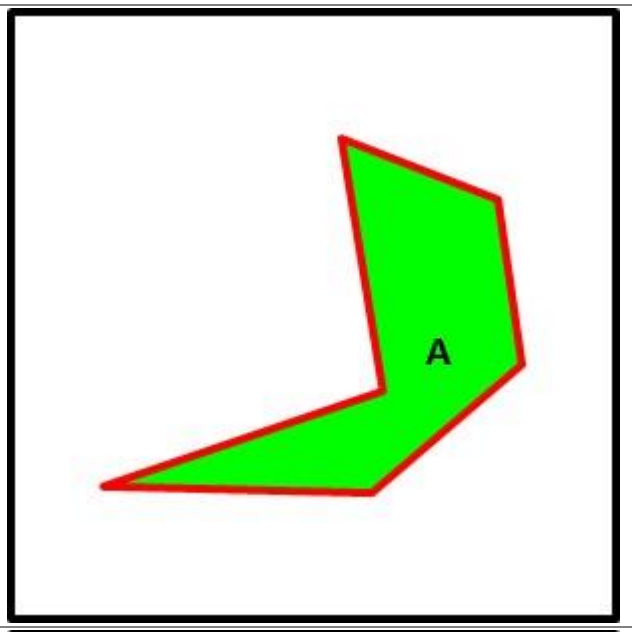
$$N = M_n$$

N : *Number of Particle Pixels*

Area

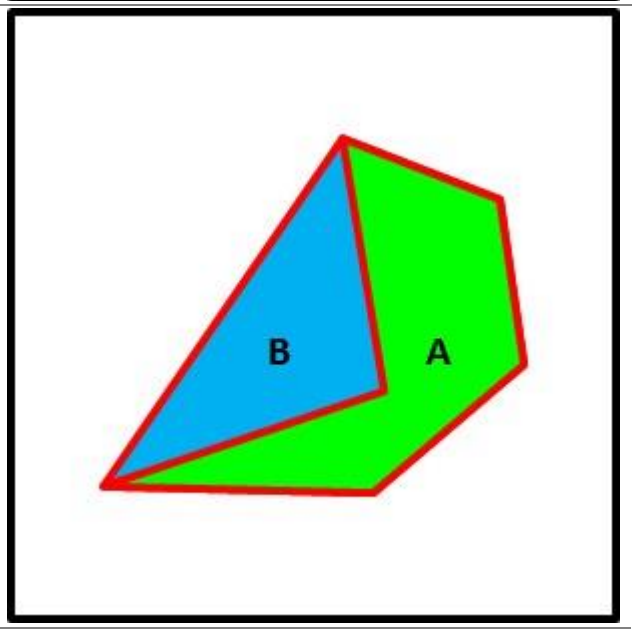
$A = a \times N$

A : *Particle Area*
a : *Pixel Area*



Convex Area

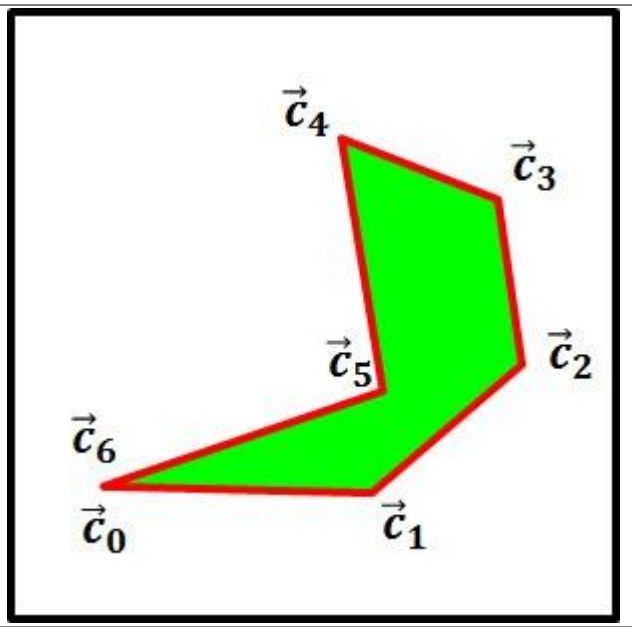
$A_{convex} = A + B$



Perimeter

$$P = \sum_{i=0}^{M_C - 1} |\vec{c}_{i+1} - \vec{c}_i|$$

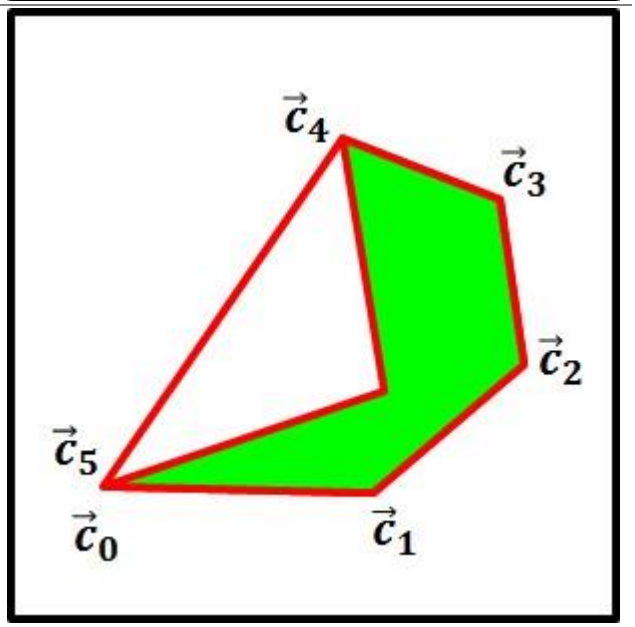
P : *Perimeter*



Convex Perimeter

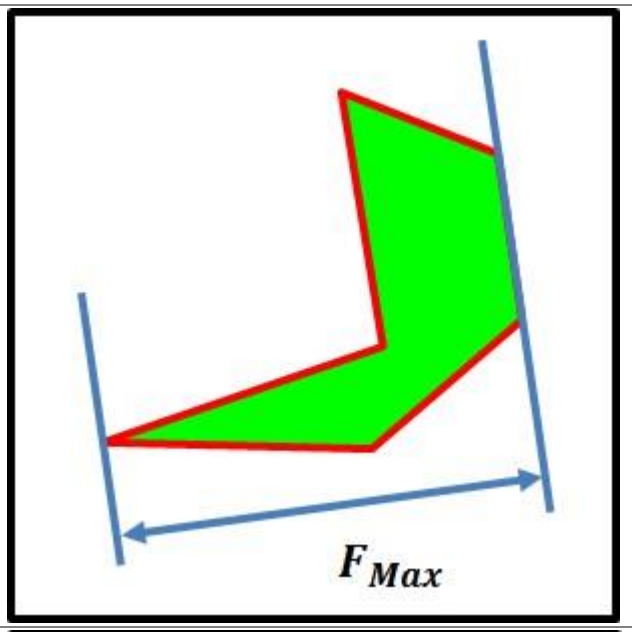
$$P_{convex} = \sum_{i=0}^{M_C - 1} |\vec{c}_{i+1} - \vec{c}_i|$$

P_{convex} : *Convex Perimeter*



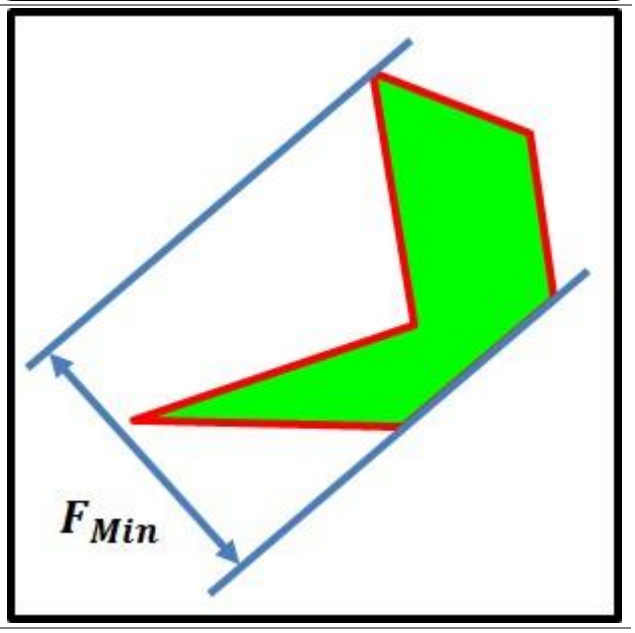
Maximum Feret Diameter

The Maximum Feret Diameter is the maximum distance that can be measured if the particle is rotated between a pair of calipers.
At least one caliper must touch a segment line of the perimeter.



Minimum Feret Diameter

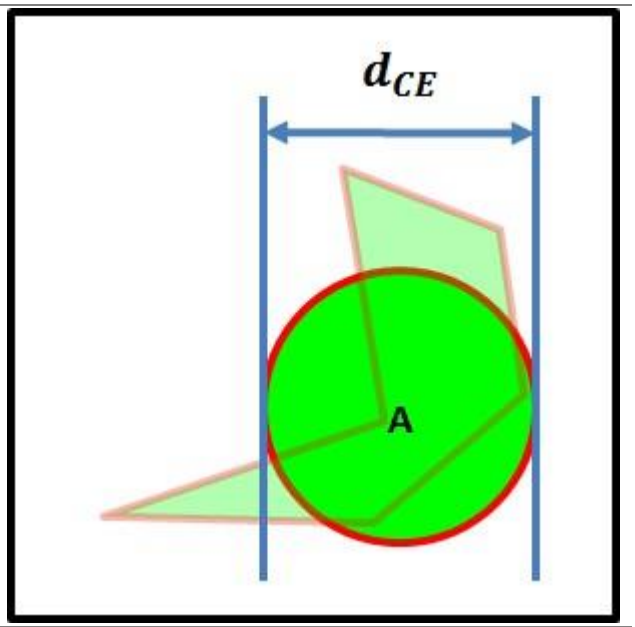
The Minimum Feret Diameter is the minimum distance that can be measured if the particle is rotated between a pair of calipers.
At least one caliper must touch a segment line of the perimeter.



Circular Equivalent Diameter

$$d_{CE} = 2\sqrt{\frac{A}{\pi}}$$

d_{CE} : *Circular Equivalent Diameter*

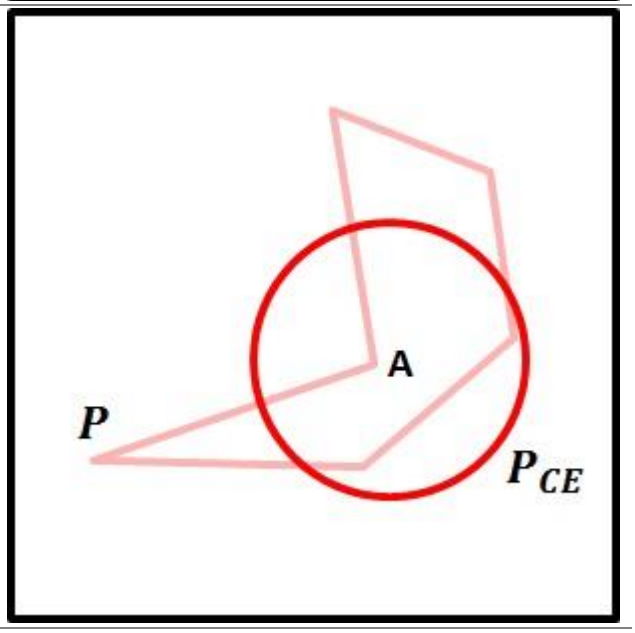


Circularity

$$C = \frac{P_{CE}}{P} = 2\frac{\sqrt{\pi A}}{P} = \frac{\pi d_{CE}}{P}$$

P_{CE} : *Circular Equivalent Perimeter*
C : *Circularity*

The Circularity range is between 0 and 1.
If the parameter is 1 the shape of the particle is a circle.



Convexity

$$Convexity = \frac{P_{convex}}{P}$$

The Convexity range is between 0 and 1.
If the parameter is 1 the shape of the particle is convex.

Solidity

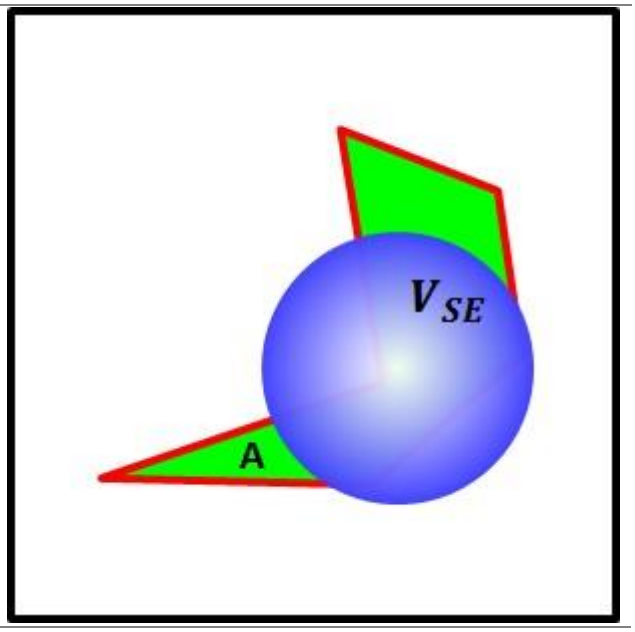
$$Solidity = \frac{A}{A_{convex}}$$

The Solidity range is between 0 and 1.
If the parameter is 1 the shape of the particle is convex.

