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Talos Basic General Information

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Document History

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Talos Basic General Information

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Chapter 3 TEM Modes & Contrast Mechanisms

Chapter 4 TEM Optimization

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Glossary

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1 Electron Microscopy Basics

Introduction to Electron Optics

While light microscopy has been known for over more than 400 years, electron microscopy is a relatively young technique that first appeared in 1931. During the last 60 years, the resolution of electron microscopes has been improved dramatically from values of around 5 nm (EM100 1949, the first commercial electron microscope from Philips) down to atomic level (Thermo Scientific™ Titan™ G2 60-300: TEM information limit, TEM point resolution and STEM resolution below 0.10 nm).

Figure 1 Comparison of TEM with Light Microscope

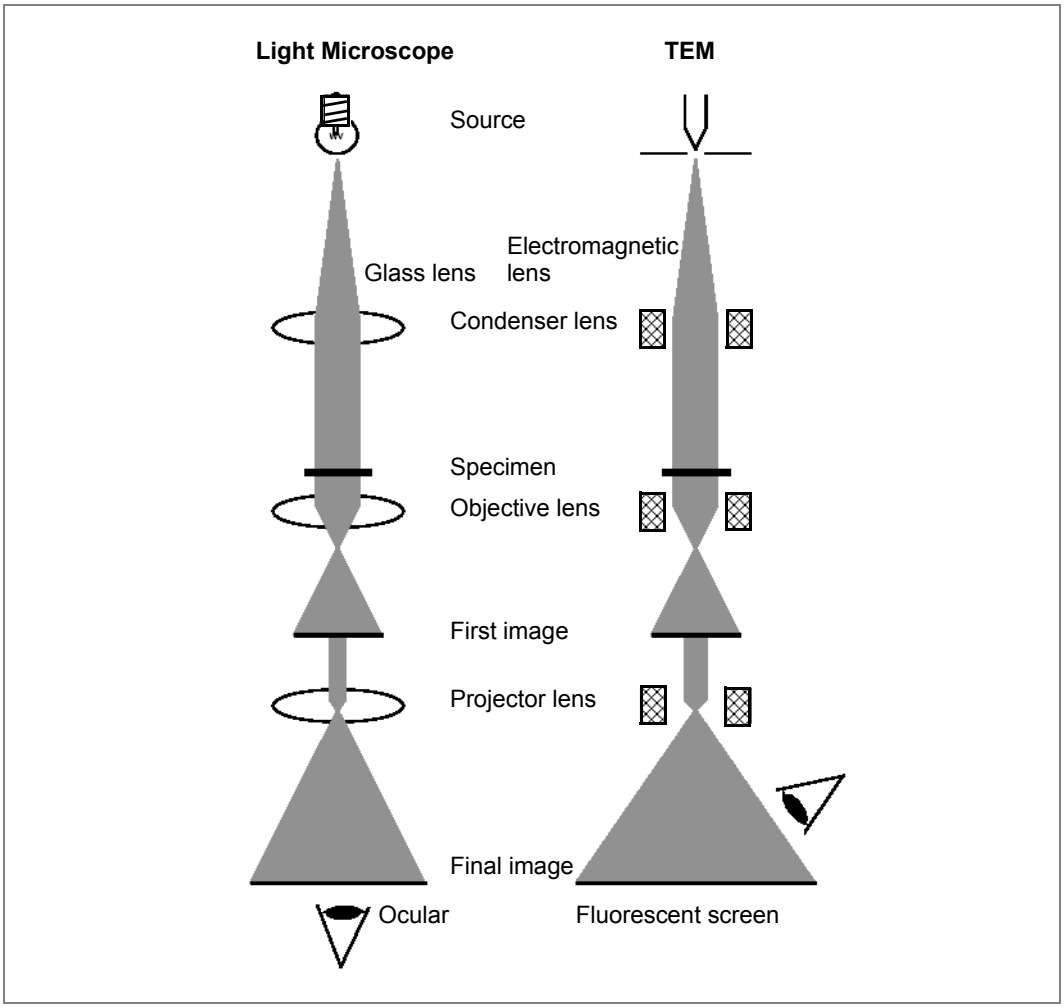


Figure 1 compares a light microscope and a transmission electron microscope. The principle is essentially the same: a source illuminates the thin specimen with the aid of a condenser lens, and then the objective lens produces an image that is subsequently further magnified by the projector lens. The three major differences are:

- The illumination source and information carrier (light or electrons)
- The lenses (glass or electromagnetic lenses)
- The way of observing the final image (direct observation or on the fluorescent screen/photographic film/digital camera).

The comparison of the two kinds of microscope is further listed in *Table 1*.

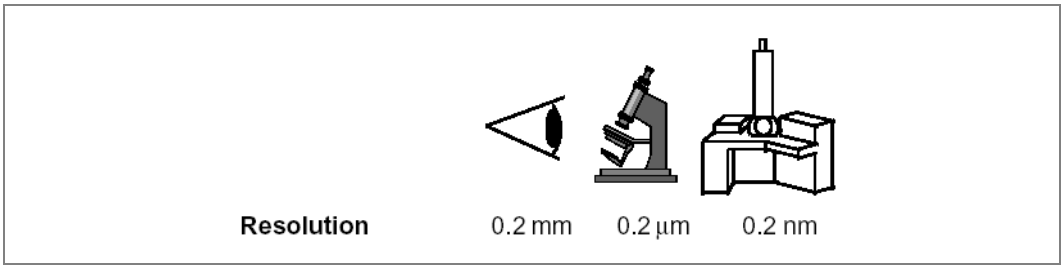
Table 1 Comparison of Characteristics of Light and Electron Microscopy

Characteristic	Light Microscopy	Electron Microscopy
Carrier	Light rays	Electrons
Wavelength	400–800 nm (visible) 200 nm (ultraviolet)	0.0037 nm (at 100 kV) 0.0020 nm (at 300 kV)
Medium	Air	Vacuum
Lenses	Glass	Electromagnets
Aperture	< 64°	0.2–0.7°
Angle observation	Direct	Via fluorescent screen or digital camera
Contrast by	Absorption, reflection, fluorescense, and phase changes	Scattering, phase changes, and diffraction
Resolving	0.2 µm (visible)	0.2 nm (point)
Focusing and alignment	Mechanically	Electronically
Depth of focus	0.1 µm–0.1 m	0.1–100,000 m
Depth of field	< 0.1 µm	< 1 µm
Information	Mass density distribution Fluorescent intensity Adsorption/reflection spectra	Mass density distribution Crystallographic and chemical Inner potential for phase contrast EM

In practice, more lenses are used in an electron microscope for a higher flexibility. For example, two or three condenser lenses not only give better flexibility of illumination, but also make switching between small spot mode and the conventional mode much easier. The final magnifying system, consisting of four or more lenses, must satisfy different demands such as a flexible range of magnifications, correction of lens errors like chromatic aberration, different lens modes, and image rotation.

The objective lens is the heart of a microscope because it provides an image of the specimen with relatively high magnification before being further enlarged by the rest of the magnifying system. The final resolution and contrast of the image is strongly determined by this lens. Many performance parameters are used to specify an objective lens, and resolution and maximum tilt angle are among them. Depending on applications, a high image contrast could be just as important as a good analytical performance, or a high flexibility for extra detectors such as secondary electron, back-scattered electron, bright-field or dark-field detectors.

Figure 2 Comparison of Resolving Power



The depth of field, i.e., the depth in the specimen plane that is in focus, depends on the wavelength of irradiation and on the objective aperture angle: a larger wavelength and a smaller aperture increase the depth in the specimen that is in focus. In electron microscopy practice, the depth of field is larger than the full specimen thickness (~ 0.1 µm); therefore, every feature in a conventional thin section will be in focus.

At the same time, the small apertures used in electron microscopes result in the projected specimen image being brought into acceptable focus over a great distance (depth of focus, see *page 16*). In other words, the final image remains in focus for a long distance along the optical axis (> 1 m). For this reason, in electron microscopy it is possible to locate multiple image-recording devices at various points beyond the projector lens because they will all be in focus.

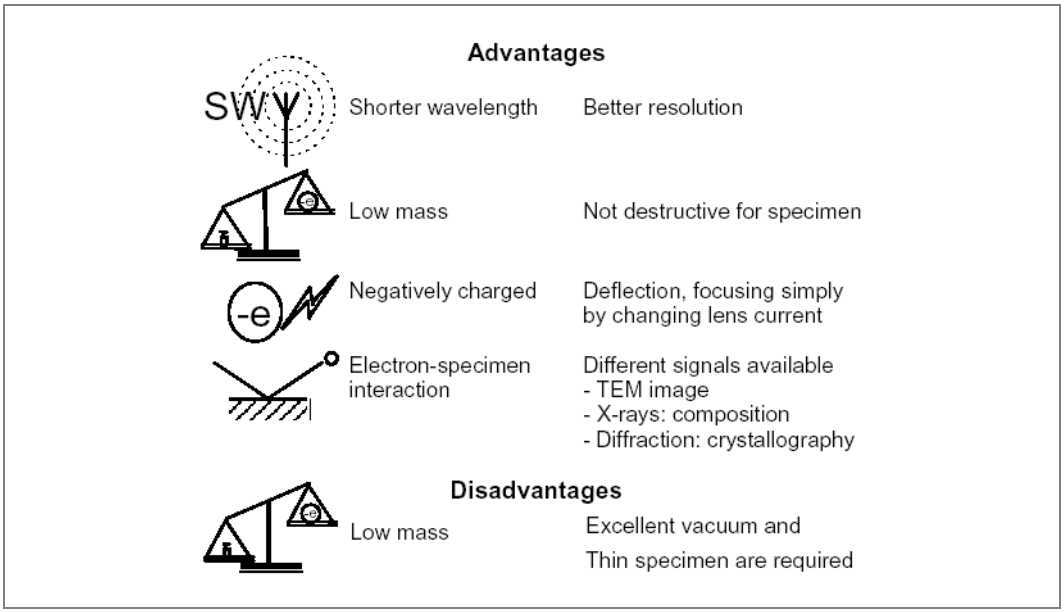
Table 2 Major Events in the Development of the Electron Microscope

Year	Event
1876	Abbé states image theory for light microscopy and discovers that wavelength is the resolution-limiting factor.
1897	Thompson describes the existence of negatively charged particle, later termed *electrons*.
1931	Ruska and colleagues build the first transmission electron microscope.
1949	Philips launches its first commercial electron microscope (EM100) with 4-lens imaging system, wobbler focusing aid, and 5 nm point resolution.

The different illumination sources lead to a difference in wavelength. Visible light has a wavelength between 400 nm (violet) and 800 nm (red). Long before the electron microscope was invented, Ernst Abbé, a German physicist, established the first imaging theory for the light microscope, taking into account the wave character of the light (*Table 2*). While up to this time microscopists thought that the quality of the glass lenses limited the resolution of the light microscopes, Abbé stated in 1876 that the nature of light itself, i.e., the relatively long wavelength, was the limiting factor. At that time he already had the idea that "*... still unknown processes ... can pass these borders...*" without knowing even the existence of electrons.

Figure 3 presents the advantages and disadvantages of using electrons, showing advantages such as better resolution, more signals, and simple focusing by changing lens current, but at the cost of requiring a high vacuum, which has consequences for specimen preparation.

Figure 3 Advantages and Disadvantages of Using Electrons



The Physics of Electrons

The electron is a charged particle and can therefore be deflected by means of magnetic and electric fields, which are the working principle of accelerators, magnetic lenses, stigmators, and deflectors. The charge of an electron is 1.6×10^{-19} Coulomb. The electron has a very small rest mass of 9.1×10^{-31} kilogram; it therefore causes much less beam damage in the specimen compared with a neutron or a proton, the mass of which is nearly 2,000 times greater.

Electrons can easily be made visible on a fluorescent screen: their energy is converted into *photons* (visible light) when they strike fluorescent material. Their energy can also be used to activate the grains in photographic material. Both mechanisms are low-noise information transmitters because one electron produces a lot of photons or a lot of activated grains in the photographic film.

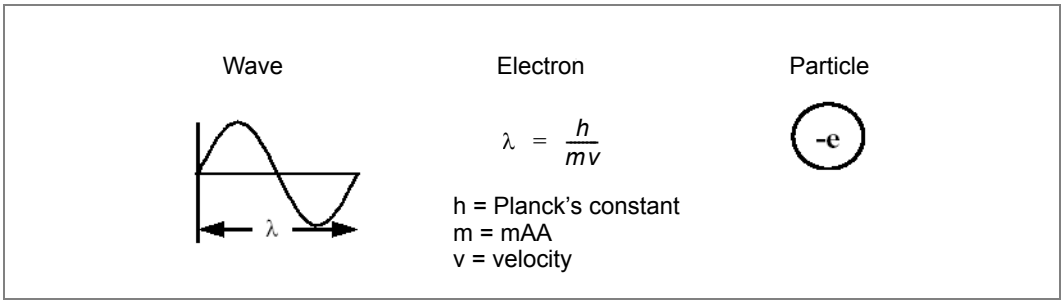
Electrons, as charged particles, interact considerably (about 10^3 times as much as x-rays) with the atoms of the specimen they are travelling through. These strong electron-specimen interactions produce specimen-specific information carried by electrons or excited X-rays. The information can be retrieved through, for example, the contrast in a TEM image, a spectrum of the excited X-rays, or a diffraction pattern.

Wave-Particle Duality

The behavior of electrons in an electron microscope is expressed using two different models. For the motion of electrons in the electromagnetic field of a lens, we treat electrons as charged particles with a mass of *m* and a velocity of *v*. In this way, we can easily compare a glass lens with an electromagnetic lens because the envelope of the electron beam in a magnetic lens is analogous to that of a light beam passing through a converging lens, except that the electrons travel in a helical path through the lens (see *"Electromagnetic Lenses" on page 13*).

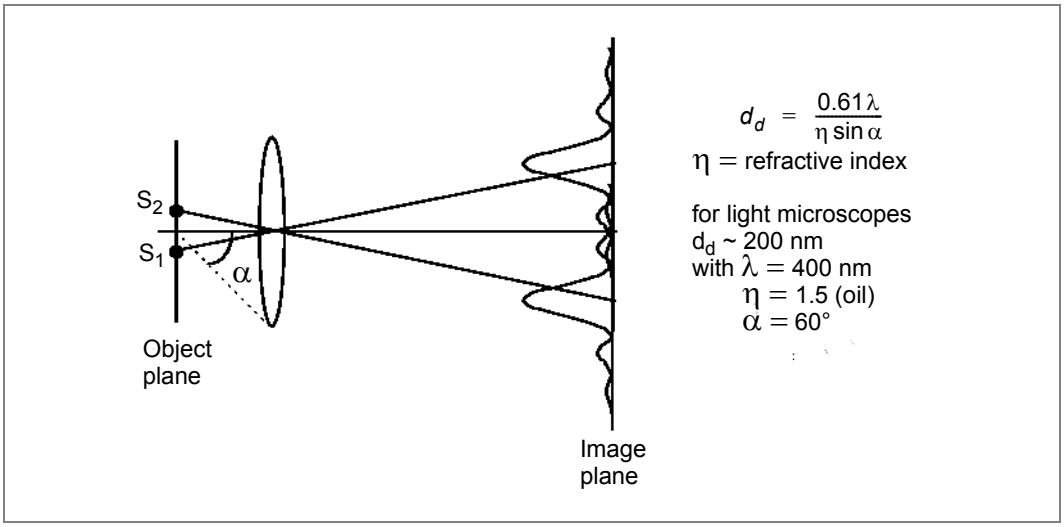
The second model is used for interpretation of the image contrast, the diffraction patterns and the resolution of microscope. This model is based on the Abbé theory for image formation and describes the electrons as waves with a certain wavelength. *Figure 4* summarizes the two models, with the De Broglie relation as a link between them.

Figure 4 Electron as Wave and as Particle



In both light and electron microscopy the ultimate obtainable resolution is limited by the wavelength. In a light microscope, small points with dimensions similar to the wavelength of light are imaged as ring patterns with an intensity distribution such as that shown in *Figure 5*.

