WiTec Confocal Raman 300R System - UC Merced - SE1 - 153A

Safety: 633 nm, 50 mW, laser class 3B is used when the beam shutter is open, however it is contained inside the microscope. For safety, use the laser shield on the stage to prevent reflection from samples redirecting laser light.

Optical fibers connect the laser to the microscope and the spectrometer to the microscope. Do NOT bend these wires.

Currently available objectives:

1. 10X: Zeiss EPIPLAN 10x/0,23 442030-9903
2. 50X: Zeiss EPIPLAN 50x/0,65 442060-9901
3. 100X: Zeiss EPIPLAN 100x/0,8

Startup Procedure:

1) Turn on the computer by turning on the Control Unit. This will power up the microscope as well. Login to ilab to start Kiosk for Raman usage.

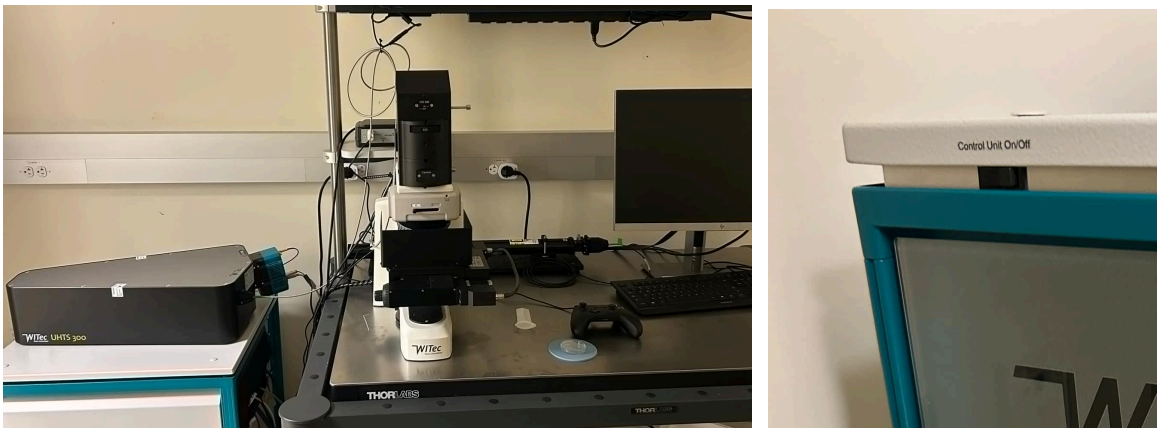

Fig. A. Microscope setup. The Control Unit Switch is located left of the microscope and computer monitor. The spectrometer is located on the Control Unit. The laser is behind the microscope.

2) Check the microscope configurations and that the microscope is on. See Figure 1a and 2a.

Fig. 1a Microscope in BF Configuration

Fig. 1b. Microscope in Raman Configuration and laser shield on sample.

When getting started, the microscope should be in BF configuration (Fig. 1a).

- VIS 300 Metal Pin should always be pulled out (==>) in both Configurations.
- 633 nm should be selected (only laser option)
- Camera Metal pin should be pulled out to view BF image (==>) and BF should be selected with the slider (<==), Fig. 1a. Note: For Raman, the Camera Metal pin will be pushed in (<==) and the slider will be pushed (==>), Fig. 1b (use sample shield when using raman).

Fig. 2 Microscope power on and Focus knob not locked.

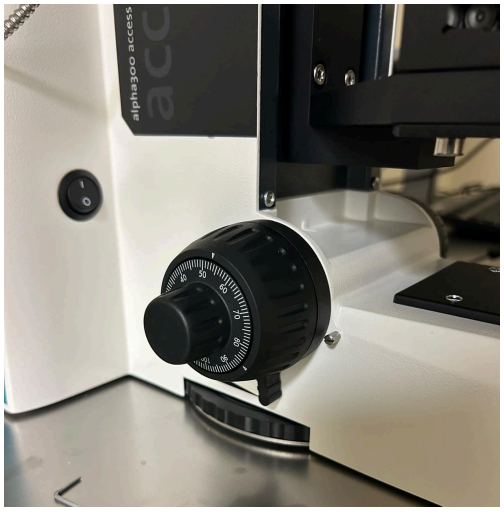

Fig. 2b. Microscope Focus locked after the sample is focused.