Spherical Equivalent Volume

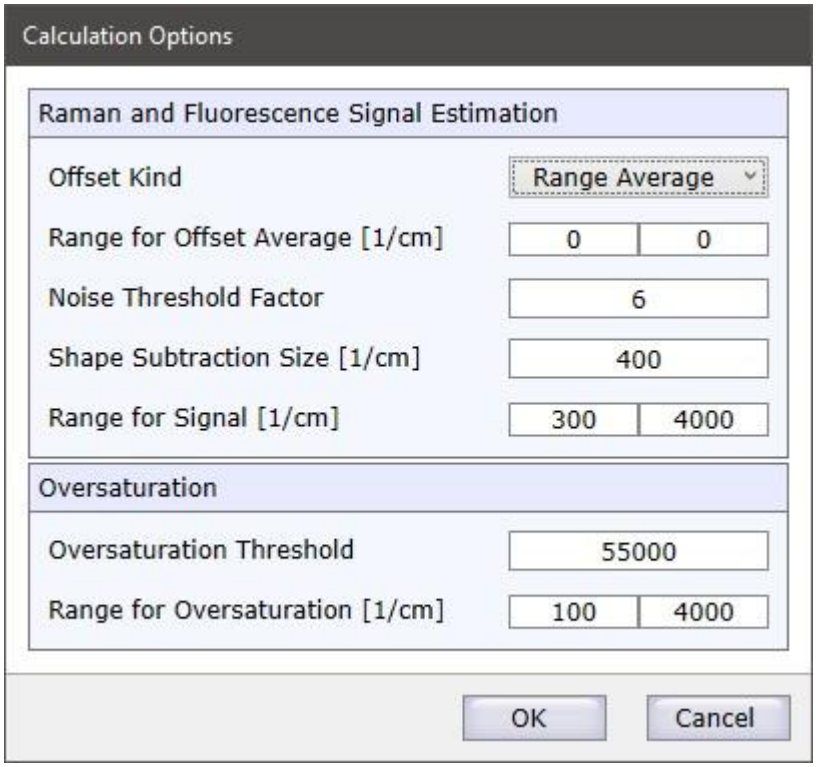
$$V_{SE} = \pi \frac{{d_{CE}}^3}{6}$$

V_{SE} : *Spherical Equivalent Volume*



Spectrum Properties

This dialog calculates an estimation of the amount of Raman and Fluorescence signal and a yes-no-info about the over-saturation for all selected particles.



Raman and Fluorescence Signal Estimation

Offset Kind
Before calculating any signal value, a horizontal offset is subtracted from the spectrum:
- Minimum: The minimum value of the spectrum is subtracted from the spectrum
- Range Average: Here you can define a spectral range that is used to calculate an average value (Parameter "Range for Offset Average"). This value is subtracted from the spectrum
- User Defined: A user defined value is subtracted from the spectrum

Range for Offset Average / User Defined Offset
Depending on the selected offset kind, you can define a range for calculating an average value used as offset or directly enter an offset value.

Noise Threshold Factor
This factor defines how much higher the spectral signal must be in comparison to a local noise in order to be detected as Raman signal.
The Raman signal value is determined by all spectral pixels that are higher than <Noise Threshold Factor> * <Local Noise>.

Shape Subtraction Size
Defines the shape size of the shape background subtraction.
The fluorescence signal value is determined by the subtracted background.

Range for Signal
Defines the spectral range that is used for the signal estimation.

Oversaturation

Oversaturation Threshold
If any spectrum pixel value within the defined range is higher than the threshold, the spectrum is marked as over-saturated.

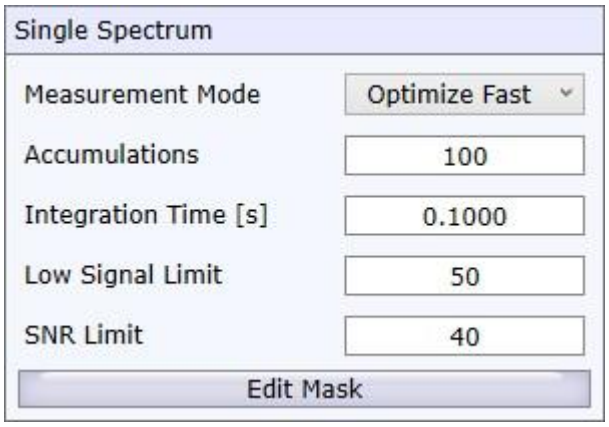
Range for Oversaturation
Defines the spectral range that is used for the over-saturation calculation.
The Rayleigh Peak might be saturated, so you can e.g. skip the Rayleigh area.

Raman Measurement
The Raman Measurement view allows to acquire a Raman spectrum for each particle.

WITec Control Configuration Setup
Before starting the Raman Measurement, you can choose one of the Raman configurations in WITec Control (e.g. "Raman CCD 1").
Its possible to measure a set of particles, then switch the WITec Control configuration to "Raman CCD 2" or change to another excitation laser, then measure other particles.

Measurement Sequence
For each particle, the following tasks are done by the software:
- The Sample Positioner is moved to the particle using the Positioning Settings
- Depending on the Z-Axis Behavior, a spectral Auto Focus is performed
- The spectrum is measured

Single Spectrum



Measurement Mode

- Normal: Uses the defined number of accumulations
- Optimize: Only accumulates spectra that improve signal to noise ratio of the Raman signal. Spectra with high fluorescence signal are rejected.
- Optimize Fast: In addition to the above option this mode will stop before the defined number of accumulations is reached:
    - if the signal to noise limit of the optimal accumulated spectrum is reached.
    - if the Raman signal of a single spectrum is too low
    - if the signal to noise limit of the optimal accumulated spectrum is not improving.

Accumulations
The number of accumulations for the spectrum measurement.
If more than 1 accumulation is used, a cosmic ray removal can be performed.

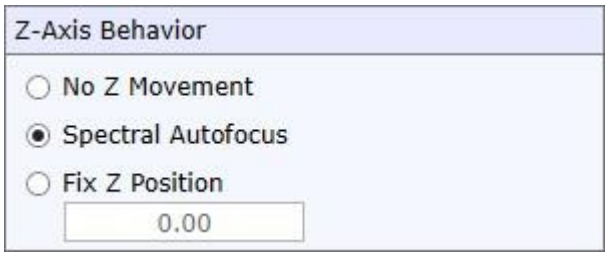
Integration Time
The integration time for each spectrum accumulation.

Low Signal Limit
Only used for Measurement Mode "Optimize Fast".
If the signal of a single spectrum is lower than this value the measurement will stop in order to save time.

SNR Limit
Only used for Measurement Mode "Optimize Fast".
If the optimal accumulated spectrum has a higher signal to noise ratio the measurement will stop in order to save time.

Edit Mask
Only used for Measurement Modes "Optimize" and "Optimize Fast".
Here you can define a spectral mask to define which parts of the spectrum should be used to calculate the signal.

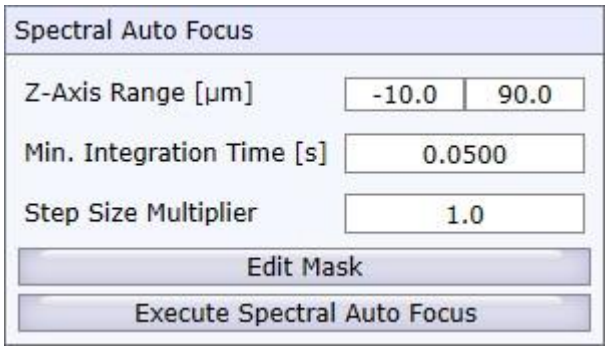
Z-Axis Behavior



- No Z Movement: user can move the Z-Axis to a desired location
- Spectral Autofocus: a spectral auto focus is performed at each particle
- Fix Z Position: lets you define an absolute Z-Position (Software controlled, limited Z-axis space)

Spectral Auto Focus

Before using the spectral Auto Focus adjust the following parameter:

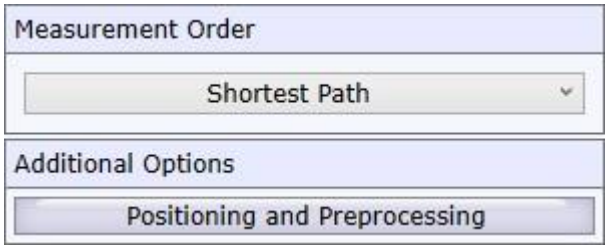


Z-Axis Range / Min. Integration Time / Step Size Multiplier
See Spectral Auto Focus documentation of WITec Control.

Edit Mask
Lets you edit a spectral mask to define which parts of the spectrum should be used for performing the auto focus.

Execute Spectral Auto Focus
Executes the spectral auto focus at the current position.
This way you can test if the spectral auto-focus works as expected.

Measurement Order and Additional Options



Measurement Order
Here you can define in which order the particles are measured:

- Shortest Path: calculates an estimation of the shortest path in order to save time while traveling to each particle.
- User Defined: uses the sort order of the list view in the Particle Manager, e.g. "Area".

Positioning and Preprocessing
See Measurement Options.

Actions

 **Use Results**
This will assign the measured spectra to the particles.

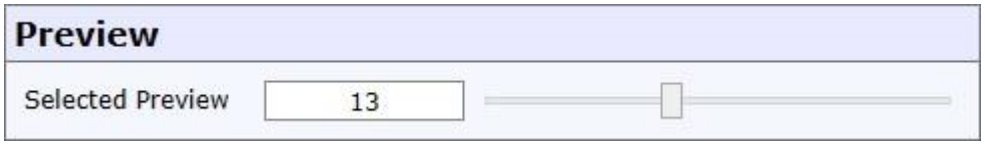
 **Start Measurement**
Starts the measurement.

 **Pause / Stop Measurement**
Stops the measurement.
You can continue at the last measured particle or measure all particles again.

Progress

Shows the progress and number of measured particles.

Preview



Here you can select which measured spectrum and corresponding particle should be shown.
If the last measured particle is selected, the preview will automatically stay at the latest measurement.

Particle Details

See Particle Details.

Material Search

The spectra of all flagged particles are used in TrueMatch in order to search for chemical components.
The names of the best component search results are assigned to the particles as material name and HQI (hit quality).
This information can be used:

- to sort, filter and select particles in the Particle Manager
- to decide if certain particles spectra should be measured again, e.g. with different measurement parameters
- to create a report with statistics

See TrueMatch Search Overview.

Actions

 **Use Results**

This will assign the best search results as material name to the particles:

- If no result-flags are set, the result with the best HQI is used
- If result-flags are set, the flagged result with the best HQI is used

If a spectrum has no search results, the user can decide to assign no material or a material named "Unknown".

Result Selection

If a multi-component search was performed, you can define which of the sub-results should be used (Component 1, 2, 3).
You can also define a minimum weight percentage for the selected component in order to avoid using results with a very low importance.

Report

The report view allows you to define a report layout with the following elements:

- Particle Image (Map) with Legend
- Typical Particles with Thumbnails and Spectra
- Bar Chart with Legend
- Pie Chart with Legend
- Table with Categories

The resulting report view can then be exported as bitmap into the windows clipboard.