Figure 5 Resolution Criterion

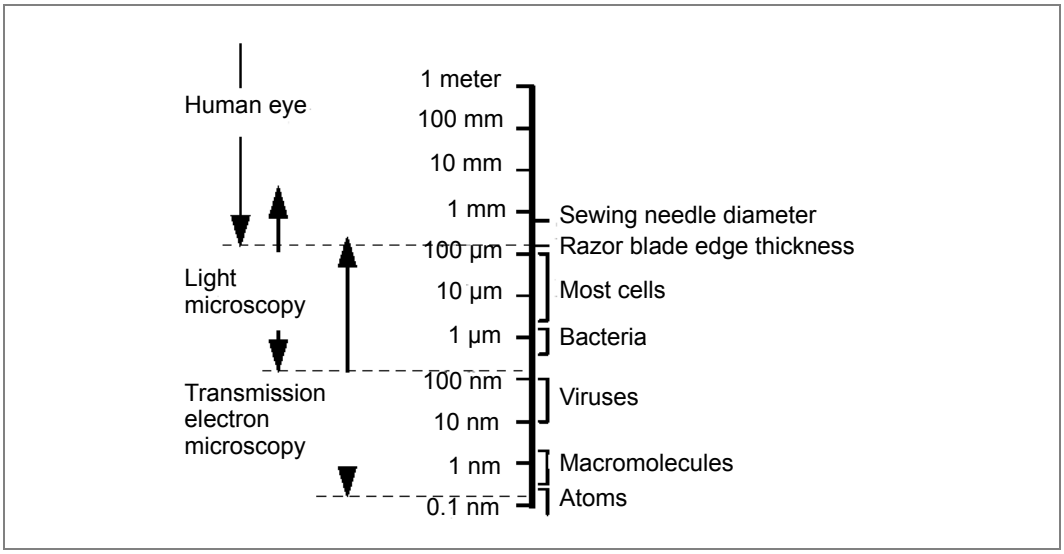


When light rays emanating from two point sources pass through a lens with semi-opening angle α, they form spots surrounded by diffuse rings, instead of two sharp bright spots. These enlarged, indistinct spots are termed *Airy discs*, named after the astronomer Sir George Airy, who described these patterns first in the 19th century. The resolution is defined as the distance (d_d) between the central maxima of two image points when the central maximum of one image point just coincides with the first minimum of intensity of the adjacent image point.

For light, the resolution is comparable to the wavelength since the other factors in the equation (*Figure 5*) are close to 1. For electrons, sin α is about 0.01, so resolution is about 100 times the wavelength.

While for light microscopy the resolution limit is about 200 nm (*Table 1*), the resolution in electron microscopy depends on the accelerating voltage and also on the objective lens. *Figure 6* compares the applications' range of light microscopy and electron microscopy.

Figure 6 Microscope Resolution and Applications



In principle, the resolution of electron microscopy can be improved by increasing the high tension, as the following relation between the wavelength (λ) and the high tension (U in volts) holds.

	U	Classic	Relativistic
$\lambda \bullet nm = \sqrt{\frac{1.50}{U(V)}}$ **De Broglie (nonrelativistic)**	100 kV	λ = 0.0039 nm	λ = 0.0037 nm
	120 kV	λ = 0.0035 nm	λ = 0.0034 nm
	200 kV	λ = 0.0027 nm	λ = 0.0025 nm
	300 kV	λ = 0.0022 nm	λ = 0.0020 nm

Recent advances in light microscopy have improved resolution beyond the diffraction limit to approximately 50 nm or better. The distance between two different fluorescent molecules can be determined with approximately 10 nm accuracy. Also, the microscope has improved TEM resolution to approximately 0.05 nm.

The relation is known as the *De Broglie equation* for the wavelength of an electron. The physical properties of the electron are summarized in *Table 3*. Relativistic effects have to be considered at high accelerating voltages, i.e., U > 100 kV, especially in high-voltage electron microscopy. Therefore, the table contains not only the classical formulae, but also their relativistic counterparts.

Table 3 Properties of Electrons

Rest mass	$m_o = 9.1091 \times 10^{-31}$ kg
Charge	$q = e = -1.602 \times 10^{-19}$ C
Kinetic energy	$E = eU$, 1 eV = 1.602×10^{-19} Nm
Velocity of light	$c = 2.9979 \times 10^{8}$ ms^{-1}
Rest energy	$E_o = m_o c^2 = 511$ keV = 0.511 MeV
Non-Relativistic ($E \ll E_o$)	Relativistic ($E \sim E_o$)
$m = m_o$	$m = m_o(1 + E / E_o)$
$E = eU = \frac{1}{2} m_o v^2$	$mc^2 = m_o c^2 + eU = E_o + E$
$\lambda = \frac{h}{p} = \frac{h}{\sqrt{2 m_o E}}$	$\lambda = \frac{hc}{\sqrt{2EE_o + E^2}}$

Although the wavelength of the electron beam at 200 kV accelerating voltage is in the order of 0.002 nm, the actual resolution of a modern electron microscope is closer to 0.2 nm. This far lower resolution is mainly due to the extremely narrow aperture angles (about 100 times smaller than for light microscopy) needed by the electromagnetic electron microscope lenses to overcome a major resolution-limiting phenomenon called *spherical aberration*. In addition, diffraction phenomena, chromatic aberration, and astigmatism all degrade the resolution capability of the electron microscope.

The correlation between resolution (ρ), wavelength (λ) and the spherical aberration of the objective lens (C_S) is given by:

$$\rho = 0.66 \times C_s^{1/4} \lambda^{3/4}$$

Resolution is a very important parameter in high resolution microscopy, e.g., in material science applications to separate individual atomic columns. For a given high tension (200 kV or 300 kV), resolution improvements are made by decreasing the C_S. With optimized lens geometrical parameters, C_S can be only reduced by decreasing the lens gap, the space between the pole pieces, which also limits the maximum tilt angle of the specimen.

Thus, high resolution lenses are always a compromise between acceptable tilt angles and resolution.

Resolution of 0.5 µm is no longer adequate for single-particle analysis; of course good contrast is very important, perhaps even more than for “normal” biological TEM.

In biological applications, the situation is different because the specimen itself is primarily the resolution-limiting factor and not the microscope. While a resolution of 0.5 nm is normally adequate, good contrast of the objective lens is much more important, especially when unstained specimens are investigated. In order to increase the contrast, the objective lens for biological applications normally has a longer focal length and a wider lens gap. The resolution is then worse than microscopes with a smaller lens gap because of the higher spherical aberration.

Electromagnetic Lenses

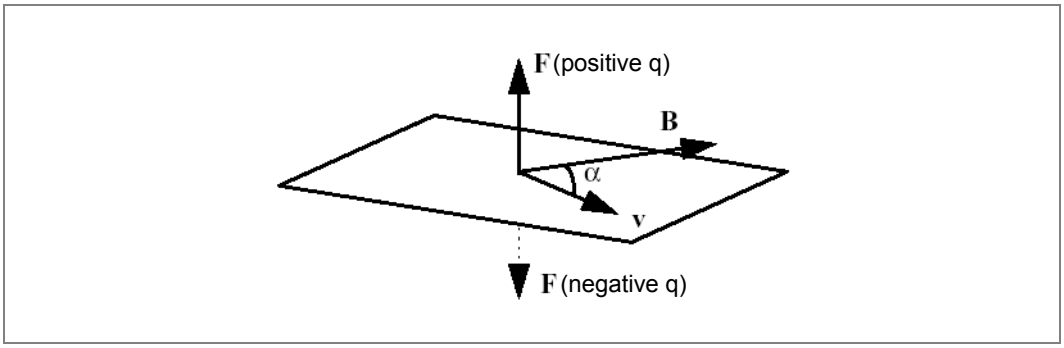
Electromagnetic lenses are based on the fact that moving electrons are deflected in magnetic fields. The electromagnetic lens can be compared with a glass lens, which makes it easy to understand the ray path within a electron microscope.

The force exerted by a magnetic field **B** on a charged particle (e.g., an electron) is proportional to its charge q and its moving velocity **v**. The direction of force **F** is perpendicular to both the velocity and the magnetic field: $\mathbf{F} = q(\mathbf{v} \times \mathbf{B})$.

Here, **v**, **B** and **F** are vectors, which have both magnitudes and directions. Because **F** is perpendicular to the velocity, its work on the particle is zero, and therefore it does not vary the kinetic energy of the electrons.

If α is the angle between **v** and **B**, the magnitude of **F** is $qv\sin\alpha$. This implies that the minimum force F=0 occurs if **v** and **B** are parallel, while F is maximum if they are perpendicular. *Figure 7* shows the relations among these vectors.

Figure 7 Relations between Vectors F, B, and v



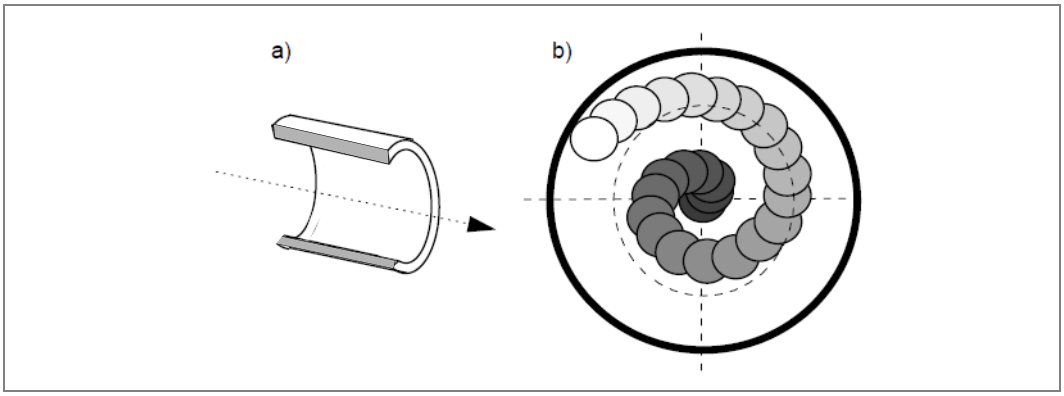
When the electron moves in a region where there is an additional electrical field **E**, the total force is the sum of the electric force and the magnetic force:

$\mathbf{F} = q(\mathbf{E} + \mathbf{v} \times \mathbf{B})$

This is called *Lorentz force* in physics, which describes the forces on a charged particle by electrical and magnetic fields. In the electron microscope, both forces are employed. The electrons are accelerated by an electric field between cathode and anode. In this way the electrons gain a kinetic energy which only depends upon the accelerating voltage. In the magnetic field of the lens coils, the electrons are only deflected (focused) without any change of the kinetic energy. However, they spiral around the optical axis due to the magnetic force on them.

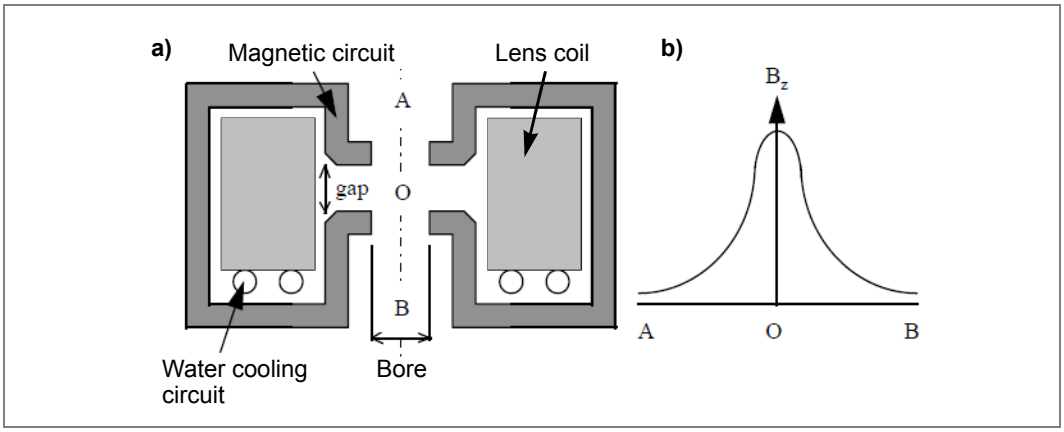
The most basic form of a magnetic lens is a coil, a section of which is shown in *Figure 8*a, together with the direction of motion of electrons. As discussed above, the trajectory of an electron is not a straight line but a spiral about the optical axis, causing an image rotation depending on the lens current. Looking down the direction of motion, the electron trajectory can be expressed as in *Figure 8*b.

Figure 8 Section of Simplest Magnetic Lens and Electron Trajectory



A more realistic lens design is given in *Figure 9*. For the high gradient of the magnetic field, which reduces the focal length and thus lens defects, the magnetic field has to be concentrated in a very small region. This is done by encasing the coil with soft iron and using pole pieces. In this way, the magnetic field is concentrated within the gap as shown in *Figure 9*b, where A, B, and O are three points on the lens axis indicated in *Figure 9*a.

Figure 9 Lens with Pole Pieces (a) and Magnetic Field Distribution (b)



With a given lens geometry, the strength of the field in the pole piece gap is proportional to the excitation of the lens, defined as the product of current I in the windings and number of turns N in the coil:

$$f = \frac{K \times U}{(NxI)^2}$$

where *f* is the focal length, *K* a constant, *U* the accelerating voltage, *N* the number of turns, and *I* the electrical current through the coil. It is easily recognized that at each different accelerating voltage, the lenses of the electron microscope must be adjusted to maintain the same focus. The image rotation ϕ can be expressed as:

$$\phi = \frac{NI}{\sqrt{U}} \times 18.6 \times 10^{-2} \; rad$$

Although electromagnetic lenses and electrons behave quite differently from glass lenses and light, the general principles of light optics can be applied and the electromagnetic lenses can be described for convenience as the lenses of light optics (ray paths, image construction, etc.).

Figure 10 Image Formation and Ray Paths of Lens

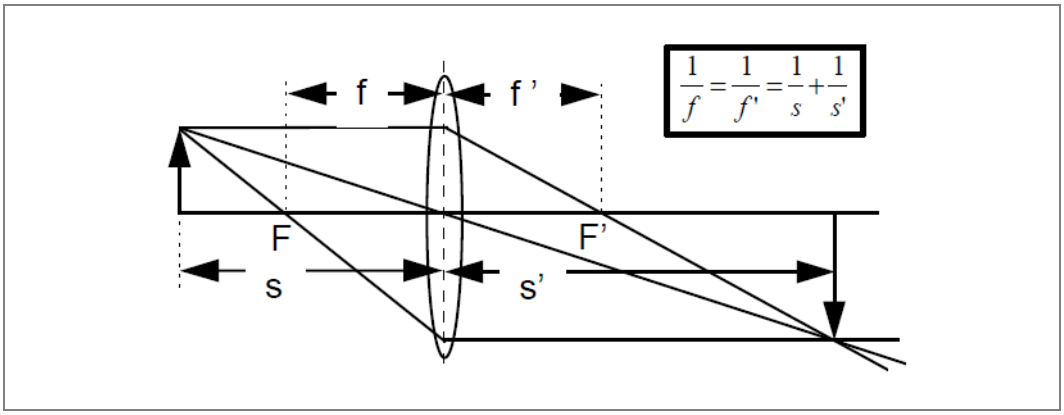
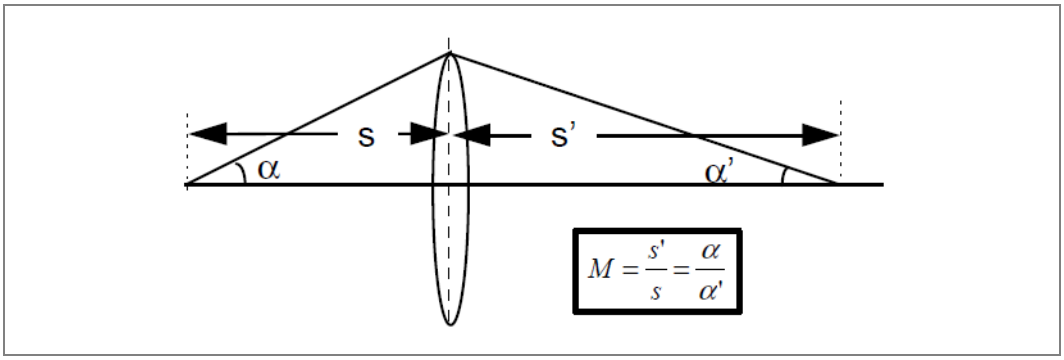


Figure 10 shows the typical ray paths and image formation analogous to a glass lens. *Figure 11* defines the magnification of the lens, M. With these similarities, two optical parameters of the electron microscope lens can be estimated: the depth of focus and the depth of field.