- Check that the Microscope switch is on the on position.
- The focus knob has a black lock switch on it. When pulled to the metal pin, it allows the z-position to change. Once you have your sample in focus, you can pull the switch down to lock the focus knob.
- Below is the illumination turret to adjust the intensity.

3) Turn the key on the laser 90 clockwise-turn to turn on the laser (I position). It is off in the O-position (Fig. 3a). For stable power, the laser should be given 3-5 mins to warm up. Make sure the Beam shutter is closed (Fig.3b) before putting in your sample.

Fig. 3a. Image of Laser power key switch. Image shows the laser in O-position (Off). Turning the key to I-position turns laser power on.

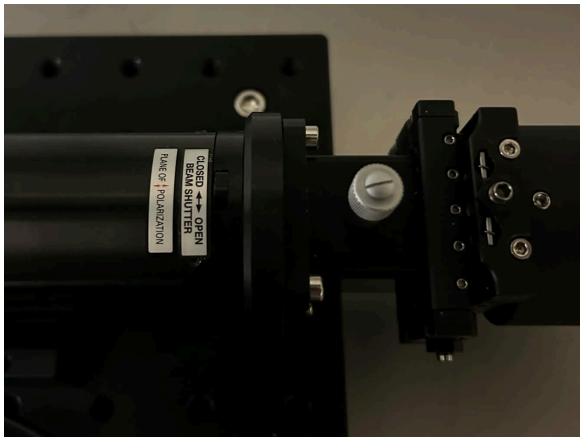

Fig. 3b. Image of Beam shutter switch and attenuator. Make sure the beam shutter is closed (switch is up) when changing samples. Put up a laser shield on the sample stage when the beam shutter is open. The silver knob is the attenuator to adjust beam intensity.

4) On the computer screen, click on the “Service Monitor” (H icon) at the bottom right corner of the Windows screen. The microscope controller and camera should be green.

5) Open the “Control FIVE” software. It should open the following windows: Main Menu, Control, Messages. Video Control, and Project Manager. (Note: There is another software called “Project FIVE” and that is for data processing, not data collection.)

6) Wait for the spectrometer to cool down to -60 degrees before starting.

Troubleshooting: If you have trouble starting up the microscope, try turning off Control FIVE, turn off the laser, turn off the computer/controller unit and then wait for 5 minutes. Then, turn back on the computer/controller unit, turn back on the laser, and open Control FIVE again.

Sample placement and scanning:

Standard Operation: Recommended dry samples on 1” x 3” glass slide.

1) Choose the 10x objective lens.

2) Mount your sample (microscope slide with mounted sample works) on the stage and pin down the sample with the stage clips. Move the objectives out of the way if you need more space. DO NOT crash the sample into the objectives. Lower the stage using the focus knob if needed.

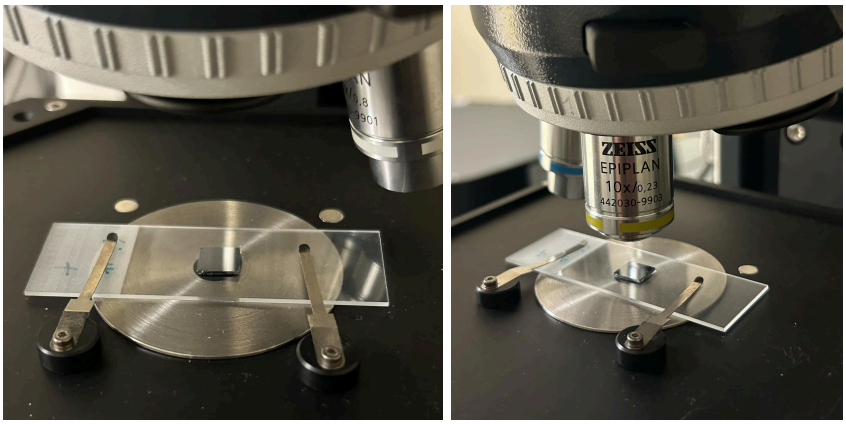

Fig. 4. Sample microscope slide secured and 10x objective selected.