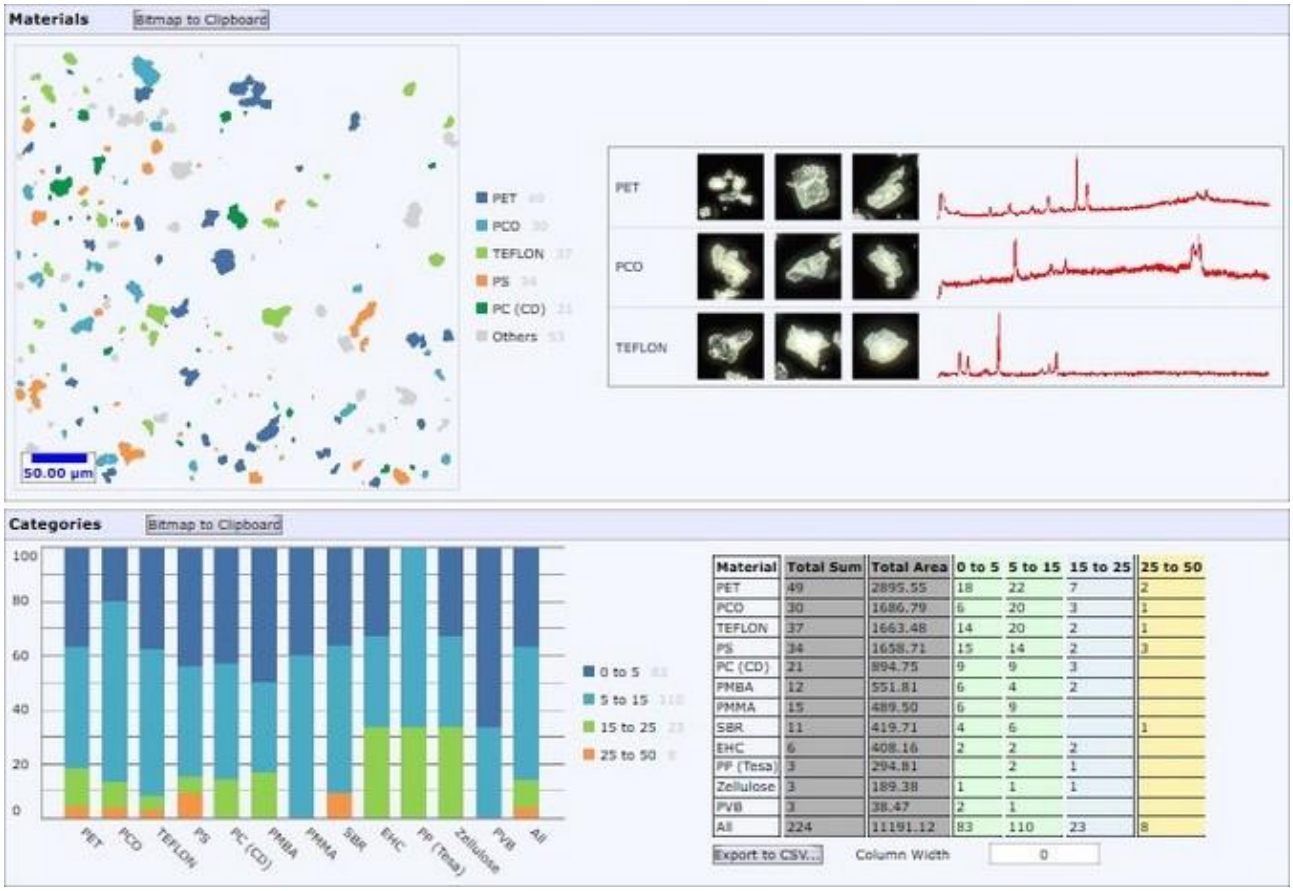
Report Settings

"Configuration 1" ComboBox
Here you can select between your saved configurations.

Options Button
Opens the report configuration window in order to define the report elements and data preparation.
See Report Configuration.

Bitmap to Clipboard
Copies the whole report as a bitmap to the clipboard.

Example:



Report Configuration

The ParticleScout Report is configured using the XML Format (Extensible Markup Language) with the following structure:

WITec
  Style
  Data
    Colors
    Groups
    Categories
    Visual Elements (Map, Thumbnail, Bars, Pie, Table)
  Data
    [...]

Press on the examples button in the software in order to create example xml code automatically.

The script must begin with
<WITec>

and end with
</WITec>

Style Tag

You can define exactly one Style Tag.
The Style Tag can be used to define some display properties:

```
<Style ScaleFactor='2'
```

```
      ShowCaptions='true'
      CaptionBackground='LightGray'
      CaptionFontSize='16'
      CaptionPlacement='Top'
      CaptionBorderThickness='1'
      CaptionBorderColor='Gray'
      ElementBackground='Yellow'
      CanvasBackground='#11000000'>
```

ScaleFactor: Defines a scale factor to scale the size of all report drawings (0.3 to 3, default: 1).
ShowCaptions: Show or hide all captions (default: true).
CaptionBackground: Defines the color of the caption background (default: #11000000).
CaptionFontSize: Defines the caption font size (default: 14).
CaptionPlacement: Can be top or bottom (default: Top).
CaptionBorderThickness: Defines the border size around the caption (default: 0).
CaptionBorderColor: Defines the color of the caption border (default: Transparent).
ElementBackground: Defines the color each elements background (default: Transparent).
CanvasBackground: Defines the color of the canvas (default: White).

Data Tag

You can define any number of data tags. At least one data tag must exist.
Within each data tag you can define colors, sorting, grouping and categorization of particle properties.
Any number of visible elements can be specified in order to define how the sorted data should be presented in the report.

```
<Data GroupCategoryName='Material'
      SortCategory='Total Area'>
  <Colors> [...]
  <DynamicGroup> [...]
  <NamedGroup> [...]
  <Category> [...]
  <Map> [...]
  <Bars> [...]
  <Pie> [...]
  <Thumbnail> [...]
  <Table> [...]
</Data>
```

GroupCategoryName: Is only used for tables. Defines the header of the group column.
SortCategory: Defines the category name of the category that should be used for sorting the data.

Colors Tag

Colors are used for maps and bar charts.
The number of defined colors will define the number of categories visible in maps and bar charts.

You can define a color by using hexadecimal (A)RGB Values or by using the string representation (please refer the System.Windows.Media.Colors class in Microsoft documentation):

```
<Colors DefaultColor='LightGray'>
  <Color Value='#4572A7' />
  <Color Value='#4BACC6' />
  <Color Value='#92D050' />
  <Color Value='#F79646' />
</Colors>
```

DefaultColor: Is only used for Table elements. Defines the group name / caption of the most left column.

DynamicGroup Tag

This will automatically create a dynamic number of groups of particles.
Each group is defined by a unique string/name which is evaluated from each particle using a string expression.

```
<DynamicGroup NameExpression='Material' Condition='NOT
Material.Equals("PET")' />
```

NameExpression: A string expression.
This expression will be automatically evaluated for each particle.
You can use any particle property here, for example Material.
All particles returning the same string will be in the same group (e.g. all particles with the Material "PMMA").
Condition: A boolean expression (optional). Only particles matching this expression will be used for creating groups.

NamedGroup Tag

This will create a group with a user defined name and condition.

Name: The name of the group. Used as table row group name.
Condition: A boolean expression. Only particles matching the expression will be part of the group.
Color: Color of the group. Used in map and map legend.
Position: Can be "Top", "Bottom" or "Default". Makes it possible to put a group at the top or bottom of all sorted groups.

Category Tag

This will create a category with a user defined name and condition. Categories can be used as columns in table presentation or in bar charts.

Name: The name of the category. Used as table column header.
Condition: A boolean expression. Only particles matching the expression will be part of the category.
Count: Allows to define how the "sum value" of the category is calculated.
A value of "1" is used, if no "Count Expression" is defined.
Must be a method call of one of the following methods:
Min, Max, Sum, Average, Median, StandardDeviation.
Usage Example: "Sum(Area)". The string in brackets is a numeric expression that can use all of the particle properties.
NormMode: Normalizes the category results for each group by dividing by the count result of all particles (None, Relative1, Relative100).
Color: Color of the category. Only used as table column background color.
StringFormat: The string representation expression for table cell content. Please refer to the Microsoft .NET string format documentation.
Example: If the number is 0.2345, then StringFormat "F0" = 0, "F1" = 0.2, "F2" = 0.23, "P2" = 23.45 %

Visual Elements

The following properties can be used in all visual elements:

Caption: Used for a surrounding group box header.
SideBySide: Set to "true" to place the element horizontally right next to the previous visual element.
ShowEmptyGroups: Set to "true" to show groups that have no matching particles.
ShowEmptyCategories: Set to "true" to show categories that have no matching particles.
InvertData: Set to "true" to display groups as categories and vice versa.

Map Tag

This will present the groups in a map using the defined colors.

```
<Map Background='Transparent'
     ImageOpacity='0.0'
     MaskOpacity='1.0'
     Width='400'
     LegendType='NameAndValue'
     ShowScaleBar='true'
     ScaleBarForeground='Blue'
     ScaleBarBackground='White'
     ScaleBarVerticalAlignment='Bottom'
     ScaleBarHorizontalAlignment='Left'/>
```

Background: The background color (used if ImageOpacity!=1, default: Transparent).
ImageOpacity: Defines the opacity of the particle image (0 to 1, default: 0).
MaskOpacity: Defines the opacity of the particle mask (0 to 1, default: 1).
Width: The width of the map in pixels (2 to 4000, default: 400).
The height is automatically adjusted using the correct ratio.
The pixel size scales with the global report scale factor.
LegendType: Visibility of the legend (None, Name, NameAndValue, default: NameAndValue).
ValueCategory: The name of a Category that defines the values for the legend (default: not defined).
ValueStringFormat: String format for the values (default: not defined).
ShowScaleBar: Set to "true" to show a scale bar on the map (default: true).
ScaleBarForeground: The scale bar color (default: Blue).
ScaleBarBackground: The scale bar background color (default: White).
ScaleBarVerticalAlignment: The scale bar vertical position (Bottom, Top, Center, default: Bottom).
ScaleBarHorizontalAlignment: The scale bar horizontal position (Left, Center, Right, default: Left).

Bars Tag

Shows a bar chart with an X/Y Axis and legend.

```
<Bars Caption="Categories"
      Width='500'
      Height='300'
      BarsSideBySide='false'
      Normalize='true'
      BarWidth='0.7'
      XAxisLabelRotation='45'
      LegendType='NameAndValue'
      InvertData='true' />
```

Width: The width of the chart, in DPI depending units (4 to 4000, default: 500).
Height: The height of the chart, in in DPI depending units (4 to 4000, default: 300).

BarsSideBySide: Set to "true" to show each group as a separate bar next to each other (default: false)
Normalize: Set to "true" to normalize categories to 100% (default: false).
BarWidth: The width of each category, as factor (0.1 to 1, default 0.7).
XAxisLabelRotation: The X Axis label angle in degrees (-180 to 180, default: 45)
LegendType: Visibility of the legend (None, Name, NameAndValue)
ValueCategory: The name of a Category that defines the values (default: not defined).
ValueStringFormat: String format for value labels (default: not defined).

Pie Tag

Shows a pie chart with legend.

```
<Pie Caption="Some Pie"
     Width='300'
     Height='300'
     OutsideLabelStyle='NameAndValue'
     InsideLabelStyle='None'
     LegendType='Name'/>
```

Width: The width of the chart, in DPI depending units (4 to 4000, default: 300).
Height: The height of the chart, in in DPI depending units (4 to 4000, default: 300).
OutsideLabelStyle: Visibility of the label outside from each pie segment (None, Name, NameAndValue, Value, default: None).
InsideLabelStyle: Visibility of the label inside each pie segment (None, Name, NameAndValue, Value, default: None).
BorderColor: Color of the border of each pie segment (default: White)
BorderThickness: Thickness of the border of each pie segment, in DPI depending units (default: 1.0)
LegendType: Visibility of the legend (None, Name, NameAndValue, default: NameAndValue)
ValueCategory: The name of a Category that defines the values (default: not defined).
ValueStringFormat: String format for value labels (default: not defined).

Thumbnail Tag

This will present a list of particle groups with name, particle thumbnails and a representing spectrum (if measured).

```
<Thumbnail NumberOfEntries='3'
           NumberOfThumbnails='3'
           ThumbnailWidth='60'
           SpectrumWidth='300'
           SpectrumHeight='60'
           ShowXAxis='false'
           ShowYAxis='false'
           XAxisTitle='1/cm'
           SortThumbnailsBy='Area'
           SortSpectraBy='HQI' />
```

NumberOfEntries: The maximum number of list entries (maximum is number of groups, default: 3).
NumberOfThumbnails: The number of thumbnails (0 to 20, default: 2).
ThumbnailWidth: The width of each thumbnail, in DPI depending units (0 to 4000, default: 60).
SpectrumWidth: The width of the spectrum, in in DPI depending units (0 to 4000, default 400).
SpectrumHeight: The height of the spectrum, in in DPI depending units (0 to 4000, default: 60).
ShowXAxis: Set to "true" to show the X Axis.
ShowYAxis: Set to "true" to show the Y Axis.
XAxisTitle: The title/caption of the X Axis.
SortThumbnailsBy: An expression returning a particle property used for sorting the thumbnails (e.g.