Figure 11 Magnification of a Lens



The depth of field is the axial distance D_{fi} within which specimen details on the axis will be focused without affecting the resolution (D) and changing the image sharpness. $D_{fi}=\rho/\alpha$, with α again the semi-opening angle. Whether the objective lens aperture α_o or the specimen illumination aperture α_i has to be used depends on the specimen. It must be realized that these apertures differ by orders of magnitude: 5 mrad $<\alpha_o <$ 20 mrad and 0.01 mrad $< \alpha_i <$ 1 mrad.

In thin specimens, which scatter only weakly, most electrons are unscattered and enter the lens with the opening angle as determined by the illumination, i.e., $\alpha = \alpha_i$. In thick specimens, the electrons are more strongly scattered. In this case, the objective lens aperture determines the opening angle, i.e., $\alpha = \alpha_o$. In a numerical example with the typical values of α =10 mrad (objective aperture) and ρ = 5 nm, the answer is $D_{fi} < 0.5$ µm. Therefore, both the upper and lower surfaces of a specimen with a thickness of < 0.5 µm will be within the focused range.

The depth of focus, on the other hand, refers to an image distance variation D_{fo}. The depth at the image plane is much larger than the depth of field due to the high magnification: $D_{fo} = \rho M^2/\alpha$. For M = 10,000, α = 10 mrad, and ρ = 5 nm, the answer is D_{fo} = 50 m, which is much more than the height of an electron microscope itself. Because of this large depth, a focused image on the fluorescent screen is also sharp on the photographic plate, which is centimeters below the view screen.

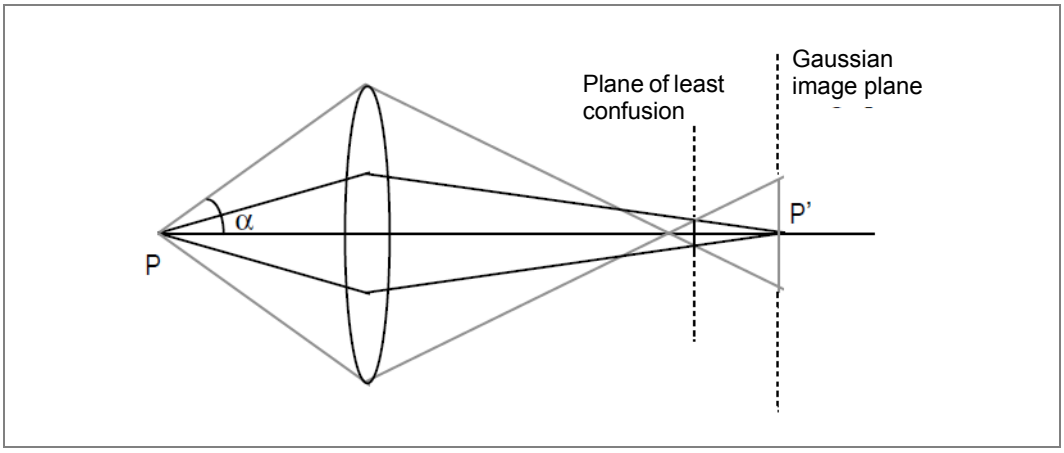
Lens Defects

Spherical Aberration

Spherical aberration is the inability of a lens to focus all incident beams from a point source to a point. Because outer zones of the lens have a greater strength, paraxial rays have a different focal point than rays from the outer part of the lens. The practical focus will appear to be at the plane where the imaging rays occupy the smallest diameter, i.e., at the plane of the circle of least confusion.

A point source P is imaged as a disc, as shown in *Figure 12*. Thus, spherical aberration causes a resolution limit, which is defined as the radius of the circle of confusion in object space, given by $d_s = C_s \alpha$. In *Figure 12*, the image of point P' for paraxial rays lies in the Gaussian image plane, while for beams with a semi angle α, defined by the objective diaphragm, the image point lies somewhere else.

Figure 12 Spherical Aberration



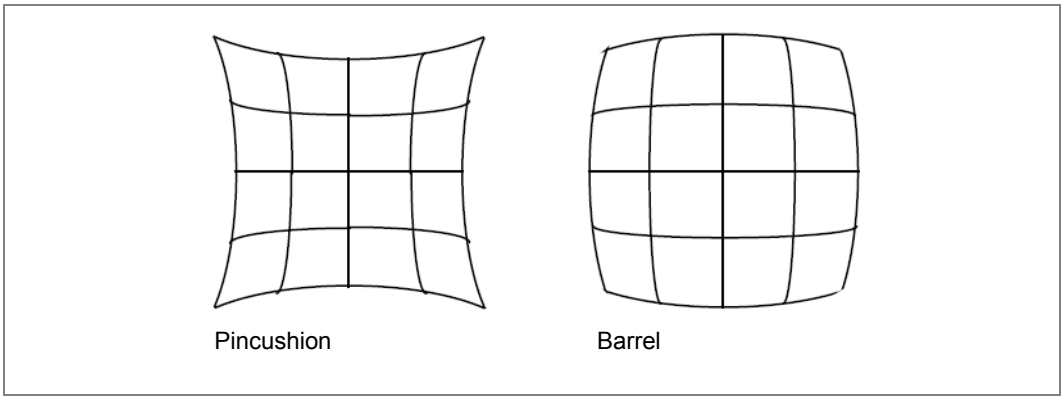
For a chosen angle α, C_s depends on the lens geometry, mainly the bore, the gap, and the angle of the pole piece. Choosing all these parameters properly, the C_s value is proportional to the focal length. Thus, decreasing the focal length or increasing the lens strength decreases the C_s value. The focal length can be influenced by the gradient of the magnetic field between the pole pieces. Reducing the distance between the pole pieces (decreasing the lens gap) and increasing the current shortens the focal length. The limit depends on the saturation magnetization of the pole piece material used. Because of their high saturation magnetization values, Fe-Co alloys with 50% Co are preferred materials for the pole pieces. A further improvement can be achieved by the use of super-conducting lenses.

While in a light-optical system, spherical aberration can be compensated by a combination of diverging and converging lenses or by so called aspherical lenses, this is not possible in electron optical systems for the objective lens. The spherical aberration of the objective lens is one of the resolution-limiting factors, while for the other magnifying lenses the spherical aberration causes image distortion that can be corrected by combining lenses.

Image Distortions

The effect of image distortion becomes visible as pincushion or barrel distortion, especially at low magnification (*Figure 13*).

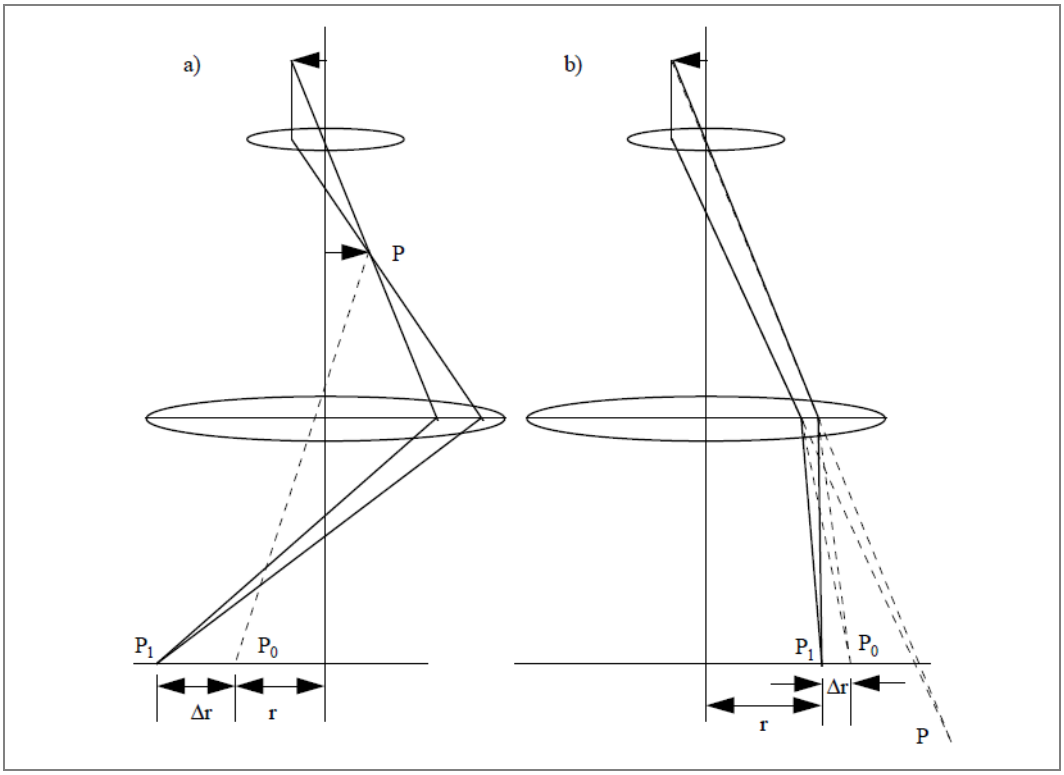
Figure 13 Image Distortions



Spherical aberration is the main reason for the image distortion found in intermediate and projector lenses operating at low magnifications. The objective lens (semi-angle α) magnifies the object by a factor of M. From *Figure 11*, we know the opening angle is demagnified to α' by a factor of 1/M. With an objective lens magnification of 30 and α = 10 mrad, α' will be 0.3 mrad. Considering the radius of the circle of least confusion is proportional to the cube of the opening angle, any further spherical aberration of the magnifying lenses (diffraction, intermediate or projector lenses) is negligible. Even for a modest magnification of 30 times at the first intermediate image, the aperture is so small that the spherical aberration of the intermediate and subsequent lenses can be neglected, even though these lenses normally have larger spherical aberration. It is the objective lens that demands the highest performance.

*Figure 14*a shows that a point P in the intermediate image is imaged into P_o without spherical aberration but into P_1 if spherical aberration is present. The deviation Δr increases with r^3, resulting in pincushion distortion of a square specimen area. The opposite situation occurs when the intermediate image lies beyond the second lens, resulting in barrel distortion (*Figure 14*b). It is possible to compensate this kind of distortion by a combination of lenses in such a way that pincushion distortion is compensated by barrel distortion at another intermediate lens.

Figure 14 Distortions Caused by Spherical Aberration of Projector Lens



Diffraction Error

A diffraction error is not really a lens defect, but a resolution-limiting factor due to diffraction phenomena at the diaphragms. As before, the resolution is limited by α and the wavelength with a relation of $\rho \sim \lambda/\alpha$.

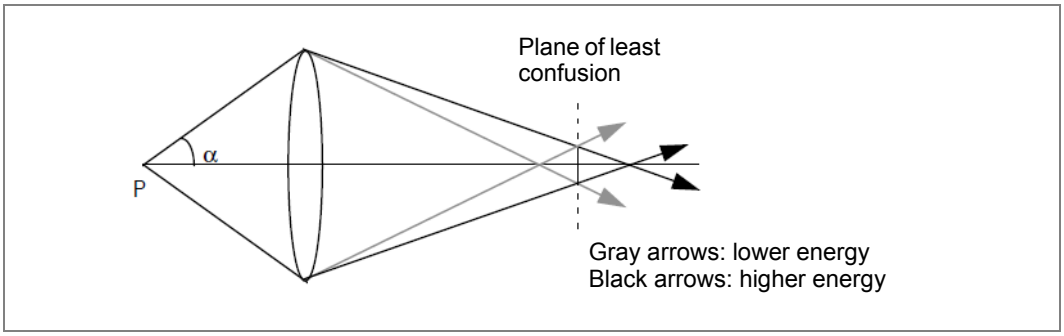
Chromatic Aberration

Only mono-energetic electrons are focused into one point if the lens has a fixed current. If the electrons have an energy spread ΔE, caused by fluctuations of the high tension, by the energy spread of the emitted electrons, or by energy losses in the specimen, and if the lens currents have a variation ΔI, then a point is imaged as a chromatic aberration disc (*Figure 15*). The radius of the circle of confusion in object space due to chromatic aberration, which is a resolution limiting factor, is given by:

$$d_c = C_c \alpha \left(\frac{\Delta E}{E} + \frac{2\Delta I}{I}\right)$$

with Cc the chromatic aberration constant.

Figure 15 Chromatic Aberration

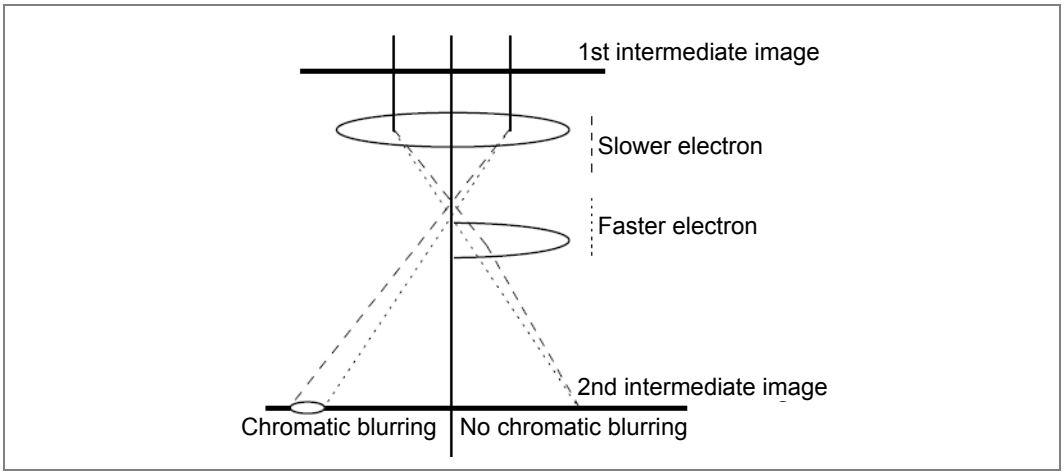


The chromatic aberration results when waves of different energies converge at different focal planes. In an electromagnetic lens, electrons with shorter wavelength, and thus more energetic, have a longer focal point than do electrons with a longer wavelength.

Because of the noncorrectable chromatic aberration of the objective lens, it is important to minimize the energy spread of the emitter (W: 3-4 eV, LaB_6: 1.5-3 eV, Schottky field emitter: 0.6-1.2 eV) and the fluctuations of the lens current and of high tension.

For the intermediate and projector lenses, the chromatic magnification error can be corrected by the correct combination of lenses, as shown in *Figure 16*. This correction is important primarily for relatively low magnification (20....40,000 X) where the chromatic aberration of the objective lens plays no important role.

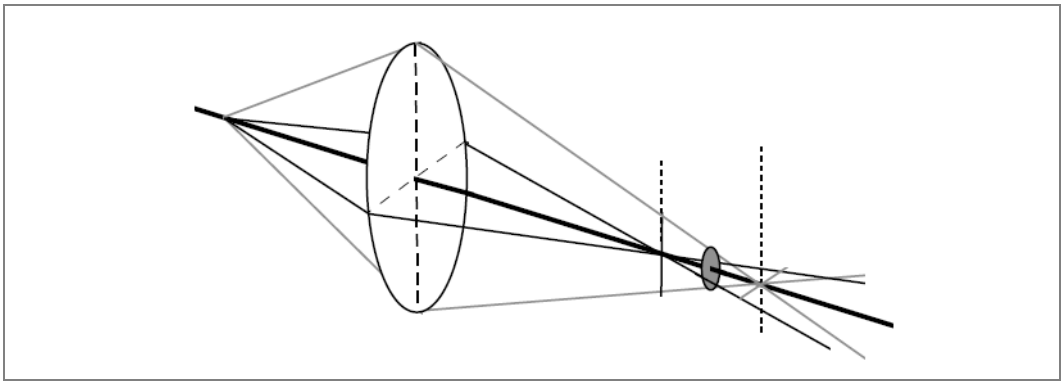
Figure 16 Correction of Chromatic Aberration by Magnifying System



Astigmatism

As shown in *Figure 17*, astigmatism is a lens defect caused by magnetic field asymmetry, resulting in different lens strength in perpendicular planes. Thus, a point is imaged at two focal lines, and the smallest circular image of the point is halfway between the lines. The amount of astigmatism of a lens is defined as the distance between two focal lines.