3) The software does not automatically update the objective. Make sure you select the 10x objective in the Video control window. Find a feature on the sample to focus.

4) Repeat focusing with the 50x and 100x objectives (remember to switch objectives in software as well). Use the field-stop diaphragm as focusing reference (Fig.5). When the edges of the F-aperture are clear, we are in focus. Use the laser shield after focusing is completed. You can also use the focus lock in Fig. 2b.

Fig. 5. On the right side of the microscope has the field-stop diaphragm controlled by the “F” to help with focusing. When the edges of the aperture are clear, the sample is in focus. The “A” is for adjusting optical density and BF image contrast.

5) Acquire a video image for reference (either video image or video stitching image). Click on the video image in the project manager to open the image. (Note: Once we are in Raman mode, we won't be able to see the sample in BF anymore, so taking an image here is important to know where you are on the sample.)

6) Change the microscope configuration to Fig. 1b. Slide from BF to the right and push the metal pin for the camera in. Make sure the laser is on and use the laser shield on the sample.

7) Make sure the 633 nm laser is selected in the Video Control window. Open the beam shutter but sliding the shutter shown Fig. 3b. Adjust the laser power with the attenuator as needed.

8) Choose the appropriate grating in Spectrograph 1 in the Control window and select a Spectral center. You can select G1 for grating and choose 2000 for Spectral Center if unsure.

9) Expand the Oscilloscope in the Control window and select parameters for Integration time:

- Start with a short time (0.05s-0.2s) and low laser power, and then slowly increase laser power (by adjusting the attenuator, silver knob as shown in Fig.3b).
- Fine focus to maximize spectral signal.

10) Expand Single Spectrum in Control window, set parameters for integration time to match oscilloscope. For Accumulation:

- Set Accumulation to smaller numbers (10-30) for samples with strong Raman signals
- Set Accumulation to larger numbers (100-150) for samples with weak Raman signals

11) Click Acc. Single Spectrum to start. You will obtain a live single acquisition and an accumulated spectrum.

12) Expand Large Area Scan and Geometry in the Control window, select the type of Listen Position/Area and then draw an area in the opened video image. Adjust parameters for width/height and number of pixels in the Control window.

13) Click Start Large Area Scan to start data collection. Four new windows will pop up:

- Filter panel window is used to add a filter to have the generated maps only display on the signal from the selected peak. (ie. Elemental map filter, background filter)
- Map window that shows maps created from filters.
- Live spectrum window. You can only move the green band in this window to select peaks in a large area spectra window.
- Large area spectra window. Purple band in this window is coupled to the green band in the live spectrum window and works as an indicator to select peaks.

14) Save the Project file.

Shutdown Procedure:

1) Double check that you have saved the project file to save all data. If you want to save individual data, you can click on the individual data sets and export. We can use Project FIVE on a project file to view, export, and analyze data afterward as well.

2) Close the Control FIVE program and wait for the spectrometer to warm up.

3) Log out of ilab and upload files to “Box”.

4) Turn off the laser by turning the key back to O-position. Close the laser beam shutter shutter. Shutdown the computer/controller unit.

5) Clean up your area and take your samples away.

References:

1) IMF training video: https://www.youtube.com/watch?v=kGydyJFVtno&t=212s

2) E1, MAY 2020, ASRC Imaging, TONG WANG, TWANG1@GC.CUNY.EDU, SHENG ZHANG, SZHANG3@GC.CUNY.EDU https://asrc.gc.cuny.edu/wp-content/uploads/media/global-assets/WITec-Raman-Microscope-alpha300R.pdf