"Area" -> the particles with the biggest areas are shown)
SortSpectraBy: An expression returning a particle property used for sorting the spectra (e.g. "Area" -> the spectrum of the particle with the biggest area is shown)

Table Tag

Shows a table with groups as rows and categories as columns.

```
<Table ColumnWidth='50'
       MasterColumnWidth='0'
       HeadingColorOpacity='0.5'
       CellBackground='LightBlue'
       CellBorderThickness='1'
       CellBorderColor='Transparent'
       CellPadding='2'/>
```

ColumnWidth: Defines the width of each category column, in DPI depending units. If 0, the width is minimal for each column (default: 0).
MasterColumnWidth: Defines the width of the most left column, in DPI depending units. If 0, the width is minimal (default: 0).
HeadingColorOpacity: Defines the opacity of the heading colors or columns and row headings (0 to 1, default: 0.5).
CellBackground: Defines the background color for each cell (default: #19000000).
CellBorderThickness: Defines the thickness of each cells border. See thickness format. (default: 1).
CellBorderColor: Defines the color of the cell border. Only visible if the thickness is not 0. (default: Transparent).
CellPadding: Defines the space around cell content. See thickness format. (default: 2).

Thickness Format

Use one number to define the same thickness for left/right/top/bottom: '2'
Use two numbers to define lef/right and top/bottom: '5 2'
Use four numbers to define left/top/right/bottom: '2 2 1 0'

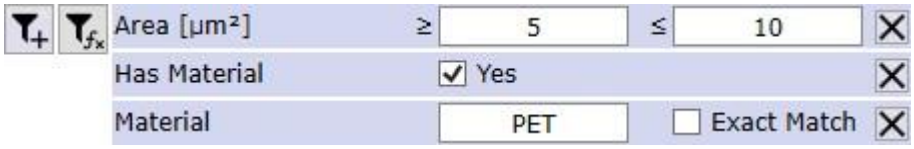
Possible Color Strings

AliceBlue	#FFF0F8FF	DarkTurquoise	#FF00CED1	LightSeaGreen	#FF20B2AA	PapayaWhip	#FFFFEFD5
AntiqueWhite	#FFFAEBD7	DarkViolet	#FF9400D3	LightSkyBlue	#FF87CEFA	PeachPuff	#FFFFDAB9
Aqua	#FF00FFFF	DeepPink	#FFFF1493	LightSlateGray	#FF778899	Peru	#FFCD853F
Aquamarine	#FF7FFFD4	DeepSkyBlue	#FF00BFFF	LightSteelBlue	#FFB0C4DE	Pink	#FFFFC0CB
Azure	#FFF0FFFF	DimGray	#FF696969	LightYellow	#FFFFFFE0	Plum	#FFDDA0DD
Beige	#FFF5F5DC	DodgerBlue	#FF1E90FF	Lime	#FF00FF00	PowderBlue	#FFB0E0E6
Bisque	#FFFFE4C4	Firebrick	#FFB22222	LimeGreen	#FF32CD32	Purple	#FF800080
Black	#FF000000	FloralWhite	#FFFFFAF0	Linen	#FFFAF0E6	Red	#FFFF0000
BlanchedAlmond	#FFFFEBCD	ForestGreen	#FF228B22	Magenta	#FFFF00FF	RosyBrown	#FFBC8F8F
Blue	#FF0000FF	Fuchsia	#FFFF00FF	Maroon	#FF800000	RoyalBlue	#FF4169E1
BlueViolet	#FF8A2BE2	Gainsboro	#FFDCDCDC	MediumAquamarine	#FF66CDAA	SaddleBrown	#FF8B4513
Brown	#FFA52A2A	GhostWhite	#FFF8F8FF	MediumBlue	#FF0000CD	Salmon	#FFFA8072
BurlyWood	#FFDEB887	Gold	#FFFFD700	MediumOrchid	#FFBA55D3	SandyBrown	#FFF4A460
CadetBlue	#FF5F9EA0	Goldenrod	#FFDAA520	MediumPurple	#FF9370DB	SeaGreen	#FF2E8B57
Chartreuse	#FF7FFF00	Gray	#FF808080	MediumSeaGreen	#FF3CB371	SeaShell	#FFFFF5EE
Chocolate	#FFD2691E	Green	#FF008000	MediumSlateBlue	#FF7B68EE	Sienna	#FFA0522D
Coral	#FFFF7F50	GreenYellow	#FFADFF2F	MediumSpringGreen	#FF00FA9A	Silver	#FFC0C0C0
CornflowerBlue	#FF6495ED	Honeydew	#FFF0FFF0	MediumTurquoise	#FF48D1CC	SkyBlue	#FF87CEEB
Cornsilk	#FFFFF8DC	HotPink	#FFFF69B4	MediumVioletRed	#FFC71585	SlateBlue	#FF6A5ACD
Crimson	#FFDC143C	IndianRed	#FFCD5C5C	MidnightBlue	#FF191970	SlateGray	#FF708090
Cyan	#FF00FFFF	Indigo	#FF4B0082	MintCream	#FFF5FFFA	Snow	#FFFFFAFA
DarkBlue	#FF00008B	Ivory	#FFFFFFF0	MistyRose	#FFFFE4E1	SpringGreen	#FF00FF7F
DarkCyan	#FF008B8B	Khaki	#FFF0E68C	Moccasin	#FFFFE4B5	SteelBlue	#FF4682B4
DarkGoldenrod	#FFB8860B	Lavender	#FFE6E6FA	NavajoWhite	#FFFFDEAD	Tan	#FFD2B48C
DarkGray	#FFA9A9A9	LavenderBlush	#FFFFF0F5	Navy	#FF000080	Teal	#FF008080
DarkGreen	#FF006400	LawnGreen	#FF7CFC00	OldLace	#FFFDF5E6	Thistle	#FFD8BFD8
DarkKhaki	#FFBDB76B	LemonChiffon	#FFFFFACD	Olive	#FF808000	Tomato	#FFFF6347
DarkMagenta	#FF8B008B	LightBlue	#FFADD8E6	OliveDrab	#FF6B8E23	Transparent	#00FFFFFF
DarkOliveGreen	#FF556B2F	LightCoral	#FFF08080	Orange	#FFFFA500	Turquoise	#FF40E0D0
DarkOrange	#FFFF8C00	LightCyan	#FFE0FFFF	OrangeRed	#FFFF4500	Violet	#FFEE82EE
DarkOrchid	#FF9932CC	LightGoldenrodYellow	#FFFAFAD2	Orchid	#FFDA70D6	Wheat	#FFF5DEB3
DarkRed	#FF8B0000	LightGray	#FFD3D3D3	PaleGoldenrod	#FFEEE8AA	White	#FFFFFFFF
DarkSalmon	#FFE9967A	LightGreen	#FF90EE90	PaleGreen	#FF98FB98	WhiteSmoke	#FFF5F5F5
DarkSeaGreen	#FF8FBC8F	LightPink	#FFFFB6C1	PaleTurquoise	#FFAFEEEE	Yellow	#FFFFFF00
DarkSlateBlue	#FF483D8B	LightSalmon	#FFFFA07A	PaleVioletRed	#FFDB7093	YellowGreen	#FF9ACD32
DarkSlateGray	#FF2F4F4F						

Filter Expression Editor

The Filter Expression Editor allows to filter particles before creating the particle list or to hide or select certain particles in the Particle Manager.

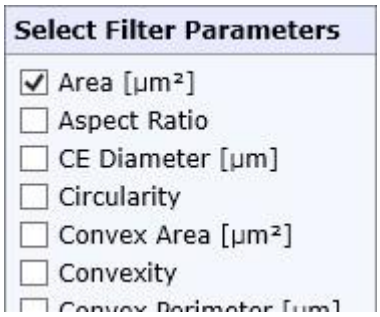
Visual Filter Editor



Here you can define a value range for the selected parameter, e.g. the area.

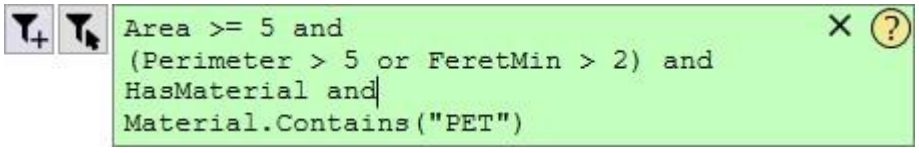
Press  to remove a filter from the list.

 Add filter parameters by selecting particle parameters:



Toggles between the simple parameter list and an custom formula editor, see below.

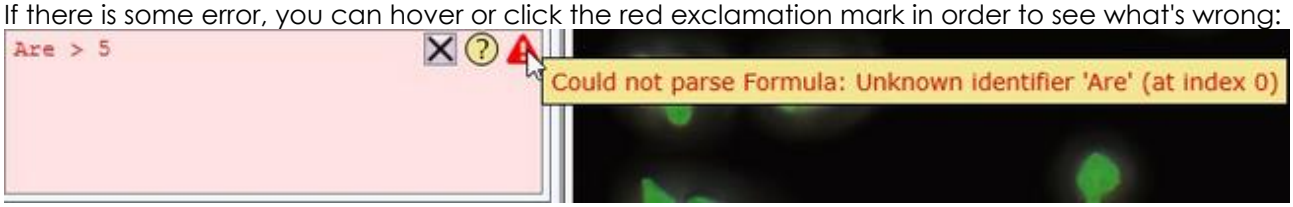
Custom Formula Editor



Lets you define a custom boolean formula that defines a filter condition.
Click on the yellow question mark in order to see all possible variables and some examples.

To clear the formula, press X.

If there is some error, you can hover or click the red exclamation mark in order to see what's wrong:



Example Formulas

`Area > 5`	Larger than condition
`Area > 5 and Area < 10`	Combination with AND
`(Area > 10 and Area < 20) or (Area > 50 and Area < 70)`	Combination with AND and OR using brackets
`Material = "Quartz"`	String comparison
`Material.Contains("Quar")`	String method call "Contains"
`FeretMin > 3 * FeretMax`	Larger than condition with multiplication
`RandomValue > 0.5`	Random selection of half of all elements (RandomValue = Random number between 0 and 1)
`IsOversaturated`	Show only particles that have over-saturated spectrum
`!IsOversaturated`	NOT operator "!": show only particles that have NO over-saturated spectrum
`Math.Sqrt(Length * Width) > 5`	Calculates the square root of Length * Width and compares larger than 5.

Please refer to the .NET Framework 4.7.1 documentation for other Math or String methods.

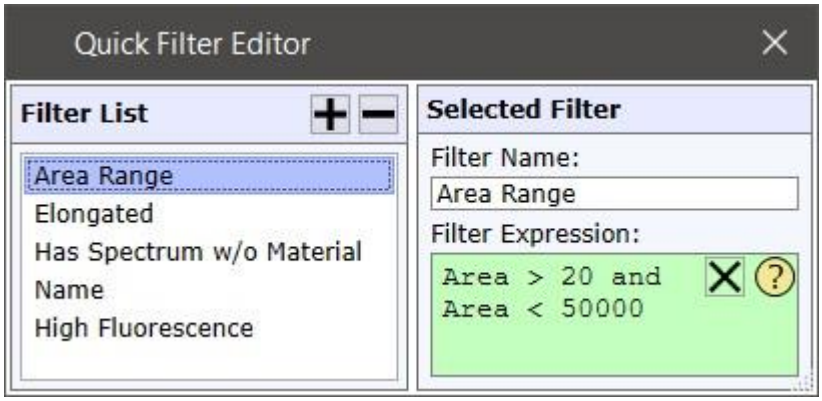
Quick Filters

You can define quick filters in order to recall a custom formula with a single mouse-click.

Click on a desired Quick Filter to use the saved formula in the filter expression editor.

In Particle Manager: click on the desired Quick Filter check mark to add/remove particle flags.

Configure Quick Filters



Here you can add, remove or edit Quick Filters.

Just add a new filter and set a name and formula.

Here you can choose between the visual filter editor and the custom formula editor.