Figure 17 Astigmatism



Astigmatism is caused by the imperfections in the objective lens: imperfection of the circularity in the bores, flatness of the pole faces, and asymmetry in the magnetic material are the main reasons. However, the specimen itself and contamination can also change the symmetry of the magnetic field and induce astigmatism. Fortunately, astigmatism can be corrected with stigmators, which compensate the asymmetry and will be described in detail later.

Summary

Most of the lens defects mentioned above cannot be changed by operators. The spherical aberration is fixed by design of the lens. The image distortion is minimized by lens combination, but at very low magnification; because of the electrons coming from the rim of a lens, sometimes the distortion occurs. The chromatic aberration from the imaging lens system is also minimized by lens combination, but the chromatic aberration from the electron source and the objective lens still exists, unless an energy filter is used. What an operator can correct is astigmatism, which will be a dominant factor at high magnifications.

Electron Optical Elements

The transmission electron microscope is made of a number of basic systems that are integrated to allow orienting and imaging of thin specimens: the illuminating system, the specimen manipulation system, and the imaging system. This section addresses the elements of these systems, i.e., the electron gun, the lens, the deflection coil, and the stigmator.

The Electron Gun

For generating electrons that are accelerated by the high tension, two common types of electron guns are used:

- Thermionic gun, see below
- Field emission gun (FEG), see *page 27*

These types differ by the mechanism by which the electrons are released.

Thermionic Gun

Within a conductor, there are many free electrons which are loosely bound to nuclei. By heating the material, the free electrons may gain energy and be speeded-up. Some electrons can overcome an energy barrier (work-function) of the material and escape into the vacuum.

The thermionic electron gun is based on this principle (see *Figure 18*). The heating of the material (filament) is achieved by running current through it. The most often used filament (emitter) materials are tungsten (W) and lanthanum hexaboride (LaB_6), due to their high melting points and low work functions.

The filament is at the negative high tension HT (-) of the microscope and is often called the *cathode*. A plate, called the *anode* at the positive HT (+) with respect to the cathode, accelerates and attracts the electrons from the hot surface of the filament. Only a small fraction of those electrons passes through a small aperture in the anode and reaches the condenser system further down the microscope column. Between the cathode and the anode is a Wehnelt cylinder, which has a potential more negative (--) than the cathode itself. Its function is to use the variable bias voltage (varied by changing the emission parameter in the Filament control panel) for controlling the emission from the filament. The bias voltage is not completely fixed, in practice, but is linked to the *emission current* (the total current emitted by the electron gun), which in turn acts as a feedback to stabilize emission and high tension.

Therefore, at the higher bias voltage (lower emission parameter), the emission comes from a smaller area with smaller total emission current and higher gun stability (both temporally and spatially). This type of gun is called a *triode gun* because of its similarity to a triode (*Figure 18*).

Figure 18 Schematic, Triode Gun Used with Thermionic Filament

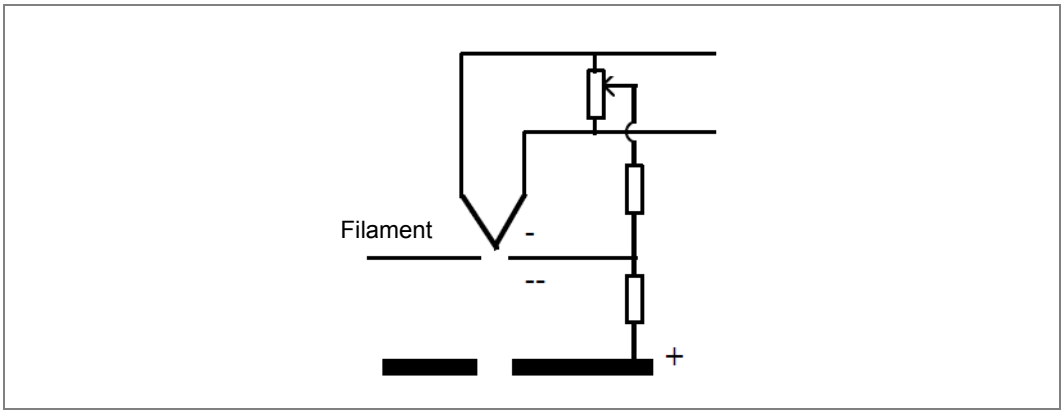
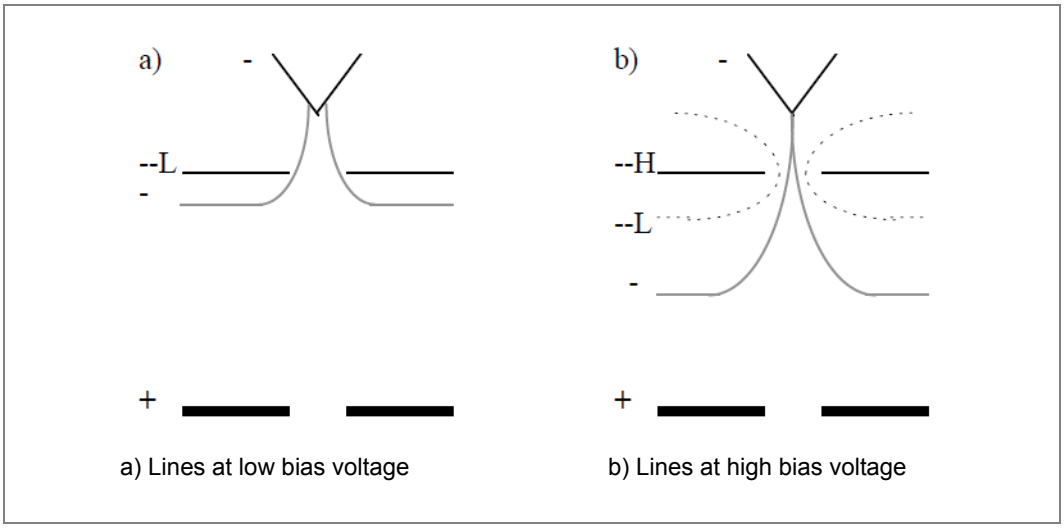


Figure 19 schematically shows a few representative iso-potential lines within a triode gun at high and low bias voltages, respectively. At low bias voltage (--L), i.e., high emission parameter, the iso-potential line of (-) is relatively closer to the Wehnelt, resulting in the emitted electrons coming from a relatively bigger area, and so the high emission current. On the contrary, the high bias voltage (--H), i.e., low emission parameter, restricts the emission to a smaller area and so the low emission current.Coherence of the beam is greater for small source size and for smaller spread in the energy of the electrons. Coherence is improved, therefore, at higher bias voltage.

Figure 19 Iso-potential Lines in Triode Gun



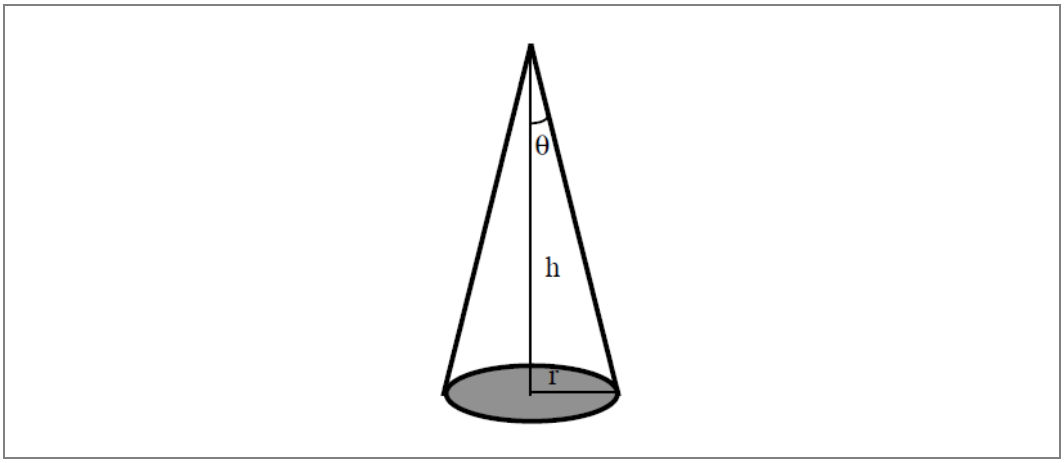
The emitted electrons that pass through the Wehnelt aperture are focused into a crossover between the Wehnelt and anode. This crossover acts as the electron source for the optics of the microscope. The size of the crossover is determined by the type of filament as well as by the bias.

Brightness

The (reduced) brightness refers to the electron current by a source with a unit area and a unit solid angle, and at a unit accelerating voltage for direct comparison of guns at different voltages. It is, perhaps, one of the most important characteristics a user encounters first.

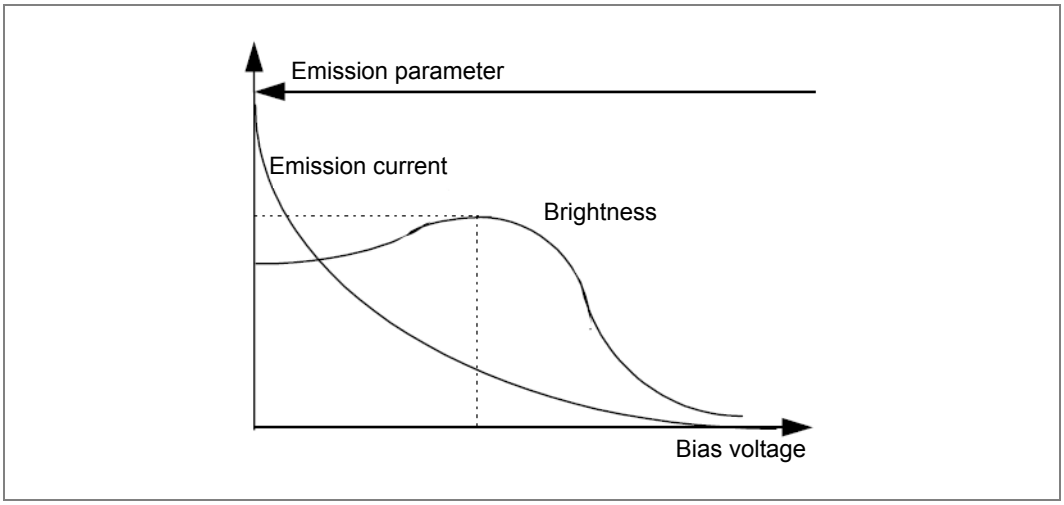
Solid angle (Ω) is defined as an area (D) on a spherical surface divided by the square of the radius (R): $\Omega = D/R^2$. Therefore, the maximum solid angle (over a whole sphere) is $4\pi R^2/R^2 = 4\pi$. A cone, with a half opening angle of θ (in radians) and a height (h) much greater than the radius of base (r), corresponds to a solid angle of $\pi r^2/h^2 \cong \pi(\theta h)^2/h^2 = \pi\theta^2$ (*Figure 20*).

Figure 20 Illuminating Cone



For a source with an emission current i_e, an area of D, and a semi-divergent angle of θ, the brightness will therefore be $i_e/D\pi\theta^2$. To increase brightness, you may increase the emission current (higher accelerating voltage, lower bias voltage), or reduce the source size and the divergent angle. However, at low bias voltage (*Figure 19*a), since electrons come from a large area of the curved filament tip, both the source size and the divergent angle will be big. Thus, a higher emission parameter does not necessarily lead to improved brightness (*Figure 21*).

Figure 21 Brightness and Emission Current vs. Bias Setting



Emission Current or Total Current

This is the total current emitted by the electron gun, which is largely intercepted by cathode and condenser aperture before reaching the specimen. Emission current is determined by the bias as shown in *Figure 21*.

Energy Spread of Primary Beam

Not all electrons leave the emitter at the same speed or with the same energy. This is not only because of the imperfection of the gun, for example, or the instability in the accelerating voltage. This statistical energy spread is also due to the surface temperature of the emitter and mutual Coulomb interaction among electrons in the beam (*Boersch effect*). The Boersch effect is more serious at higher emission current because of the greater number of electrons. So, a high emission parameter will increase the energy spread. The energy spread determines the temporal coherence that, in turn, affects the ultimate resolution of a microscope. In other words, the smaller the energy spread, the better the resolution. This is in addition to the effect of chromatic aberration of the lenses.

Virtual Spot Size or Source Spot Size

The virtual or source spot size refers to the geometrical size of the crossover between the Wehnelt and anode, previously mentioned. The typical size is 30 µm for W and 5 µm for LaB6 emitter. This size can be further demagnified by the illumination system, making the probe size even smaller on the specimen.

High Tension

This is the acceleration voltage applied to the electrons coming from the emitter.

Probe Current/Specimen Current

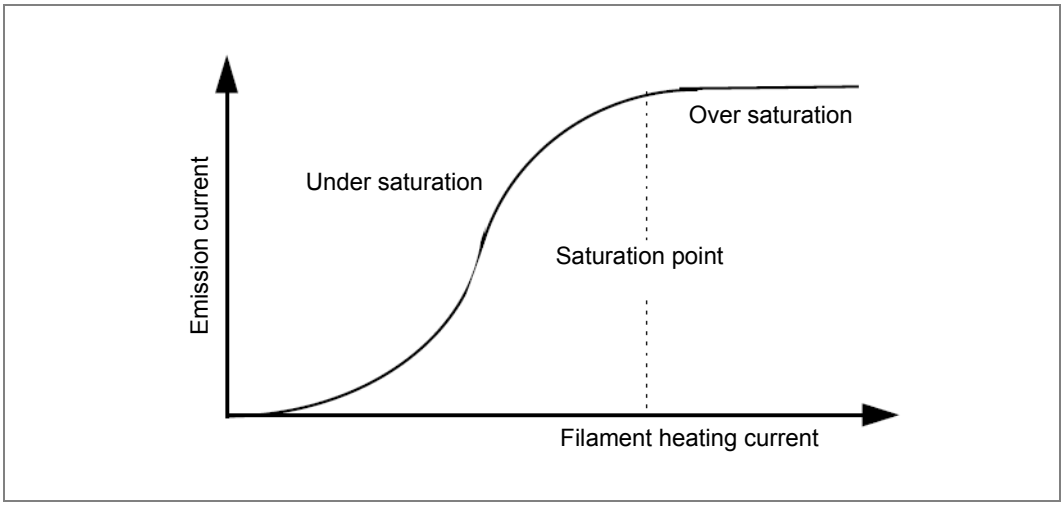
This is the actual current reaching the specimen.

Saturation Point

The saturation point is the temperature (filament heating current) at which further increase of the filament current, for a given gun emission parameter (bias setting) and high tension, will not result in an increase of emission current (*Figure 22*). The saturation point is the working point of a filament.

NOTE	Increasing the heat of the filament beyond the saturation point will not increase the brightness of the gun, but will considerably shorten the filament life. On the other hand, undersaturation of the filament may lead to instabilities and heterogeneities in the illumination. If a filament is undersaturated, the beam spot is not uniform.

Figure 22 Emission Current vs. Filament Heating Current



Emitter Lifetime

This may be the question of most concern. The most severe factor is the sublimation of the emitter. Avoiding unnecessarily high heating temperature, e.g., by oversaturating the filament, can greatly improve the lifetime. Chemical etching of the filament is also a factor. This etching can be largely reduced by operating at as good vacuum as possible. In addition, reducing and/or stabilizing emission current can make limited improvement as well.

Especially for LaB_6, slow increases, and to a lesser extent, decreases in temperature avoid thermal stresses, which can abruptly destroy a filament.

Field Emission Gun (FEG)

In a field emission gun (FEG), electrons are "sucked out" by a strong external electric field. The field emission cathode is usually a wire of single-crystal tungsten fashioned into a very sharp point. The significance of the small tip radius, about 1 µm (Schottky) or less, is that the electric field is concentrated to an extreme level. If the tip is held at a negative 3-5 kV relative to the extraction anode, the applied electrical field at the tip is so strong (> 10^7 V/cm) that the potential barrier for electrons becomes narrow in width as well as reduced in height by the Schottky effect. The narrow barrier allows electrons to "tunnel" directly through the barrier and leave the cathode. The Schottky tip is coated with a layer of ZrO_2 to reduce the work-function of tungsten. Unlike the thermionic gun, the FEG does not produce a small crossover directly below the emitter. Instead, the electron trajectories seemingly originate inside the tip from a very small virtual source.

The major advantages of a field emission gun over a thermionic gun include its relatively high brightness, small virtual source size, and low energy spread. Some electron gun parameters are listed in *Table 4* for comparison.

Two different types of FEG emitters can be found on the Thermo Scientific™ Talos™ platform: S-FEG and X-FEG.

- The Schottky FEG emitter (S-FEG) has been already used on Talos platform for more than 20 years and is characterized by its high total current, stability and a long lifetime. The X-FEG module combines these benefits with a considerably increased brightness. Its brightness equals that of C-FEG emission (cold field emission gun), but still provides a symmetric energy distribution for EELS applications, and a significantly higher total current, which ensures good illumination intensities at midrange magnifications.
- The X-FEG makes use of optimized Schottky field emission technology, known for its robustness and reliability. This makes it an easy to use module with a long life time, which is designed for a higher throughput. The illumination current is ultra-stable over time and the emission tip does not require flashing or resetting. These are key benefits for experiments that are very sensitive to any emission fluctuations, such as tomography, focus series reconstruction, EFTEM or chemical mapping applications.

Table 4 Comparison of Three Major Filament Types

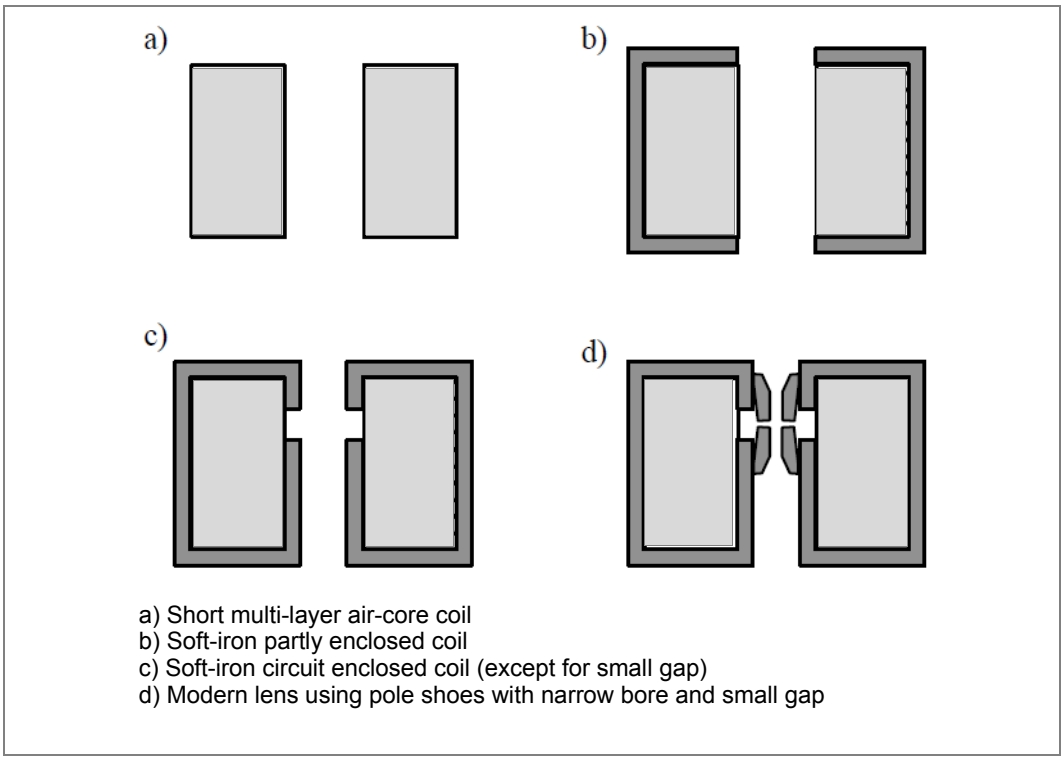
Characteristic	W	LaB_6	Schottky FEG	X-FEG
Maximum current (nA)	1000	500	300	50
Normalized Brightness (-)	1	10-30	2500	25000
Energy spread (eV)	3-4	1.5-3	0.6-1.2	0.8-1.1
Required Vacuum (Pa)	10^{-3}	10^{-5}	10^{-7}	10^{-7}
Temperature (K)	2700	2000	1800	1800
Lifetime (hr)	60-200	1000	>2000	>2000
Normalized price (-)	1	10	100	>100

Lens

The action of a lens magnetic field on electrons may be compared to the way in which a tornado lifts up an object. The “tornado” is generated by the current through the coils, and the electrons passing through it are caught in the “tornado” field and spiralled towards the axis. The lens current direction is the reverse of the “tornado” spinning direction, due to the negatively charged electrons. The “tornado” field or magnetic field can be concentrated by an iron “magnetic circuit.” The magnetic field is captured within the iron, thus producing a stronger and more localized magnetic field at the optical axis. The parts close to the beam are called *pole-pieces*.

Figure 23 shows the evolution of the electromagnetic lens design. The corresponding magnetic field (Z component) along the optical axis is higher and sharper with this development.

Figure 23 Lens Design Evolution

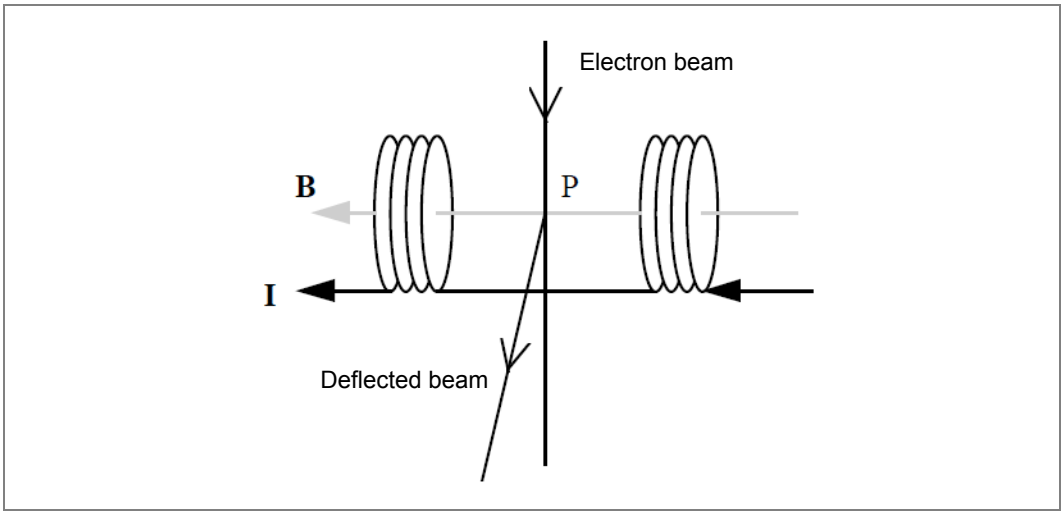


One of the most important features of an electromagnetic lens is the fact that its focal length can be changed simply by changing the lens current. This enables you to adjust the lens system, i.e., focusing and magnification, without any mechanical change.

Deflection Coil

Deflection coils deflect the electron beam. These coils are used for microscope alignment and some special applications. *Figure 24* depicts a simple form of a pair of deflection coils.

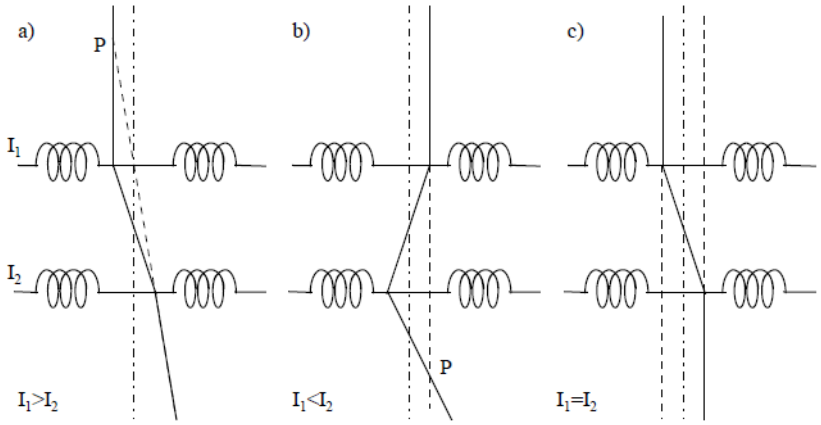
Figure 24 Basic Principle of Deflection Coils



In principle, a more or less uniform magnetic field B, perpendicular to the incident electron beam and generated by the current through the coils, acts on the electrons and deflects them. The combination of two such coil pairs perpendicular to each other can deflect a beam in any direction. The point where the direction of the beam is changed is called a *pivot point*, and the beam "pivots" or "tilts" at the point.

For a single pair coils like that in *Figure 24*, the pivot point is at the height of coils itself (marked P). However, in some cases it is desirable to pivot or tilt the electron beam at a point that is not accessible for deflection coils. The combination of two pairs of coils can achieve this (*Figure 25*).

Figure 25 Combination of Two Pairs of Coils



The combination of two pairs of deflection coils can situate the pivot point either:
a) above the coils (e.g., guntilt)
b) below the coils (e.g., STEM)
c) in infinity (e.g., gunshift).

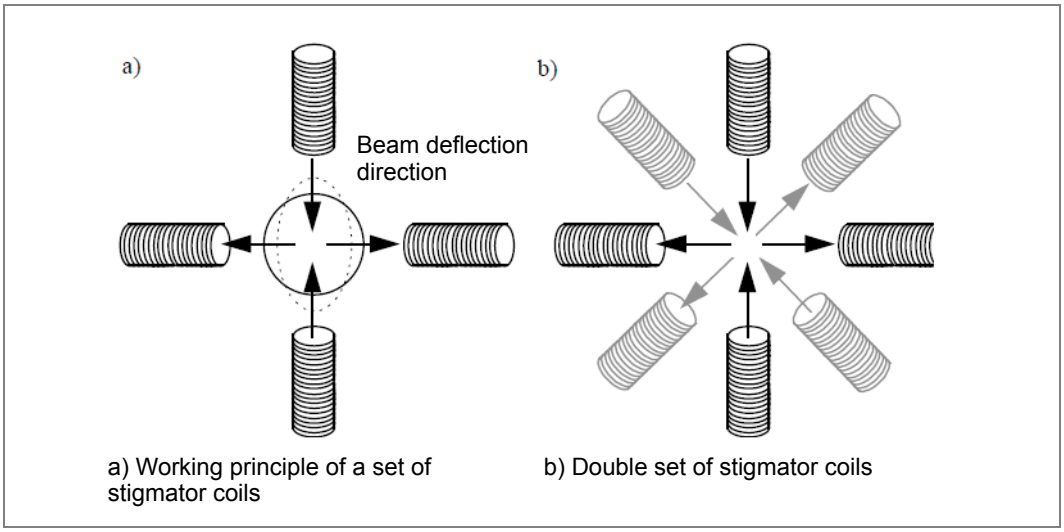
These sets of coils are used, for example, in aligning the objective lens, in dark-field imaging, and in the Scanning mode. The image coils are underneath the objective lens, which shifts the image and the diffraction pattern. They are employed in image shift, diffraction alignment, and detector alignment. Besides these three double-coil sets, there is one simple one-direction coil, i.e., the shutter coil above the projection chamber, which deflects the beam away/back for exposure of the negative films.

Stigmator

Astigmatism is one of the imperfections of the electromagnetic lens that reduces the resolution. It results when a lens field is not symmetrical in strength, but is stronger in one plane and weaker in another so that only part of the image (direction or orientation) will be in focus at one time. A point can appear to be a line or worse for very severe astigmatism, where the Thon rings can appear to be hyperbolas.

Consequently, a point will appear elliptical. To correct the lens astigmatism, stigmators act on the electron beam by pressing it from opposite sides and pulling it from the both sides in the perpendicular direction. Therefore, stigmators can modify an ellipse into a circle as shown in *Figure 26*a.

Figure 26 Stigmator Coils



When the current through the coils changes its direction, the beam deflection direction also changes. Thus, the pulling force becomes the pressing force and so on, and an ellipse perpendicular to that shown in *Figure 26*a can be modified to a circle as well. Unlike the deflection coils, the opposite coils in a stigmator have a reversed current; furthermore, a single set consists of four coils and the second set has 45° rotation difference (*Figure 26*b). A double set stigmator can correct astigmatism in all directions.

Residual 3-fold and higher order astigmatism cannot be corrected by a double set of coils, but this is usually so small as not to limit the resolution of an image.

The Talos microscope contains: (1) a condenser stigmator to correct for beam astigmatism, (2) a objective stigmator to correct for astigmatism in high magnification image and low magnification diffraction, and (3) a diffraction stigmator to correct for astigmatism in high magnification diffraction and low magnification image. These stigmators are closely positioned to the corresponding lenses.

All these stigmators must be properly aligned to avoid the side effects of beam shift. In the Talos microscope, shifts due to imperfect positioning are compensated by identical but opposite shifts. The condenser stigmator is aligned using the beam deflection coils, whereas the objective and diffraction stigmators are aligned using the image deflection coils. Dialog box controlled procedures can be followed to correctly align these stigmators.

Electron Beam Principles of Interactions and Chemical Information

Interactions and Interaction Volume

When an electron enters the specimen it may "meet" the atoms in the specimen. If it travels close to an atom, the presence of the atomic and nuclear electrostatic potential will affect the speed and/or direction of the travelling electron, the electron is scattered. If only the direction has been changed, the scattering is called elastic. For inelastic scattering, a change of speed is involved. In other words, the incident electron does not pass its energy to the atom during an elastic scattering process, while in the inelastic scattering process, there is an energy exchange between the incident electron and the atoms in the specimen.

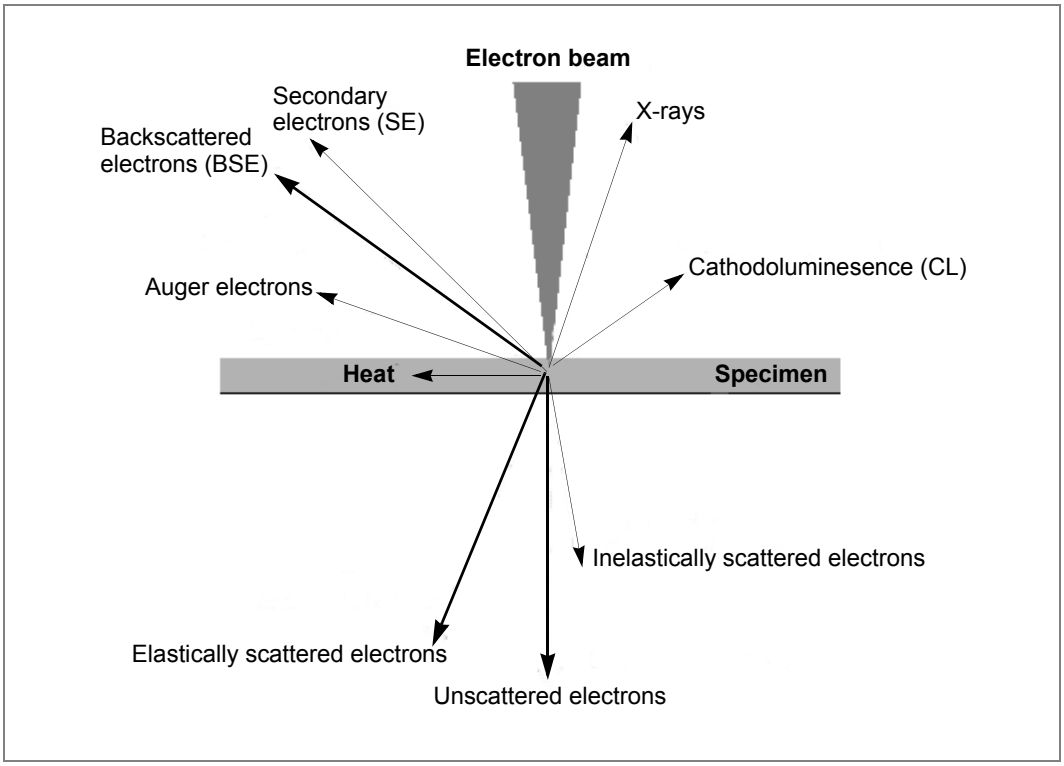
Beneath the specimen, there are three kinds of transmitted electron:

- Unscattered electrons that pass through the specimen without being involved in any interaction
- Elastically scattered electrons
- Inelastically scattered electrons

All of these contribute to the formation of a normal TEM image. The backscattered electrons (BSE) are defined as those electrons undergoing a number of wide-angle scattering events. The cumulative change in directions may be sufficient to cause a scattering angle greater than 90°. Conventionally, it is also supposed that the energy either reserves or reduces very little, and hence this is considered to be an elastic process.