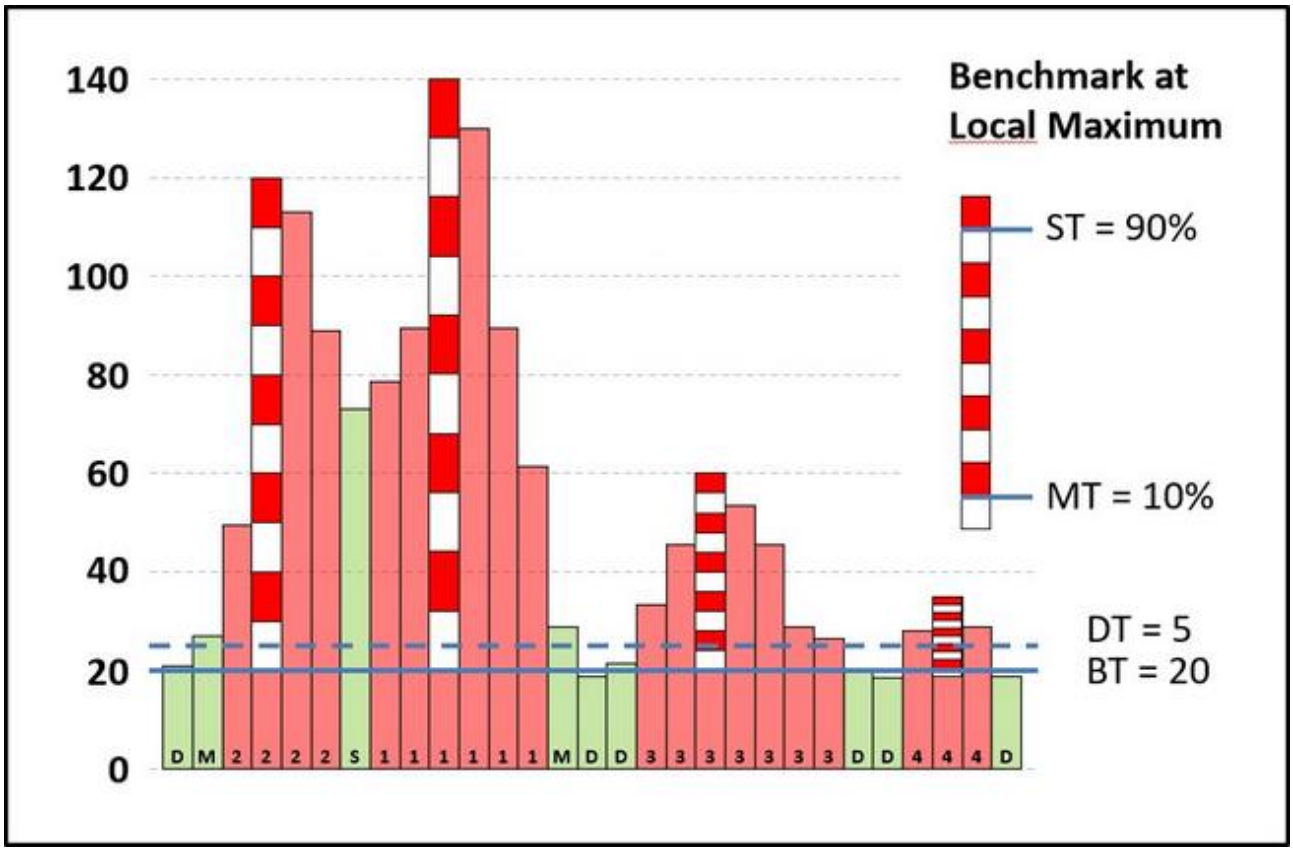
Watershed Examples

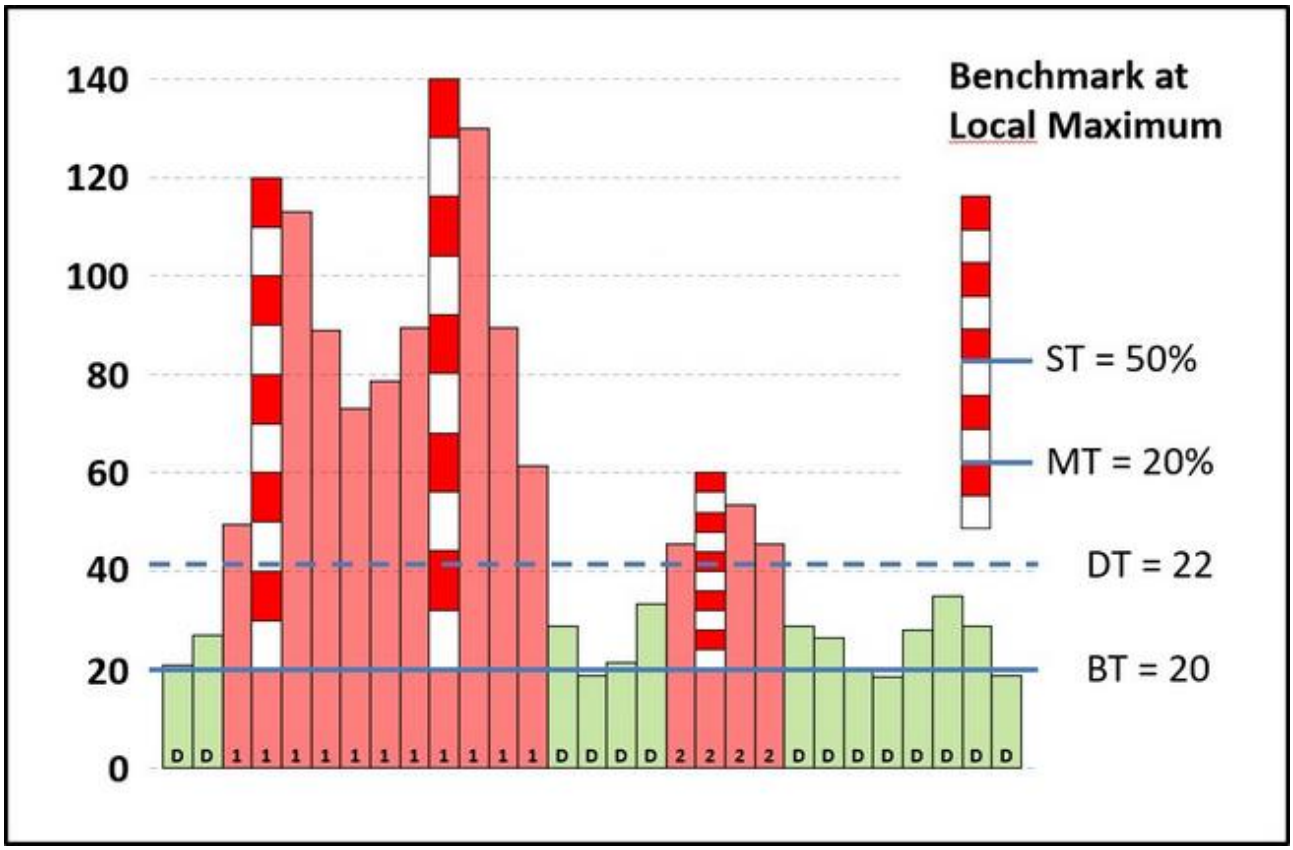
Legend

BT = Background Threshold
DS = Detection Threshold
ST = Separation Threshold
MT = Mask Threshold

Pixel Description

D = Not marked because of Detection Threshold
S = Not marked because of Separation Threshold
M = Not marked because of Mask Threshold
1,2,3... = Region Index




140
120
100
80
60
40
20
0
Benchmark at
Local Maximum
ST = 50%
MT = 20%
DT = 22
BT = 20
D D 1 1 1 1 1 1 1 1 1 1 D D D D 2 2 2 2 D D D D D D D D

User Management

User Management Overview

The user management provides the possibility to allow or deny certain users certain features of WITec software components.

Please start with the Setup, then Manage User Rights.

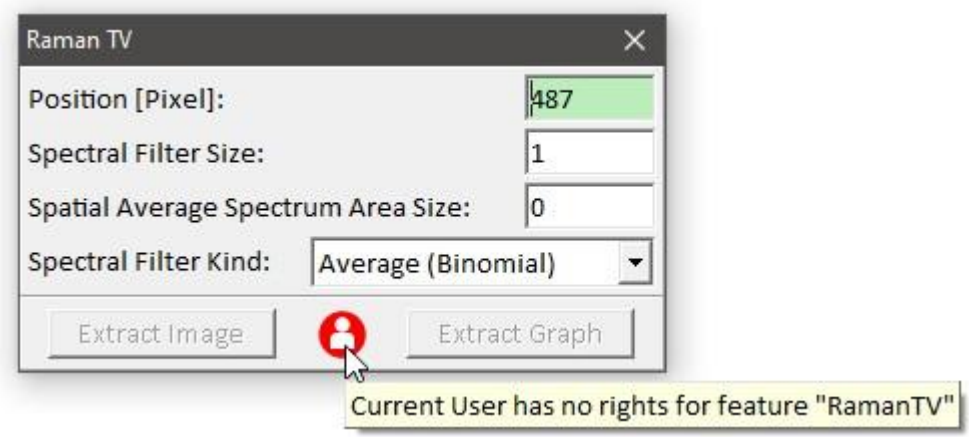
Licensing

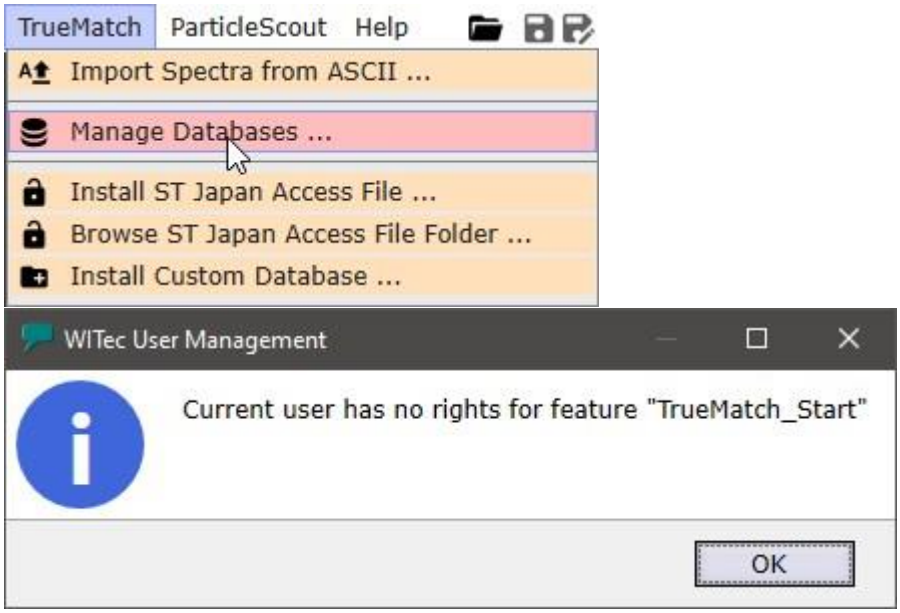
A special user management license is needed to use it. Once you have ordered the user management license feature, it is included in the WITec Project license and can be used on any number of computers.

Missing User Rights Indication

The software itself indicates missing user rights by

- showing a red icon with person
- using orange/red background color for menu items
- showing a dialog (e.g. when clicking a menu item)





User Management Setup

Enable / Disable User Rights on a computer

The default installation of WITec software components does not use any user management.

The administrator must explicitly activate and configure user management.

To activate the user management, just start the WITec User Manager application which is installed with any WITec Software.

Please make sure to start the correct version, matching the WITec Suite you want to change.

Access Rights / Security

After activating the user rights, the administrator must set up the folder access rights for this folder:

```
C:\ProgramData\WITec\WITec Suite 6.X\Configs\Common Configs\WITecUserRights\
```

- Make sure that all WITec Software Users can read this folder and all files in it
- Make sure that only the desired Administrators can write to files in this folder in order to manage the user rights
- To check if everything is set up correctly:
  - just start the WITec User Manager Application as administrator (can change and save the user rights)
  - and as "normal user" (gets a proper error message when starting the Application).

Use User Rights in Windows Network

It is possible to define the user rights on a server share (e.g. mapped network drive).

Before doing this, please note the following points:

- All computers that use the same user rights must use the same exact WITec Suite Version
- If a newer version of the WITec User Manager Application saves the user rights, it might not

be readable by older WITec Suite software

- If the network path is not accessible, WITec Suite software will not allow any features

To define a custom path for storing the user rights, please first activate the user rights by starting the WITec User Rights Manager Application.
Then edit the following file:

```
C:\ProgramData\WITec\WITec Suite 6.X\Configs\Common
Configs\WITecUserRights\UserRightsPath.txt
```

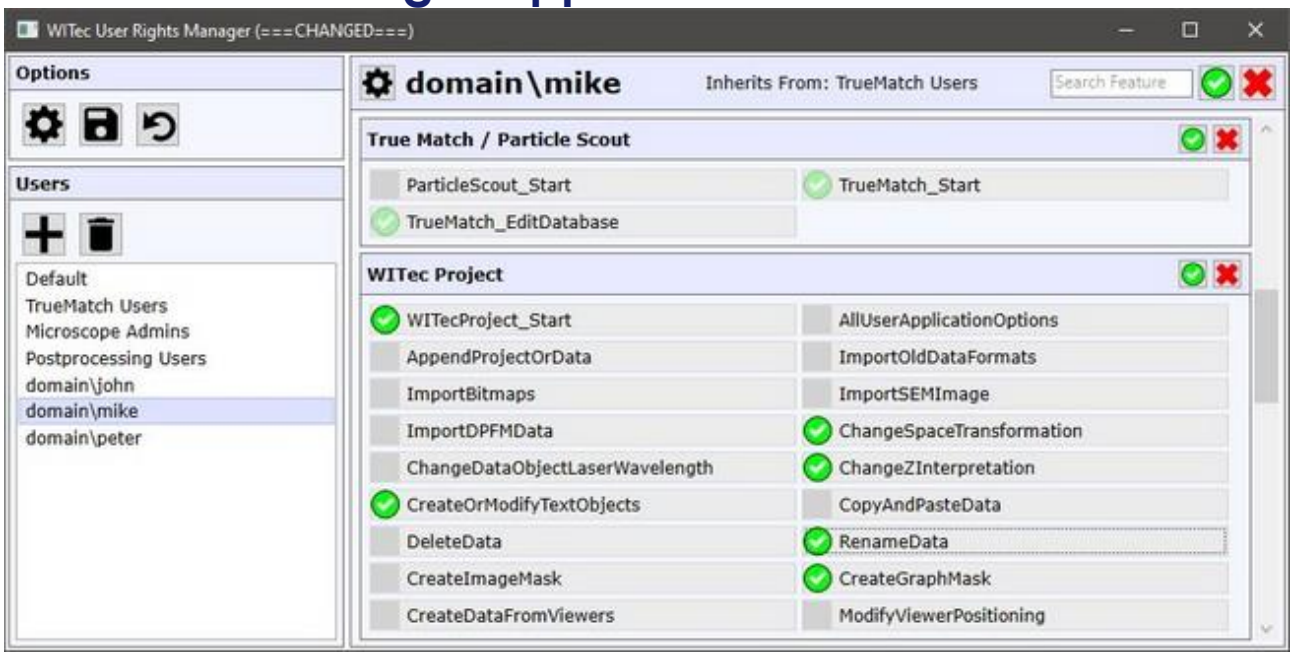
The file content must contain a valid file path, e.g.

```
N:\MyNetworkPath\WITecUserRights 6.X\
```

This must be done on all computers that should use the network user rights.
We recommend adding the WITec Suite version number in the path name.