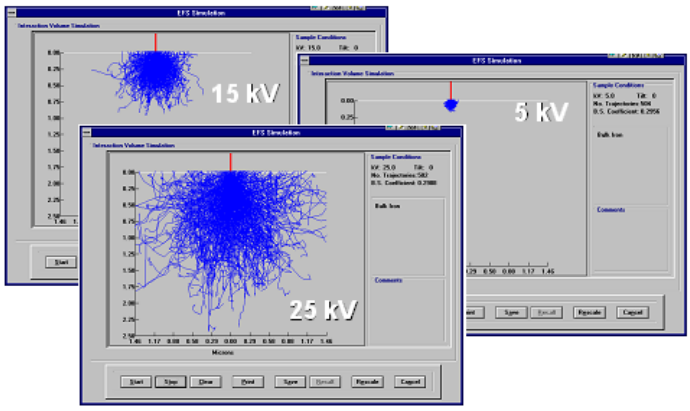
As mentioned above, the inelastically scattered electrons transfer their energy to the specimen and thus have less energy than originally. Various types of inelastic scattering may happen. The energy passed to the specimen is then released in different ways and, if detectable, different signals may be collected. *Figure 27* outlines some possible forms for energy release.

Figure 27 Electron Beam Specimen Interaction



One result of scattering is beam broadening. The beam size at the bottom surface of a sample is larger than the size at the entrance surface. The size difference depends on the scattering within the sample and the specimen thickness. For a thick sample, an interaction volume is produced by beam broadening, as shown in *Figure 28*.

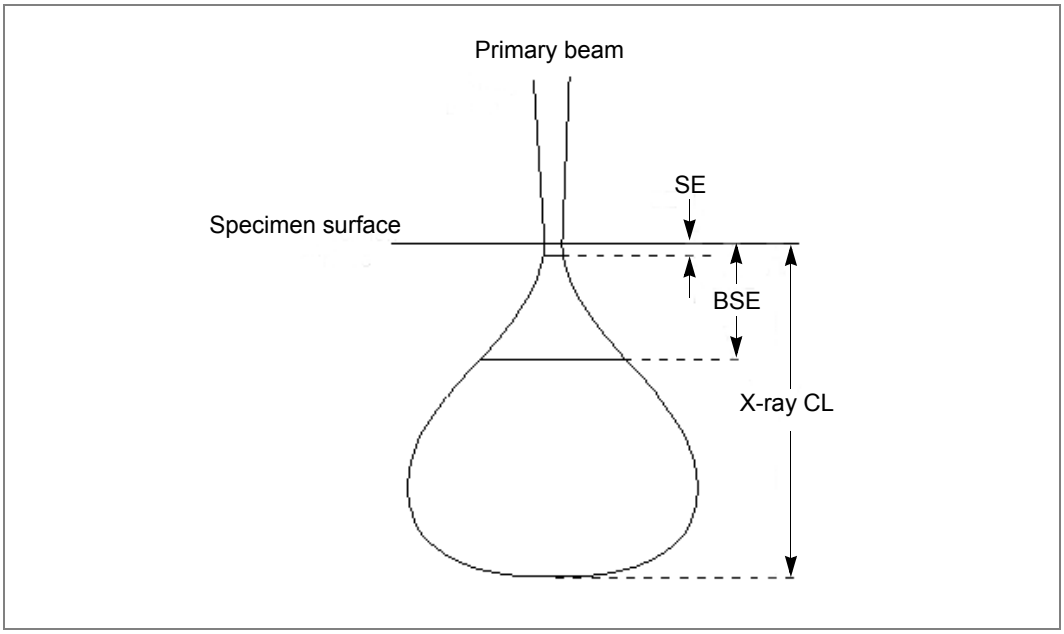
Figure 28 Influence of Scattering Voltage on Electron-Specimen Interaction Volumes



It is from Monte-Carlo simulations, which take account of both elastic and inelastic multiple scattering and model the trajectory of a single electron based on the probability of scattering angles and energy transfer.

A simplified interaction volume in a thick sample is shown in *Figure 29*. It is clear from it that a direct effect of beam broadening is the degrading of the spatial resolution in analytical mode, i.e., the small probe applications such as STEM and EDS. The resolution degrading for some signals is shown schematically.

Figure 29 Interaction Volume & Its Influence on Spatial Resolution



However, for electron transparent specimen, the thickness is so small that the beam is only slightly broadened before it comes out of the sample. The same argument holds for the applications using the transmitted electrons, such as EELS, CBED and STEM. Because of this, beam broadening, a serious problem for spatial resolution in SEM is not very crucial in TEM as long as the specimen is thin.

Elastic Scattering of Electrons

The elastic scattering of the incident electrons by the atomic potential is a Coulomb interaction, also known as Rutherford scattering. The force acting on the electrons is proportional to the charge of the nuclei, or the atomic number Z. It is important to realize that a scattering process is a statistical event, i.e., during scattering an electron has certain probability to be scattered to a particular angle. The probability that a particular scattering happens is called *cross section*, denoted by σ. The cross section for Rutherford scattering by a single atom is approximately.

$$\sigma \propto \frac{Z^2}{U^2}\frac{1}{\sin^4(\theta)}$$

with U the accelerating voltage of the incident electrons and 2θ the scattering angle measured relative to the incident direction ($0 \le 2\theta \le \pi$). The general behavior of the cross section, or the Rutherford scattering power, is that it increases with the square of the atomic number. At the same time, it decreases with the incident electron energy and the scattering angle. Considering the specimen has a finite thickness and the possibility of plural scattering, the scattering power also increases with the specimen thickness.

If the specimen is crystalline, the scattering happens differently. The phenomenon is called Bragg diffraction, and it is based on Rutherford scattering by regularly arranged individual atoms. To understand this, it is easier to use the wave nature of the electron beam. The waves scattered by the individual atoms are coherent, which means that when those waves meet, the effect may be constructive or destructive, leading to excessive or deficient intensity. In a diffraction pattern, there are sharp spots at certain scattering angles (constructive) and very low intensity at the others (destructive).

The backscattered electrons are generally considered to be caused by cumulative elastic scattering. According to equation above, it is easy to see the image intensity is Z-dependent, though the total signal is quite small due to the angular distribution at very high scattering angle. The very restricted space within a TEM column determines that it is not easy to mount a BSE detector in a TEM. In addition, the very thin specimen used in TEM results in such a low BSE signal that the images are generally noisy and of poor quality. Because of these facts, together with the resolution improvement in SEM, it is not common now to have a BSE detector mounted in the TEM, although it is possible.

From the forward Rutherford scattered electrons, a Z-contrast image is also possible, but the much stronger transmitted beam and coherent Bragg diffraction must be excluded. This is achieved by using an annular detector excluding the transmitted beam and all the coherent scattered electrons. This is the working principle of the high angle annular dark-field (HAADF) detector in STEM.

It should be noted that during the scattering processes the nucleus is hardly affected as a result of its heavier mass compared to an electron. The mass of a hydrogen nucleus is 1830 times of that of an electron.

Inelastic Scattering of Electrons

An electron entering the material can also interact with the electron cloud around each nucleus, and transfer part of its energy to the electrons of the material. As one of the results, electrons in the atomic shells will gain sufficient energy to escape from the bonding of the nucleus. Thus, the atom is being ionized. Though mostly the outer shell electrons are involved, because of the relatively low energy required, the inner shell electrons can also be knocked out if the energy is high enough. If this ionization occurs near the surface of the specimen and the electrons have acquired sufficient energy to overcome the work-function of the material, they may escape from it. The escaped electrons are called *secondary electrons* and are one of the primary signal sources in SEM. For the same reasons as for backscattered electrons, SE detectors in TEM are also not very common.

When ionization happens, the atom is left with an electron vacancy in one of its shells. The atom is said to be excited and has excess energy. One of the ways for the atom to get rid of the excess energy is to transfer it to an electron in another shell, thus generate electrons with characteristic energies for that element. These electrons, called *Auger electrons*, having energies up to 1 or 2 keV, may escape from the material within a depth of only around 1 nm and may be detected by a suitable detector. Although not the primary sources in electron microscopy, they are employed frequently in surface studies involving other techniques.

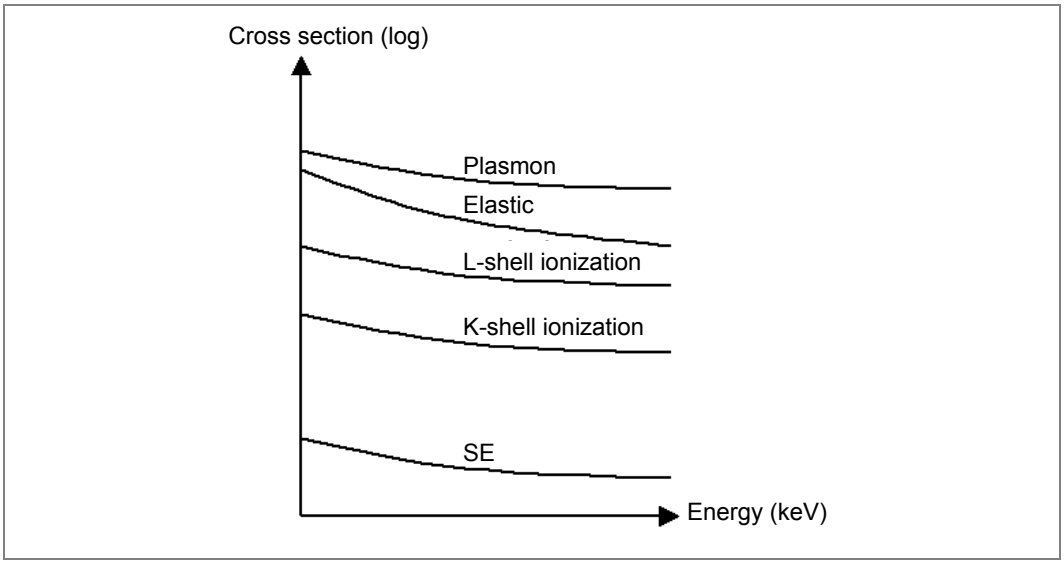
Another way for an ionized atom to fill its electron vacancy is to catch one of the electrons from an outer shell. This electron with higher energy jumps into an inner shell and the difference in energy is released as emission of an X-ray quantum. Since the electron energy levels of the atomic shells are distinctive and since the allowed jumps from one shell to another are subjected to quantum mechanical rules, the energy of the emitted X-ray quantum is characteristic for the element. These X-rays are one of important signals in analytical electron microscopy and an obvious advantage over its optical counterpart. The technique to detect the emitted X-ray is *X-ray energy dispersive spectroscopy* (EDS).

The energy transferred to the sample by the electron beam may also be released as light (photon) emission. The light is generated by electronic transitions at low energy difference, e.g., by transitions from the conduction band to the valence band or by de-excitation of atoms and molecules. In this light-emitting process, which is called *cathodoluminescence*, the outer-shell electrons are involved. Whether or not a material will emit light under electron irradiation depends on its structure and transparency for the involved wavelength. Materials that typically show a strong luminescence are minerals and semiconductors where the luminescence intensity strongly depends on the dopants. In practice, due to its poor spatial resolution and the size of the cathodoluminescence collector, which is actually a concave mirror, it is rarely used in TEMs, but in SEMs it is.

Plasmons refer to the collective oscillation of weakly bound outer-shell electrons, commonly the conduction electrons existing in a metal. The energy causing the oscillation is transferred from the incident electrons when their Coulomb fields disturb the stability of this electron gas. In electron microscopy, plasmons are not measured directly but indirectly through the transmitted electrons that excite plasmons and lose the corresponding energy.

The incident electrons may also pass energy to the nuclei in the sample, causing them to vibrate about their equilibrium locations. The more energy the atoms gain, the more violent they vibrate and the higher the specimen temperature is. This is called thermal vibration or *phonons*. The phonons are present in all materials and are most significant in crystals. They carry no useful information and are not measured in electron microscopy. However, transmitted electrons, that lose energy due to the excitation of phonons might contribute to the diffuse background in electron diffraction patterns and the contrast mismatch of simulated high-resolution images to the experimental ones. On the other hand, the energy loss due to phonon excitation is very small (< 0.1 eV), even less than the typical energy spread of an electron gun (0.6 – 2.0 eV). Basically, they are indistinguishable from the purely elastically scattered electrons. Therefore, the transmitted electrons with phonon energy-loss are often considered part of the elastically scattered electrons. *Figure 30* schematically shows the probability (cross section) of some inelastic processes against incident electron energy using aluminium as an example. For comparison, the elastic scattering cross section has also been plotted. The figure gives an overview of the possibility that one process happens. For example, among all processes, plasmon scattering is the most probable one.

Figure 30 Comparison of Scattering Cross Sections



Transmitted Electrons

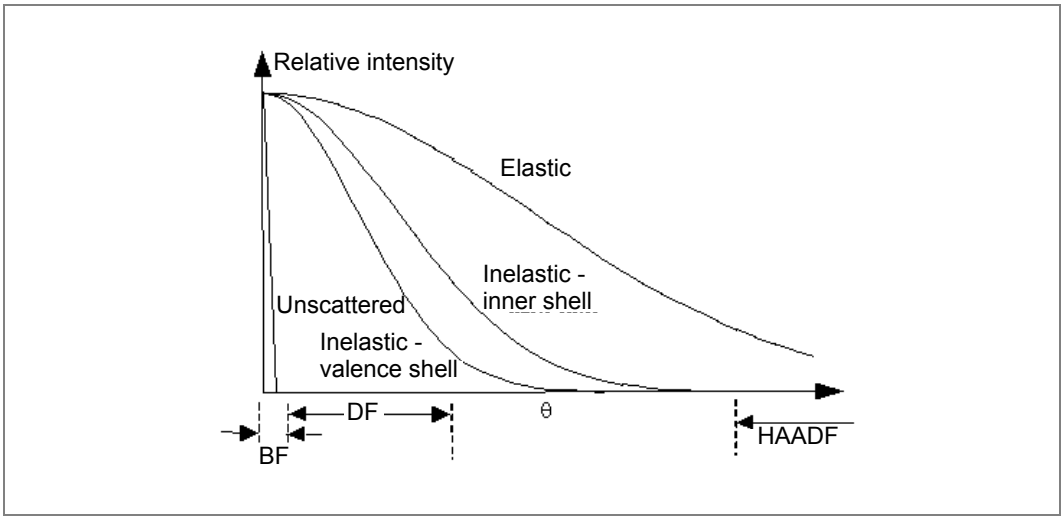
According to their directions, there are two kinds of transmitted electrons: the unscattered and the scattered. The incoming primary electrons will traverse a specimen and leave it either without (unscattered) or with change of direction (scattered).

In normal TEM operation, those unscattered or scattered electrons form the bright field or dark field images, respectively, with the help of an objective aperture. In STEM mode the selection of unscattered and scattered electrons is achieved by locating BF/DF detectors at different positions. The signals detected are then displayed on the computer monitor as BF/DF STEM images.

With a high angle annular dark-field (HAADF) STEM detector the highly scattered electron are collected and form a Z-contrast STEM image. Furthermore, the scattered electrons contain both elastically scattered and inelastically scattered electrons. The latter are scattered through relatively small angles compared to the elastically scattered ones, and the scattering angles tends to be larger for electrons that have lost more energy.

The angular distributions of transmitted electrons are plotted schematically in *Figure 31*, where the angular ranges for bright-field, dark-field and high-angle-annular dark-field are also roughly outlined. The plot is based on amorphous materials. For crystalline materials, the angular distribution is also peaked at Bragg angles. This distribution is what is seen in a diffraction pattern, except it is a sum of all the curves in the figure since they are indistinguishable.

Figure 31 Relative Angular Distribution of Transmitted Electrons

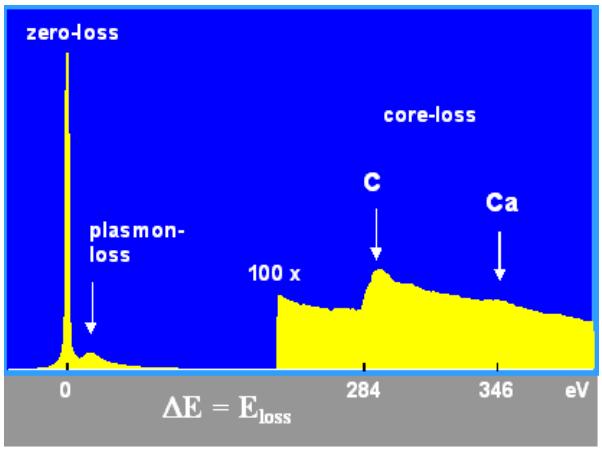


It is clear from *Figure 31* that without any discrimination, the bright-field signal consists of the unscattered and elastically/inelastically scattered electrons with small scattering angles. It is also true for a normal TEM image (angular range BF + DF). The objective aperture acts a geometric selector, allowing electrons in a certain angular range to contribute to the image and rejecting others. So do the STEM detectors. A similar effect can be achieved with an energy selector, called energy filter. One can form an image by only using unscattered electrons, elastically scattered electrons or electrons with a particular energy loss.

From an energy point of view, the transmitted electrons have energies from the incident energy down to zero, depending on the specimen thickness. The thicker the specimen is, the more interactions happen, the more energy the incident electron loses and the smaller the average energy is. The energy distribution of the transmitted electrons can be collected with an energy-loss spectrometer, which disperses electrons according to their energy.

Figure 32 shows a typical energy distribution of electrons transmitted from a thin specimen, an electron energy-loss spectrum.

Figure 32 Typical Energy Distribution of Transmitted Electrons Obtained by Electron Energy-Loss Spectrometer



It shows the number of electrons detected as a function of the energy lost within the specimen due to the various interactions as outlined before. The major peak consists of unscattered (mostly), elastically scattered and inelastic electrons with minor energy losses. The next peak is from the electrons causing plasmon excitations, which have a high probability as discussed previously. The core-loss electrons refer to those ionizing atoms in the material and therefore they show element characteristic energy losses. The core-loss signal is very low compared to the other two, partly due to the small cross section, and the vertical scale is enlarged 100 times in the figure.

X-Ray Signals

The ionized atom can be de-excited by moving an outer-shell electron to the vacancy in an inner shell where one electron is missing. The associated decrease of energy may lead to the emission of an X-ray. The energy of this X-ray is equal to the transition energy between the two atomic shells and is characteristic for this atom. By analyzing the energy spectrum of X-rays generated when an electron beam impinges on a certain location of a specimen, chemical composition information is obtained.

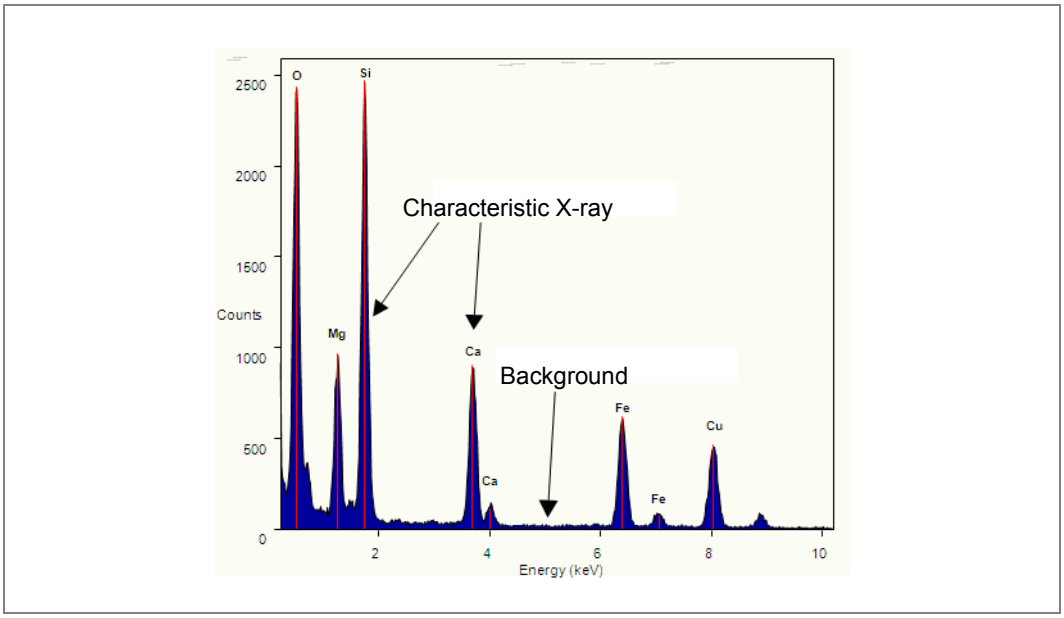
When an electron probe is scanned across a specimen linearly or rectangularly in STEM mode, the X-ray signal at each pixel can be collected. The amount of characteristic X-rays of a certain chemical element may be displayed as plotted curves or as pixel brightness in a two-dimensional presentation. The display will show the relative distribution of that specific element along a line or within the region. This is called X-ray line-profile (sometimes X-ray line-scan) or X-ray mapping, and it is a very useful technique in microanalysis.

The energy range of characteristic X-rays depends on the nature of the sample. The theoretical upper energy limit equals the incident electron energy. However, the probabilities of emission and detection at this limit are very low for a modern TEM. As a rule of thumb, for an efficient analysis the upper energy limit is probably 20 – 30 keV if the microscope accelerating voltage is 100 kV or more. The lower limit of X-ray radiation is around 0.1 keV. Below this value, the emission will be cathodoluminescence.

Apart from the characteristic X-rays, there is a continuous X-ray background radiation. It is produced when a fast moving electron is decelerated through interaction with the atomic Coulomb field in the specimen. An electromagnetic wave may be generated during this. The energy of the emitted quantum is between zero and the energy of the incident electron. The continuum radiation is called Bremsstrahlung (German for *braking radiation*). Its intensity monotonously decreases with energy from zero. Bremsstrahlung is the major source of background in X-ray energy dispersive spectra. Fortunately, it is relatively low compared to the characteristic X-ray lines for a thin specimen in TEM.

Figure 33 is an example of an X-ray energy spectrum obtained from a TEM. There are a few high characteristic X-ray peaks corresponding to certain elements. There is also a continuous background signal, which is much lower than in the SEM.

Figure 33 Example of X-ray Energy Dispersive Spectrum



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2 Talos Electron Microscope

Overview

This chapter discusses the design of the column, the mechanical, electron optical, and vacuum construction, and the user interface that controls the microscope.

Introduction

Talos microscopes make imaging and analysis workflows accessible to a broader community of scientists, with a friendly digital user interface and class-leading ergonomics (*Figure 34*).

The accessories that may be fitted to these systems have largely been embedded into this UI, meaning that operators can utilize the full functionality of the total microscope system through one coherent interface, allowing all the capabilities of the system to be easily controlled by operators of different experience levels.

The field emission Talos microscopes are a culmination of the best electron optical designs based on resolution performance, stability, specimen movement, as well as mode flexibility. The lens types that are available for the Talos series allow you to choose the optimal combination of tilt/resolution and analytical performance required for your application. The Talos series allow the high tension to be switched between the maximum and any other value with one mouse click, and allow imaging with an aligned column in any mode (TEM or STEM) at any other pre-aligned chosen voltage.

An unlimited number of user alignments (including field emission gun presets) can be stored on the microscope. This allows for rapid switching and optimization for different users.

The PC digitally controls all microscope functionality through a TEMserver service, which can be remotely accessed and which can be integrated with special service software for remote diagnosis. Mode changes of the microscope system are trivial; it is straightforward to go from a high resolution TEM image to a high resolution STEM image in seconds, and vice versa.

Talos series microscopes have, in addition, a very large selection of application software developed by Thermo Fisher, such as automated focus reconstruction (TrueImage™), automated tomography (Xplore3D™), automated EDX-Tomography as well as a low-dose package that sets the standard for imaging dose-sensitive samples.

The Microscope

Figure 34 shows a typical Talos TEM.

Figure 34 Talos F200-X Equipped with CMOS Digital Camera, EDX, and Four STEM Detectors

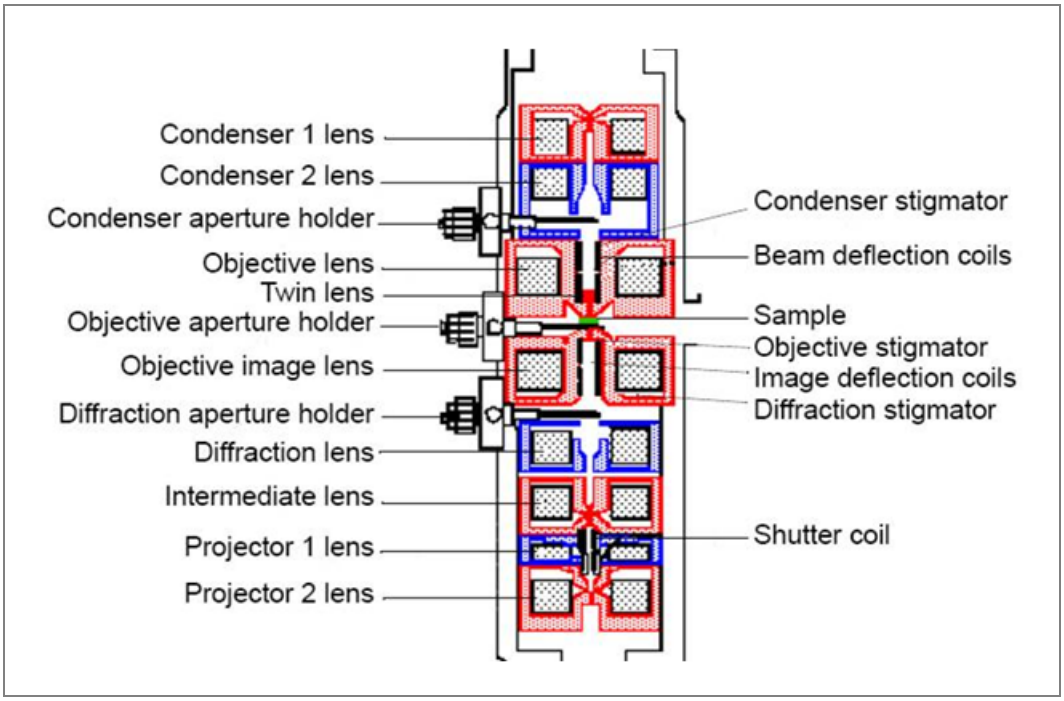
Basically, the microscope consists of the following elements, which are also noted in *Figure 34*:

- Electron gun (1)
- Upper part of the column containing the condenser lenses (2)
- Specimen area with CompuStage (3)
- Lower part of the column with the image-forming lenses (4) and the projection chamber (5).

The microscope controls are situated on two movable control panels. The microscope and detector systems, including STEM, digital cameras, EDX and EELS detectors, are embedded into one single system, with two monitors, one keyboard and one mouse. The X-ray detectors for energy dispersive spectroscopy are at the specimen area and are cooled with liquid nitrogen using the same cooling tank as the anti-contamination device. A wide angle digital camera, HAADF detector and SmartCam are located in the upper part of the Image Detection Unit (5). Bright filed (BF) and dark field (DF) detectors for STEM, high resolution digital camera and the EELS detector are located in the lower part of the IDU (6).

Figure 35 is a schematic cross-section of the microscope column, including the positions of all the lenses, deflectors, stigmators, apertures, specimen and viewing screens.

Figure 35 Cross Section of Electron Column



Apertures and Aperture Holders

Holders

There are two types of (motorized) aperture holders:

- Holders used for the diffraction and objective lens, which are made of copper-aluminium
- A thicker holder used for condenser lens (C2) made of copper

The holders are not interchangeable, even when mechanically identical. The C1 aperture cannot be removed from the column to avoid any unwanted X-ray leakage from the column.

Apertures

Each holder contains four (or up to eight) separate and individually changeable apertures. All the apertures must be mounted with their flat side towards the incoming beam. To avoid contamination or damage to the apertures, retract the apertures before releasing air into the column, such as for filament changing. The apertures can be selected or retracted from the apertures tab at the user interface.

Normally, there are three types of apertures, which are listed in *Table 5*.

Table 5 Aperture Materials and Applications

Type	Lens	Advantages	Disadvantages
Platinum, normal	• Objective • Diffraction • Condenser	• No electron leakage • Low cost • Low contamination	• Edge not well defined • Cleaning needed
Platinum, thick	Condenser 2 (X-ray work)	Stops all unwanted electrons & hard X-rays	*See above*
Thin foil	Objective	• Remain clean • Well-defined edge • Many sizes available • Long life	• Electron leakage at high voltage and loss of contrast • White spot effect at low magnification

Apart from the materials, various sizes of apertures are available for different applications. In general:

- The larger the second condenser aperture, the higher the intensity on the viewing screen and the worse the electron beam coherence.
- The smaller the objective aperture, the higher the contrast and the lower the resolution.
- The larger the diffraction aperture (or selected area [SA] aperture), the bigger the area that contributes to a diffraction pattern or the lower the LM-mode contrast.

Specimen Area and CompuStage

The specimen, fixed to a 3 mm diameter grid, is mounted in a side-entry specimen holder. The holder fits exactly in the eucentric goniometer (CompuStage). The goniometer is called “eucentric” because, when a specimen is at the eucentric height, it can be shifted in the X- and Y-directions, tilted, and rotated without loss of focus. At the same time, rotating and tilting the sample does not change the field of view since the axes of tilt and rotation coincide with the optical axis of the objective lens.

After changing the specimen, or field of view, the specimen must be readjusted to the eucentric height more precisely. This is needed to bring the specimen exactly to the optimum optical position relative to the objective lens.

Accurate adjustment of the specimen height has the following advantages:

- Optimum TEM image
- Magnification and spot size are indicated for this height
- Measuring is calibrated for this height
- Autofocus and wobbler are calibrated for this height
- Objective and selected area apertures are located optimally
- X-ray collimation for EDX analysis is optimal for this plane
- Maximum tilt can be obtained
- Eucentric specimen movements

The last point is at the same time an easy criterion for exact height adjustment since it is only at this height that the center of the field of view will remain at the center of the screen during tilting of the specimen.

The CompuStage is a eucentric, highly stable side-entry goniometer that provides computer-controlled movement of the specimen on up to five axes (X, Y, Z, and rotation α and β). For safety, the CompuStage is equipped with a number of special features, such as the MaxiTilt system, which allows maximum use of the available space between the pole piece for tilting while guarding against damage of the holder, pole pieces, and objective aperture holder. Another feature is the sEntry system, which guards against the insertion of holders that are incompatible with the objective lens configuration of the microscope. Also, among many advantages are: accurate 5D recall and no sliding of the holder O-ring, no after-movement drift, and no vacuum leakage.

As an option, a new Piezo stage extension enables ultra-precise movement in X and Y directions. Accurate stage movement facilitates easy navigation on the atomic scale with a 20 pm finest step, which is mandatory to find the right area and center it precisely in the field of view; especially working at high magnifications. The continuous jogging mode enables both linear drift compensation, beneficial for dynamic experiments, or fastest time to data acquisition after specimen loading. The Piezo elements are integrated in the software, and can be controlled with the joystick and multifunction knobs for precise navigation.

Specimen Holders

Many specimen holders are available for the Talos microscopes. They all fit correctly in the eucentric goniometer stage and can be exchanged rapidly because of the pre-pumped airlock without switching off the high tension supply or filament emission. In general, the following specimen holders are available:

- Single tilt (standard)
- High visibility, low background, double tilt holder for Super-X
- Single tilt liquid nitrogen cryo-transfer holder
- Heating holder
- High field of view single axis tomography holder
- Dual-axis tomography holder
- Analytical tomography holder

Vacuum System

There are at least four reasons for keeping the column under high vacuum:

- In a microscope, electrons travel from the gun to the specimen and then to the screen/detector. A collision between an electron and any gas molecule in the column will either keep the electron from imaging or reduce the image quality. A mean free-path length for electrons (travelling distance between two collisions) of 10–1000 times the column length is a condition for a good imaging process in a TEM, which requires a vacuum of 10^{-3} to 10^{-5} Pa.
- A high vacuum in the electron gun prevents both filament etching and high tension breakthroughs. Vacuums of 10^{-3} (W), 10^{-5} ($LaB_{6)}$ and 10^{-8} Pa (field emission) are typical.
- The prevention of specimen etching and/or specimen surface contamination is essential for investigation.
- High vacuum reduces the contamination of column parts, such as apertures, and therefore reduces the need for routine maintenance.