The windows folder access settings of your network folder must be set up the same way than the local folder, see above.

WITec User Manager Application



Options

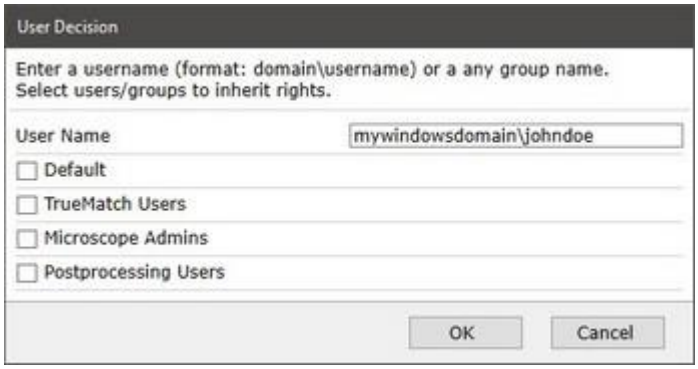
Advanced
Here you can deactivate the whole user right management.
The target path where the user management file is located is also shown here.

Save
Saves the current user management settings.

Reload / Undo
Reloads the user management settings from file, undoing all changes.

Users

Add



With this dialog you can add or edit a user.
The user name can either be the name of a windows logon user with domain and logon name (e.g. mycompany\johndoe), or a custom group name which can be used for right inheritance (e.g. to define a group "TrueMatch Users" whose members will inherit its rights).
To inherit rights from another user or group, just check or uncheck the check-boxes below the user name.

Delete
Deletes a user or group.

Default
This is the default user. All users that are not explicitly defined in the user rights, will get the default rights.

User Details (Right Side)

Edit User
See Add.

Inherits From
Shows the users or groups that the current displayed user inherits rights from.

Search Feature
Type a search text to find any user right feature.

All/None
Click on All/None to allow/deny all features of the user or a certain category.

Features
Click on any single user right feature to allow or deny this feature.

Version History

WITec Suite 6.2

- WITec Control / Video Window
    - New Workflow Manager
        - Allows to create a list of actions that are sequentially executed
        - Supports almost all measurement types and spectral auto focus
        - Supports absolute and relative positioning of sample positioner, scan table and Mic. Z
        - Supports save project, set data object name
        - Supports Laser Power, Analyzer / Polarizer, Adjustment + Calibration Coupler, TSMk3
        - Supports load/save of workflow
        - Separate License needed (demo mode allows to add 3 single spectra and one action of each measurement type)
    - All Nikon Objectives added to objective database
    - Better COM / Labview support:
        - State Setter / State Resetter can now be controlled
        - Adjustment Sample Coupler can now be controlled
        - Spectrometer Calibration Lamp can now be controlled (permanently on/off)
        - Parameter "Video | Autofocus | Execute" no longer opens dialog windows
        - True Surface Mk3 control parameters can now be queried
            - Focus Shift (float), Min Value (float), I gain (float), P gain (float), Laser Intensity (int), Gain (enum), Use Automatic Gain (boolean)
    - Possibility to manually couple Adjustment Sample Coupler permanently
    - Line Scan new parameters
        - Center / Start / Stop at current position
        - Listen Mode for Line / Start / End / Center (once and multiple)
    - Supports fast line scan (depth scan)
    - Large Area Scan Stepwise and Line Scan now have separate number of accumulations and integration time

- WITec Project
    - New Advanced Cosmic Ray Removal Dialog (Plus license needed)
    - Raman TV Drop Action now can create average spectra using mask regions
    - New Waterfall graph display in Export Manager
        - Supports waterfall of spectra along time/a line
        - Supports multiple single spectra
    - New Drop Actions for "Database Export" and "Export Manager"
    - New Import for Oxford Instruments H5OINA Files (EDS Images, Electron Images, Layered Images)
    - Data Stitching Drop Action supports reordering and removing items
    - Image Viewer scale bar now automatically switches to unit (nm / µm / mm / m) to avoid exponential display

- Image Viewer: Sync Zoom Mode now also synchronizes 3D Camera rotation / tilt / zoom

- Particle Scout
  - New User Interface for creating filters
    - Filters can now be added via menu
    - Custom Formula Editor can still be activated
    - Used in "Particle Manager" and "Find Particles: Filter"
  - Supports shape background subtraction and display range for all spectra displays. Used in
    - Particle Detail
    - Particle Manager Thumbnail
    - Report
  - True Match Multi Component Result Names
    - Now support percentages
    - Show "Unexplained" instead of material for negative weighting factors

- True Match
  - Possibility to export database spectra to clipboard directly from Database Editor Tab

WITec Suite 6.1

- True Match / Particle Scout
  - Possibility to merge two databases (copy samples between databases)
  - Direct Import of Spectra
    - RRUFF (*.txt, select all files to import)
    - JCAMP (*.dx, *.jdx, only FIX and PAC format)
    - SPC (*.spc, only newer format)
    - Simple "Multi-Columns" (*.txt, first column = X Data, next columns = Y Data)
  - Particle Scout: Possibility to create Microplate Image

- WITec Control / Video Window
  - New spectroscopy dialog
  - State Manager
  - Portable Coordinate System
    - Enables to correlate measurments of the sample used in different microscopes
  - Improved Auto Beam Output Adjustment
  - True Power Laser Correction Factor
  - New automatic Z axis focus finder / sample approximation using the Easy Link controller (hold "X" and move down)
  - Auxiliary Inertial Drive Options now allow to invert and assign hardware axes
  - Inverted Microscope Listen Position via Top Camera
  - Use default objective calibration also if tables present
  - New COM Parameters
    - Sample Positioner and Z-Stage now fully controllable via COM
    - Objective Turret controllable via COM

- User Configuration Menu now in Control Form

- WITec Project
  - New automatic window positioning
  - New Data Export using "WITec Export Manager"
    - Exports to new WITec HDF5 File Format
    - Exports to Zeiss CZI File Format
    - Exports Graph Viewer Bitmap (Single Spectra with Axes and Legend)
  - TrueComponent Analysis:
    Possibility to extract normalized percentage images
  - Image Viewer
    - Draw Tools: Possibility to create "Region Mask" (nearby masked pixels define one region)
      - Region Mask can be dropped onto Average Spectrum Drop Action in order to get one average spectrum for each region
    - Show scan geometry permanently (Shortcut Control-G)
  - Advanced Fitting Tool now supports Multi Peak Pseudo Voigt
  - More Extract and Next Step possibilities for image / AFM drop action dialogs

WITec Suite 6.0

- Suite
  - New User Rights Management (Beta Version)

- True Match
  - Fast loading without ST Japan Dongle
  - New Database Selection Dialog
  - Possibility to include / exclude samples by name
  - After Simple 1-Component-Search, a result can be used (right-click) to perform a 2/3-Component-Search using the result as one defined component
  - More tolerant ASCII import

- Particle Scout
  - Improved Image Viewer
    - Cross hair, Snap to nearest Particle
    - Listen Sample Position Mode
  - Export Particle Image to WITec Control / Project via Clipboard (Menu - Particle Scout)
  - Quick Filter Dialog now has more clear user interface
  - Find Particles shows number of used particles

- Video Window
  - RISE startup mode with all light sources turned off
  - Improved Objective Compensation
    - Hysteresis correction
    - Better behavior when switching between top / bottom camera
  - Better support for AFM and SNOM modes

  - Improved Inverted Microscope Support
    - Listen Mode
    - Possibility to restore Z distance between bottom objective and Z stage (for SNOM Tip Approach)
    - Detector / Probe compensation with inverted objective now possible
  - Support for TrueSurface Mk3 via COM
  - More clear main menu
  - Manual gain mode for TrueSurface Mk3

- WITec Project
  - New Filter Viewer User Interface with larger Icons and Font
  - New Graph Demixer User Interface similar to Demix-Tab in TCA (with wheel-controls, subtract mode, reset)
  - "Add Text Object" in project manager has new Shortcut (Ctrl-T) and now automatically focuses the object name for editing
  - Menu Options - "Save / Load Window Positions" moved to Viewer Options
  - Menu "Tools" removed, items are now in Menu "Options"

- WITec Control
  - Automatically created Text Objects are now read only (loaded Text Objects from old WIP files are always editable)
  - Estimated scan time now displayed in control form (only Raman configurations)
  - Sequencer Scan Line now shows line length as parameter
  - Image Viewer: "Skew" line scan positions now also shown (as projection or as cross for vertical lines)

WITec Suite 5.3

- Suite
  - New Splash
  - WITec Control Help integrated in this Help

- True Match
  - Automatically created search sets for each database
  - New Search Option "Optimize HQI" to improve HQI calculation of noisy spectra
  - New Search Option "Subtract 2nd Spectrum" to improve multi component search HQI calculation of mixed spectra (e.g. subtract big portion of substrate to see small portion of "interesting" material - HQI is otherwise dominated by substrate)
  - Jump to next/previous material buttons if columns are sorted by material
  - CSV Export can now be sorted by original list instead of using the display sort
  - Save/Load Spectral Masks

- Particle Scout
  - New Reporting Features (Exportable as bitmap into the clipboard)
    - Map with Legend (Image with Particle Mask)
    - Bar Chart with Legend

        - Pie Chart with Legend
        - Particle Thumbnails with Spectrum
        - Table with styling possibilities
    - New Specturm Measurement Options
        - Signal optimization using automatic signal to noise ratio detector
        - Auto focus and signal optimization masks can now be edited in the particle scout GUI (using some installed default spectra selectable from a list)
    - New "Watershed" Mask Algorithm to find particles
    - Add particles to the current project
        - Possibility to find and add particles from the same image to the current particle project (e.g. dark particles, then bright particles)
        - Possibility to load multiple images and find and add particles to the current project
        - Possibility to export multiple bitmaps from WITec Project into particle scout
        - The first image is always the master image. Only the master image will be displayed and saved in the project.
        - Possibility to define a material name for each exported bitmap
    - Possibility to import a custom particle mask from file
    - Improved performance and higher resolution of image and mask previews
    - Improved performance for loading and saving
    - Attach results from True Match new features
        - For each search spectrum, the first flagged result is now used, if any is flagged
        - Multi Component Search: User can select which component should be used
        - Possibility to set minimum weight limit if a sub component is selected
    - Jump to next/previous material buttons if particle list is sorted by material
    - Now supports big images
    - Material can now be attached to a particle without having a spectrum
    - Unified CSV Export brackets for units
    - Save/Load Spectral Masks

- Video Window
    - New Particle Scout Stitching Tab: Supports High-Resolution Images
      Possibility to define stitching mask (circle, pie, cross)
    - Video Image Vignetting Correction with automatic calibration
    - Support for new automated Output-Multi-Coupler and automatic laser output adjustment using adjustment plates
    - TSM3 User Interface now integrated in Video Window (shares place with AFM user interface)
    - Speed Limit for Sample Positioner now available in options next to joystick
    - Illumination / Brightness now also controllable via gamepad in auxiliary device modes

- WITec Project
    - New Look / Icons in Main Window
    - Graph Viewer: Shortcut Control-C now only copies bitmap. Control-Shift-C copies

ASCII Data
    - TrueComponent Analysis: User can now select which component spectra should be shown in the graph viewer
    - Filter Viewer now shows new filters automatically

- WITec Control
    - New Icons for Start Sequencer Buttons
    - Spectral Auto Focus: now a mask can be defined instead of a single range
    - KPFM now logs more values in text object
    - Improvement of multiple styles and parameter names
    - Automatic Software Z mode after successful signal stabilization

WITec Suite 5.2

- New Software: WITec ParticleScout
    - Find particles on (stitching-) video image
    - Manage and filter particles
    - ASCII / CSV Export of particles and spectra
    - Measure Raman spectrum on all particles
    - Calculate and attach spectrum properties to particle (Raman signal, Fluorescence signal, Oversaturation)
    - Search in WITec TrueMatch spectral database and attach found material name + HQI to particle
    - Simple statistical analysis (group by material and desired particle property, e.g. area)
    - Start via
        - Video Image Stitching Dialog button
        - Project Manager: Context menu of bitmap

- Microscope Control Application (Video Window)
    - Support for new automated optics
        - Field Stop and Aperture Stop
        - Polarizer and Analyzer (Polarizer and Analyzer)
        - Possibility for auto-link desired angle between polarizer and analyzer

- WITec Control
    - New data object names for new measurement data objects
    - Spectrometer Calibration File is automatically selected

- WITec Project
    - TrueComponent Analysis
        - Can now show and extract "only positive values" / positive weighting factors
        - Can now extract Pearson correlation coefficient as an image (Plus version)
    - Spectrum Normalization now available in Graph Background Subtraction dialog
    - Graph Viewer: Order of graph objects can be changed (e.g. for cascade display mode)

  - Binary export of spectral data objects (raw 32 bit float format, much faster than ASCII)
  - Graph and Image Viewer with all their settings can now be duplicated

- True Match
  - Spectral mask is now a real mask (set and clear multiple ranges allowed)
  - Search algorithm now corrects x-axis / wave-number shift (± 10 1/cm)
  - The following sorting and grouping of result columns now available:
    - No Sort
    - Sort by Best HQI
    - Sort and group by Best Material Name
    - Sort by Best HQI and group by Material Name
  - Improved web search of sample properties
  - HQI and PDF-Report available when comparing a search spectrum with a database spectrum in the database tab

WITec Project 5.1

- Image Viewer Features
  - 3D Lighting effects (use shortcut "L")
  - Animation Editor. Enables 3D Video Animations with full customizeable animation steps
  - Better mouse handling (no cursor in 3D mode, 3D rotate / tilt with left mouse button)
  - 3D panning via "Shift" + Middle Mouse Drag and "Shift" + Mouse Wheel. (Can also be used for animation "flight")
  - Automatic Color Scale of last changed line now has separate button in status bar
  - Background color can now be defined by user (also default options)
  - Color Scale Export: precision of numbers now can be changed
  - Separate buttons for exporting bitmap in original resolution
  - New Button for Automatic 3D Scale with correct aspect ratio for topography images (Shortcut Ctrl-A)
- Multi Bitmap Export
  - Available Formats: Multiple Bitmap Files, Multi Frame TIFF, AVI Video
  - Support for automatically opening a program after export
  - Used in Image Stack Export, Image Overlay Morph, Image Viewer Animation
- Image Stack Export Dialog
  - Now with previews for X/Y, X/Z, Y/Z planes
  - Automatic pixel interpolation in all directions
  - Support for intensity correction in z direction
  - Support for cutting planes, e.g. "opening" a cube for volume view now possible
- Image Overlay Morph now exportable as AVI video
- Shape Background Subtraction: improved performance, noise threshold parameter was removed
- Image Combination Changes
  - Supports automatic masks, thus avoiding over saturation
  - Supports extracting all images with their color table, also with color scale bar (extract using viewer export settings)

- TrueComponent Analysis
  - Supports Undo/Reset in Average tab
  - Now calculates better initial residual image
  - Multiple Auto Find now adds components without clearing existing components
  - Demix parameters now stay untouched when adding / removing components
- Graph Viewer Auto Zoom Enlarge Only (Status bar button)
- Drop Action Intensity Correction now available for removing Filter or Etaloning artifacts
- Main Menu removed "Add" menu. "Add Text" now available via project manager context menu
- Graph Viewer Peak Labelling now supports ignoring the Rayleigh area, configurable default options for line length, number of digits and better mouse handling
- Data Stitching now allows stitching of bitmap objects (works in combination with new Image Combination export option)
- Average Spectrum now supports dropping multiple single spectra objects
- Drop Action "Weighted Spectrum Subtraction" now integrated in Graph Background Subtraction Constant Tab, using a check box that enables the weighting factor mask
- "Reset Viewer Positions" now sets Project Dialog positions to screen center
- Improved performance when loading a WIP file with many data objects
- Improved performance when calculating image spectra datasets with memory extension / memory mapped file
- Progress Dialog now shows number of calculating cores instead of lots of sub-progress bars
- Project manager now always present (last one can not be closed)
- Load/Append Project now cleans unused objects in project

WITec Project 5.0

- Now supports using up to 4Gb of memory without memory extension (on 64bit Windows Operating Systems)
- New Analysis Dialog: **TrueComponent Analysis**
  - Replaces Basis Analysis Dialog
  - Basis Spectra can be dropped, Dialog can be used like normal basis analysis
  - Plus Version Features:
    - Add basis spectra (manually, automatically)
    - Average spectra (using manual mask, threshold mask, automatic mask)
    - Demix spectra (manually using wheel controls or edits)
- New Dialog "Wizards" (Next Step buttons) and Raman Start Analysis button
- Software News / Did you know upon first program start
- Graph Viewer Features
  - Mouse wheel can zoom all spectra in cascade mode
  - Scale Edit Boxes for X-Axis also displayed in Graph List
  - Possibility to create and manipulate masks without any dialog
  - Configurable visibility of coordinate axes and ticks
  - Rayleigh area is automatically removed from masks (range configurable in Graph Viewer Options)
- Image Viewer Features
  - Equalize color scale (non linear assignment of colors for displaying the maximal

contrast for all dynamics)
    - Controls for Contrast and Brightness (changes top and bottom color value automatically)
    - Contrast and Brightness can now be adjusted in color bitmaps
    - Draw Field / Mask: Erode and Dilate, "Minimum Structure Size" for threshold auto mask
    - Smoother display of pixels if only few pixels are displayed
    - Configurable visibility of coordinate axes and ticks
    - Improved bitmap export (wysiwyg in 3D mode)
- New colors in graph viewer and image combination dialog (now colors should fit to each other)
- Sum images from Filter Viewer or RamanTV now have spectral range in meta data. Press "G" in image viewer to show spectral range.
- Graph Background Subtraction: possibility to show the background subtracted spectrum as preview
- Average Spectra: possibility to do an average of multiple single spectra
- Peak Find: Edit peaks with mouse, show if peak finds are displayed (even if dialog is closed, e.g. after load project)
- Time for show geometry is now configurable
- Image Combination: Auto scale button
- Cluster Analysis Dialog: New Extract buttons, simpler user interface
- Possibility to display "um" instead of "µm" (for asian operating systems that can't display "µ", see Display Options)
- Image Calculator: works with images from different measurements
- Table export: header can be exported

WITec Project 4.1

- Graph Viewer: Zoom out Rayleigh Peak (Shortcut "R", e.g. for calibration purposes)
- Show Scan Position / Geometry
    - Press "G" in Graph or Image Viewer to show the Geometry/Scan Position of the displayed measurement
- Larger Font Size in most windows of WITec Project and WITec Control
- Create Stack Slice / Depth Image from many stack images (drop onto Cross Section Dialog !)
- Graph Viewer fast zoom-in:
    - With control-key hold down "draw" region
    - Press space to zoom into peak at mouse position
    - Zoom out x also zooms out y-axis, if auto y axis zoom is turned on
- Project Manager Multiple Rename Tool: Possibility to replace only those objects that contain a search string
- Graph Viewer can create Mask without any Drop Action Dialog (Circle Menu "Misc Visuals" -> Create Mask or Control-M)
- Advanced Fitting Tool: New result table for single spectra batch processing
- Shape Background Subtraction: Mask can now be used to do a simple line interpolation
- Graph Viewer Peak Labels
    - Now all graphs in viewer are labelled
    - Peaks can be added/removed manually (note: change graph / space position does

not work if peaks were manually added/removed !)
    - All Peaks and Display Settings are now saved for each graph viewer in the WIP File
- Graph Viewer: "Export view as bitmap to project" now available in Export Menu
- Show Scan Position / Geometry
    - Press "G" in Graph or Image Viewer to show the Geometry/Scan Position of the displayed measurement
- ASCII Data Export: File Suffix can be changed

WITec Project 4.0

- Look and Feel
    - New Splash/About Window
    - New Application Shortcut Icons on Desktop and Start Menu, new WIP File Icons
    - New Shortcut Viewer: Shows all the Shortcuts of the Software, see Menu Help -> Show Shortcuts
    - New Drop Action Icons
    - User is now notified if an action needs too much memory (some Drop Actions creating large graph objects, Copy/Paste of data objects in project manager)
    - Main Window:
        - New Tool Button for "Close All Viewers and Managers" next to "Reset Viewer Positions" Button
        - Recent WIP Files directly accessable in File Menu (no sub menu), better Structure of Menus
    - New Icon for Mask Image Objects (Extracted Draw Field Image or extracted calculator image with boolean unit name)
    - Automatic load of correct window settings (works for 1 monitor)
- New Program Options Window (Menu Options -> Program Options)
    - Replaces Viewer Positioning options, Export options and adds some additional, new options
    - OpenGL Graphic Acceleration can be turned on or off in this new Program Options (has effect on WITec Project AND WITec Control of the same version)
- Memory Extension
    - New Memory Option "Memory Mapped File" that allows the program to work with more than 2GB of data objects (e.g. for large Raman images or stacks; see Menu Options -> Memory Options)
    - 64-Bit Operating System with >=16GB of Memory as well as a Fast SSD Hard Drive as Memory Mapped File Location is recommended
- Cursor Manager:
    - The Cursor Manager is no longer a Viewer, but a Tool Window whose visibility can be toggled
    - The Cursor Manager Window is not visible by default due to new status information in viewers (see below)
    - The Cursor Manager Window can temporarily be shown via the Shortcut "P" in all Viewers
- Drop Action Window:
    - The Drop Action Plus Window is no longer present. All Plus Drop Actions are shown in the standard Drop Action Window (if Plus is activated)
    - The Drop Action Window will automatically show up near the mouse position upon

starting a drag Action in the Project Manager

- Inclusion Area Button is no longer present. Each Dialog supporting a Mask now shows an Image Viewer. A mask can be drawn there or a previously defined mask can be assigned (dropped)

- Viewers General
  - Automatic Mouse Mode
    - Graph Viewers with Masks automatically have the mark region mouse mode
    - If Listen is turned on, Graph and Image Viewers automatically select the correct mouse mode and switch back to move mode when listen is turned off.
  - Viewer Positioning Changes
    - If the last viewer of a viewer kind (e.g. Image Viewers) is closed, the positioning for this viewer kind will be reset
    - All Viewers are automatically repositioned after loading a project. There is an option to turn this feature on/off in the Viewer Positioning Options
    - Automatic Array: if there is not enough space for a new graph or image viewer, the size is automatically adjusted
  - New Status Bar in Graph/Image/Video Control Windows
    - The Status Bar now also shows a second line with more information (Graph Viewer: X Cursor Position, Image Viewer and Video Control: Space Cursor Distance)
    - If cursor is listening, the label in the status bar is shown red. A click on the red label will turn off listen mode for this cursor
    - Some frequently used features can be found in the new status bar (e.g. Image Automatic Color Scale, Graph Zoom Functions)
  - Cross Hair has now red color when someone listens to one of the axes of a viewer
  - Extraction of currently shown data now works in all Graph/Image Viewers and it works always (also in Drop Action Previews and e.g. Hardware Spectrum Viewer). Shortcut "Control + E"
  - Text Viewer: Text objects can be toggled in the same viewer (via arrow buttons or shortcuts F5/F6)
- New Filter Viewer (will replace old Filter Manager, also in WITec Control)
  - Filters are no longer project data objects
  - Filters are stored with the Viewer
  - Possibility to store a set of filters into a file for quick access (selectable for the current user or for all users)
  - Filter Range is defined by Position and Width
  - Filter Position/Width can be listened: Move Mouse Mode will change the Position, Mark Region Mouse Mode will change Position and Width
  - Preview Graph Window
    - Shows local average graph (like Spectrum to Images) and highlighted filter positions
    - Highlighted filter positions can be moved directly with the mouse move mode
    - Mark Region Tool opens popup that can create or change new filters at marked range
  - Single Graphs have single-value-preview in status bar
  - Also works with Line Graphs and Graphs that have a spatial X-Transformation (Cross Section)
  - Input Graph can be changed (only if it has the same size and X-Transformation Unit

kind)
    - Drop another Graph onto Title Bar
    - Use the Previous/Next Buttons in the Status Bar
    - Press Page Up / Page Down in an Filter Result Image Preview Window
  - Filter Preview can be toggled by selecting a filter. Checkboxes in Filter List allow to show more than 1 filter simultaneously. If no preview is opened, a double-click on filter will open a preview.
  - Batch Calculation via Drag & Drop onto Extract Buttons (Dropping onto "All" Button will calculate and extract all filters in the list)
  - New Data Objects are only created upon pressing one of the Extract Actions
- Graph Viewer
  - New Zoom Modes
    - Automatic Y-Axis Zoom, Cascade and Synchronized Zoom is turned on by default (you can change the defaults in the Graph Viewer Program Options)
    - Improved Cascade function with new cascade distance parameter
    - Synchronized Zoom for zooming all graph objects without using the same Y-Axis
  - New Feature: Peak Labels can be shown (using peak find algorithm). Shortcut "F"
  - Graph Mask Data Object can now be dropped directly on a Mask in a Graph Viewer to set the Mask
  - Graph Mask can be hidden. Shortcut "H"
  - New Follow Data Mode: The Cursor will follow the exact supporting points of the currently shown graph
  - New Histogram Drawing avoids gaps and aliasing drawing problems (only if the X-Transformation has equidistant points); supports cascade now
  - New Shortcut: Page Up / Page Down toggles through the Graph Objects in the project, if only 1 Graph is displayed
  - Graph Drawing Options can now be synchronized (e.g. set width for all graph objects in the same viewer)
  - Default Graph Drawing Width can be defined in the new Graph Viewer Program Options
- Image Viewer
  - Image Viewer also shows Bitmaps. Bitmap Viewer is no longer needed
  - Image Viewer can show Bitmaps and Images that don't have a Space Transformation
  - New Feature: Synchronized Zoom. Will zoom all image viewers to the same spatial bounding box. Zoom Out in Synchronize Mode will zoom out all viewers. Shortcut "S"
  - Revised New Display Modes:
    - 3 Slots for Input Images: Color Image, 3D-Z Image, Brightness Image; all assignable via drag drop
    - 3D Mode (toggle, Shortcut "3"), Brightness Mode (toggle)
    - 3D Image Mode now automatically uses perspective projection and side view
    - Free Camera Mode (toggle, automatically used in 3D Mode): Image can be tilted and rotated by moving the mouse while holding down the middle mouse button (wheel), Free Camera Distance can be defined using the Mousewheel
  - Crosshair can be hidden (e.g. for nice presentations)
  - Draw Field / Mask can be hidden. Shortcut "H"
  - Draw Field: Pen Width for Draw Point mode can be changed

- Image can be animated (change the animation speed in Image Viewer Program Options)
- Contour Lines can be drawn (Misc Visuals Circle Menu)
- Nice Mesh Borders will close the borders in 3D Mode (Visuals Circle Menu)
- Line Correction can be changed in the right bottom corner in the new statusbar
- Automatic Color Scale (and toggle with rightclick) can be found in the right bottom corner in the new statusbar
- Color Scale:
  - Bottom/Top Color Scale can now be swapped
  - Bottom/Top Color scale can now be changed synchronously
  - In the new Image Viewer Program Options the behavior of the automatic color scale algorithm can be adjusted
- Scalebar now has transparent background
- Dropping multiple objects on the viewer for showing the positions in one step is now possible

- Image and Graph Tools
  - Tool Windows are no longer present
  - New Circle Menus in Image and Graph Viewer:
    - Hold right mouse button down to see circle menu and then select one of the circle menus by moving the mouse in the right direction
  - All features can be found in the new circle menus, also those who previously only could be found in the normal context menu
  - Start Drop Action Dialogs directly from an image/graph viewer circle menu
- Project Manager
  - New Circle Menu for toggling visibility and sort of objects and for starting drop action data analysis
  - Only Graph, Image/Bitmap, Text Objects are shown (Advanced Mode shows all objects)
  - New Multiple Rename Tool with rename mask and search and replace functionality integrated
  - Project Managers now highlight all data objects that are used by another viewer when this viewer is activated/selected. There is an option to turn this feature on/off in the Viewer Positioning Options
  - Project Managers are not loaded when appending a project. There is an option to turn this feature on/off in the Viewer Positioning Options
- Drop Action Dialogs:
  - New Dialog "Image Histogram and Statistics": Image Histogram Dialog now also shows Image Statistics and Roughness parameters
  - New Dialog "Image Transform and Overlay" (Plus Feature): allows to change the spatial transformation of images and creates overlay bitmaps, supporting advanced transformations
  - New Dialog "Graph Repair" (Plus Feature): allows to interpolate manually selected spectral pixels or ranges for certain or all spectra in any kind of graph data object
  - New Dialog "Graph and Image Data Stitching" (Plus Feature): allows to stitch/merge a stack of images or graph images into one new bigger data object
  - New Dialog "Image Correlation" (Plus Feature): shows correlation points according to changeable mask in image viewer
  - New Cluster Analysis User Interface: only one window, simplified handling

- Revised Background Subtraction Dialog
  - New Subtraction Mode "Minimum". Subtracts the minimum of all spectral pixels (using the mask)
  - New Subtraction Mode "Constant". A constant value or a constant spectrum can be subtracted
  - New Subtraction Mode "Shape" (Plus Feature). A rounded shape is fitted on the spectrum, leading to a excellent background subtraction, even for fluorescence offsets
  - Now supports cross section graph objects
- Image Cross Section Dialog: Simplified Dialog, Start/Stop Coordinates can now be swapped
- Image Color Combination:
  - Redesign with Simple/Advanced Mode
  - New mode "Allow Color Mixing"
  - Listenable Edits, Color-Scale-Synchronization with Image Viewer, wheels for changing the color scale
  - Improved transparency smooth parameter
- Spectrum to Images: Improved Listen mode
- Graph Basis Analysis: Simplified Dialog
- Advanced Fitting Tool: Simplified Dialog
- Image Filter Dialogs no longer show error images, but have a new checkbox for showing the original image temporarily (Image Filter, Image FFT 2D, Image Background Subtraction)
- Data Binning Dialog is now called Data Cropping and Reduction. Simplified Dialog; now allows to crop bitmaps too
  - New in Version 4.0.12.9: Batch mode via Drag & Drop
- Graph Demixer now allows to use the current mix as new source graphs; allows graphs with different x transformations objects that are equal
- Only one Calculator Dialog for Images and/or Graph Objects
- Image Filter Dialog: new image smoothing algorithm: "Anisotropic Smoothing"

- Similar Drop Actions are now merged into one Dialog
  - Image Statistics/Roughness Drop Actions are merged into the new "Histogram and Statistics" Dialog (Image Histogram, Image Statistics, Image Roughness)
  - Graph Filter Dialogs are merged into one dialog (Graph Average, Graph Median, Graph Savitzky Golay, Graph CRR)
  - Image Filter Dialogs are merged into one dialog (Image Median, Image Average, Plus Version Image Filters like Sobel, ...)
  - Image Background Subtraction Dialogs are merged into one dialog (N-Order Line Subtraction, N-Order Surface Subtraction)
  - Image Stack Dialogs are merged into one Dialog (Image Auto Focus, Image Extended Focus)
  - Image FFT Filters are merged into one Dialog (Image FFT Amplitude, Image Filter FFT Line, Image FFT 2D)
  - Image Line Interpolation and Image Remove Bad Data Dialoges are merged into one dialog
- Import/Export
  - New Bitmap Import: Bitmaps can be imported via Menu File -> Import -> Bitmap or use Paste in Project Manager (Strg-V) if Bitmap is in clipboard

- Tescan TIF Import (for SEM images, needs special license)
- Export to Matlab
- Batch Export for Text Objects (ASCII and RTF)

WITec Project 2.10

- New Drop Action: Inverse Basis Analysis (the right button next to the Basis Analysis Button):
  - Uses an image graph together with Images (which should be background subtracted) that belong to the image graph
  - Calculates offset and de-mixed component spectra for each image
- Image and Bitmap Viewer: Drag and Drop of Graph and Image Objects onto the Viewers now open a Popup Menu for selecting several actions:
  - Show Position (Transformation) -> It's no longer necessary to search for the transformation of an image graph or image or bitmap to show it in a viewer
  - Use as Main Image/Bitmap
  - Use as second Brightness/Color Image (Image or Bitmap on Image Viewer only)
  - Use Bitmap as Texture (Bitmap on Image Viewer Only)
  - Use as Draw field (Image on Image Viewer Only)
- Drop Action Advanced Fitting Tool:
  - Batch calculation now available through Drag Drop onto the "Extract All" Button
  - Now some additional information for the current fit is shown (coefficient of determination, reduced chi square, average error)
  - Replica of multi peak functions can now be shown and extracted (Gauss, Lorentz)
  - Fit curves of all pixels can now be calculated and extracted
  - Text Result (single fit extraction) now shows more information
  - Works now with Cross Section Graph Objects (3 Dimensional X-Transformations)
- Spectrum To Image:
  - Drop Action as well as Online Algorithm now support different Filters for calculating image values: Sum, Variance, Average Binomial, Average Box, Average minus Minimum
  - Drop Action now works with 1-Dimensional Graph Objects (Time Series, Cross Section Graph, ...)
- Auto Scale on Data Change Function in Graph- and Image Viewers
  - It's now possible to right-click on the image tool window button "Automatic Color Scale" (or on the graph tool window buttons for zooming out) in order to turn on/off the automatic scaling if the data changes
  - Additional Option in Graph Viewer Popup Menu: "Auto Cascade"
- Drop Action Image Cross Section: It's now possible to define a horizontal or vertical line that uses the exact pixel supporting points as cross section values
- Graph Viewer Data Export (e.g. to ACDLABS / UV/IR-Manager): the user can now define the program path in "Menu -> Options -> Export -> Graph ASCII to External Program" instead of editing a text file
- New Bitmap Export: It's now possible to export a couple of bitmap data objects at once into jpg, png, and bmp files. (Project Manager "Popup Menu -> Export")
- ASCII Export of Graph/Image/Bitmap Data is now available through Project Manager "Popup Menu -> Export" and offers some export options
- New in Version 2.10.3: SPC Export now also works with Image Spectrum Data (2D)

How to create Support ZIP Files

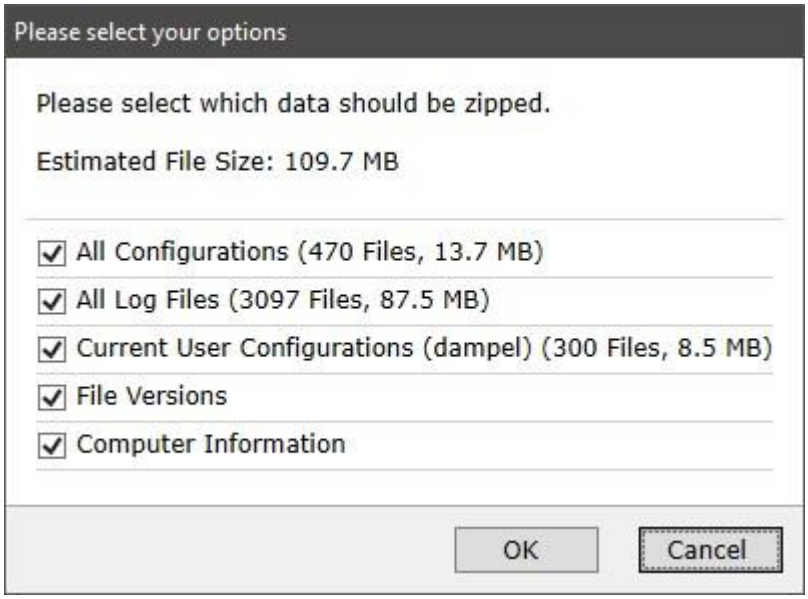
You can create a support ZIP file containing all log files and computer information in order to help a WITec support employee finding software problems.

To create the support ZIP file, just go to the Main-Menu of WITec Project/Control/TrueMatch and select

Help -> Create Support ZIP File

(On microscope computers it's also possible to use the WITec Service Monitor)

Here you can select what data should be zipped. For the best support, please send us all data:



Now press OK. A new ZIP File will be created. Please send this ZIP file to your WITec support contact.

Support and Contacts

WITec Support Team

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WITec Microscope Series

alpha300 S: Scanning Near-field Optical Microscope

alpha300 A: Atomic Force Microscope

alpha300 R: Confocal Raman Microscope

alpha300 Ri: Inverted Confocal Raman Microscope

RISE: Raman Imaging – Scanning Electron Microscope

alpha300 *apyron*: Automated Confocal Raman Microscope

alpha300 *access*: Confocal Micro-Raman System

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