For these reasons, each microscope has a vacuum system with the degree of sophistication of the vacuum system depending on the requirements. Imaging of thin biological sections is much less demanding than cryo applications or small-probe analysis. Also, field emission guns (FEG) must be operated at higher vacuum than thermionic guns.

The most basic vacuum system consists of a vessel connected to a pump that removes the air. The vacuum system of an electron microscope is considerably more complicated, containing a number of vessels, pumps, valves (to separate different vessels), and gauges (to measure vacuum pressures). Beginning at the bottom, there are four vessels in the vacuum system:

- Buffer tank
- Image detection unit (IDU, also called projection chamber)
- Column (specimen area)
- Electron gun area

These are not pumped by a single pump because there is no pump available that handles the full range in vacuum from air pressure (as present after a vessel has been vented) to ultra-high vacuum (in the specimen area or gun). The microscope can be divided in two parts, separated by a very small aperture (200 micrometers):

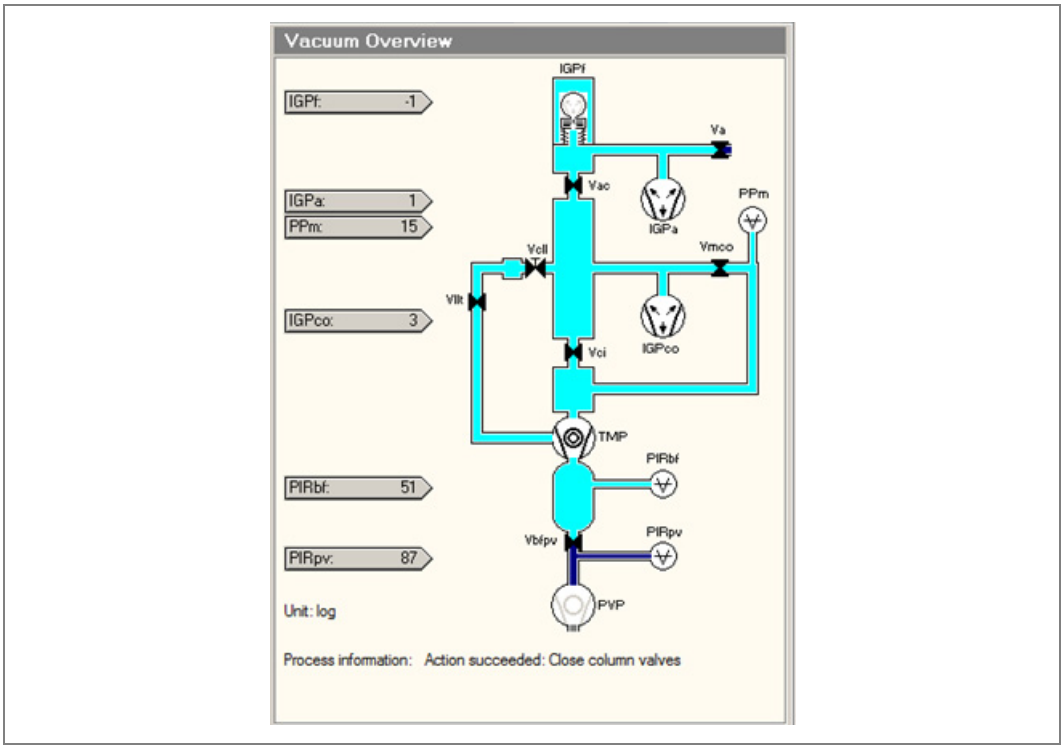
- The differential pumping aperture, located between the projection chamber and the column. This aperture keeps the column under high vacuum. Even when the camera is exposed to atmospheric pressure, such as for changing negative films, high vacuum can be restored rapidly, ready for further work.
- In some microscopes, there is another such aperture separating the gun from the column below. When the filament needs to be replaced, there is no need to expose the column to the atmosphere. Or the specimen area can be used or a higher vacuum pressure for *in situ* investigations without reducing the lifetime of the filament.

The lower part consists of the projection chamber where most of the detectors are located. This is pumped by a turbo molecular pump (TMP). Behind this pump is a rotary, or prevacuum pump (PVP), which is noisy, so it is only running when needed (not continuously). In order to have continuous backing of the TMP, there is a buffer tank in between them, which is filled by the TMP. When its pressure becomes high, it is automatically emptied by the rotary pump

The upper part consists of the specimen and gun areas, which are pumped by one or more ion getter pumps (IGPs). These pumps use no oil and are therefore clean. They also achieve higher vacuum than the TMP. The number of ion getter pumps may range from one to three. Initial pumping of the column and gun on many systems is done by the rotary and turbo molecular pumps.

Figure 36 shows the vacuum system of the Talos 200X, a FEG microscope.

Figure 36 Vacuum System, Field Emission Gun

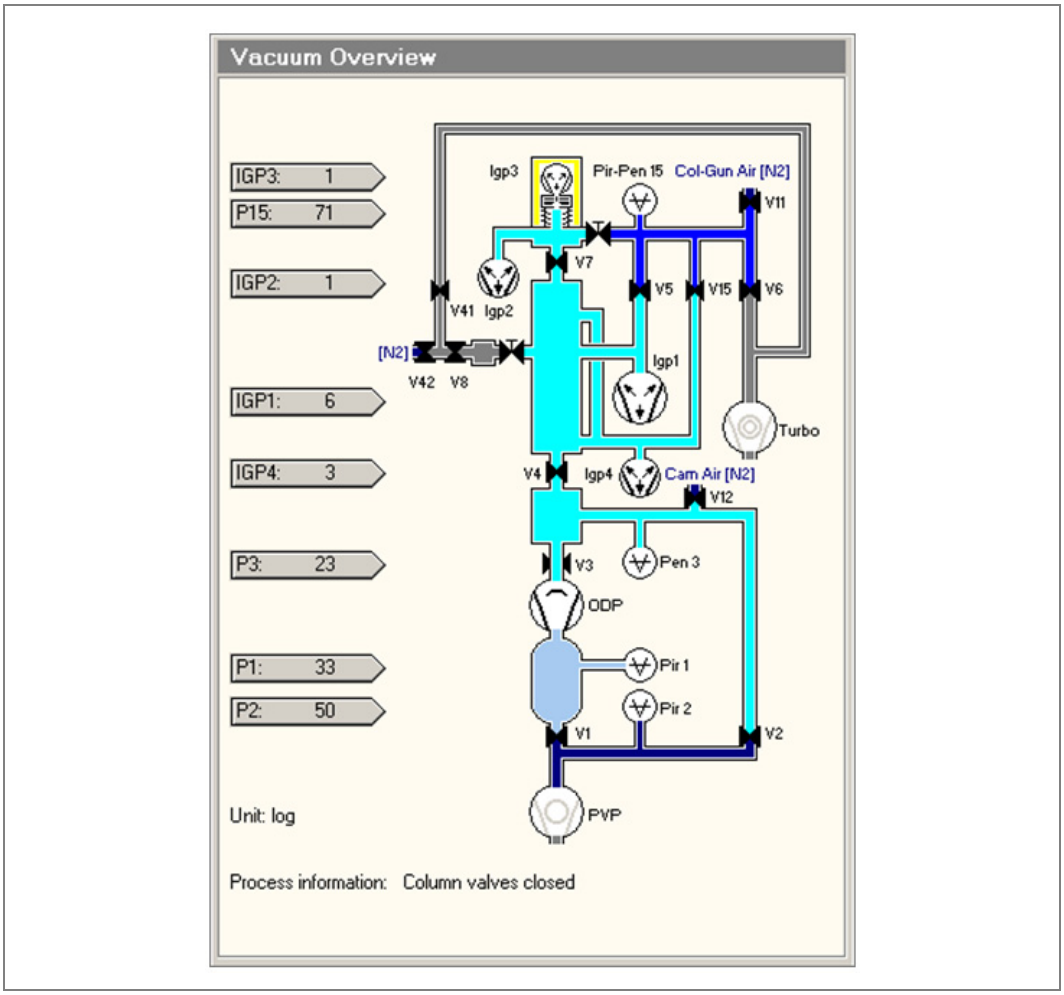


At the bottom, it starts with the rotary pump (PVP). Above the PVP is the buffer tank that is separated from the PVP by a valve (Vbfpv). Pir (Pirpv and Pirbf) are Pirani vacuum-pressure measuring gauges. Valve Vci separates the projection chamber from the upper part of the column.

Pressures are also measured on the basis of the current running in the IGPs. The specimen area is pumped by a main ion-getter pump (IGPco). The specimen holder airlock is operated with valve Vllt. In the vacuum overview, running pumps are colored black, inactive pumps are gray. The PVP only works intermittently to avoid vibration for high resolution work. During an exposure, the pump is suppressed until the exposure has been completed. Further, the electron gun (FEG) is pumped by IGPa (gun area) and a small IGPf (emitter area).

The vacuum system of field emission gun (FEG) Talos microscopes is more sophisticated than that of the thermionic gun microscopes (see *Figure 37*). The system is equipped with three extra ion-getter pumps.

Figure 37 Vacuum System, FEG



One extra pump (IGP4) evacuates the liner tubes that connect the projection chamber to the specimen area and the specimen area to the gun. Further, the electron gun (FEG) is pumped by IGP2 (gun area) and a small IGP3 (emitter area). FEG microscopes are also equipped with a TMP to pre-pump the column and to pump on the specimen air-lock.

Lens Systems

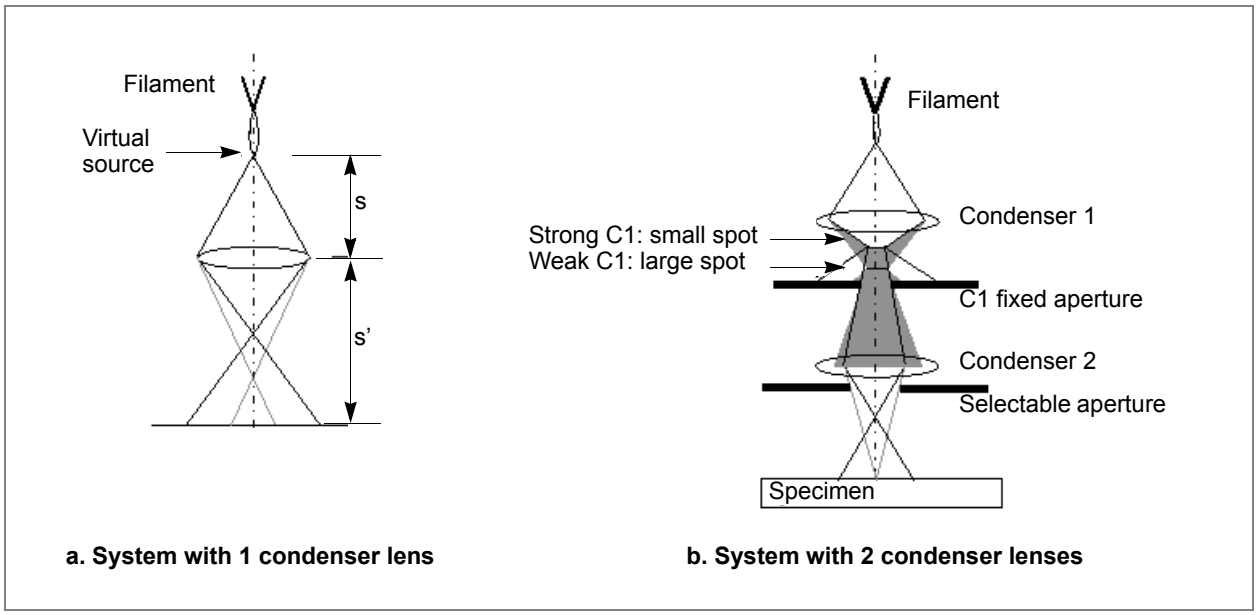
The heart of an electron microscope is its lens system. Essentially, it has the following functions for observing an enlarged image of the object: illuminating the object, imaging the object, and enlarging this image. In a modern microscope, these are achieved by three independent optical systems within the column.

The Illumination System (Condenser)

Without any lens, a specimen can still be illuminated directly by an electron gun, though with very limited performance. Both the illuminated area and the illumination intensity are fixed.

When one condenser lens is used, the illumination can be varied by changing the excitation of the lens (*Figure 38*a). This illumination flexibility is essential for different applications. The minimum spot size is determined by the magnification of the virtual source size D by the lens: D s'/s.

Figure 38 Illumination Systems



a. System with 1 condenser lens **b. System with 2 condenser lenses**

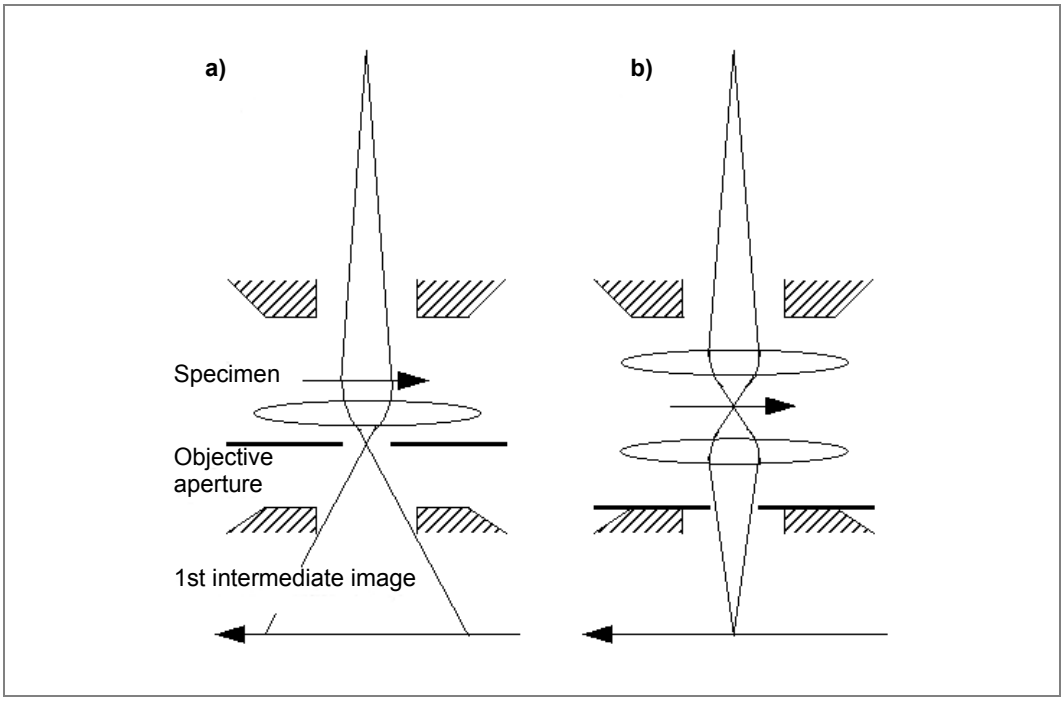
For many applications, microscopists use various spot sizes. This is achieved by working with another condenser lens (*Figure 38*b).

Changing the excitation of the first condenser lens results in different spot sizes; by changing the excitation of the second condenser lens, one focuses or defocuses the illumination on the specimen. Typically, C1 is a strong lens, while C2 is a weak lens. In the Talos microscopes, they are controlled by "spot size number" and the **Intensity** knob, respectively. The first condenser lens is a demagnifying lens that decreases the size of the gun cross-over. The second condenser enlarges the C1 spot. The overall effect of both lenses is to control precisely the amount of illumination on the specimen. When studying *Figure 38* it becomes clear why smaller spot sizes are inextricably dimmer. The maximum size of the C2 aperture is limited by the spherical aberration of the lens. Smaller apertures sometimes are selected for better coherent illumination.

Objective Lens

The objective lens produces the first intermediate image of the object, which will be further enlarged by lenses downwards in the column. Because of the fact that the objective lens errors are magnified by the total magnification, this lens is the most critical/important lens in a microscope. In general, the smaller the focal length, the smaller the lens aberration constants and the better the resolving power of the microscope. The objective lens is used primarily to focus the image. All other lenses (intermediate and projector) are used to magnify this image. Typically, the objective lens is least variable when adjusting magnification of the image. It is maintained at very short focal lengths to ensure high resolution.

Figure 39 Objective Lenses, Conventional (a) and Symmetrical (b)

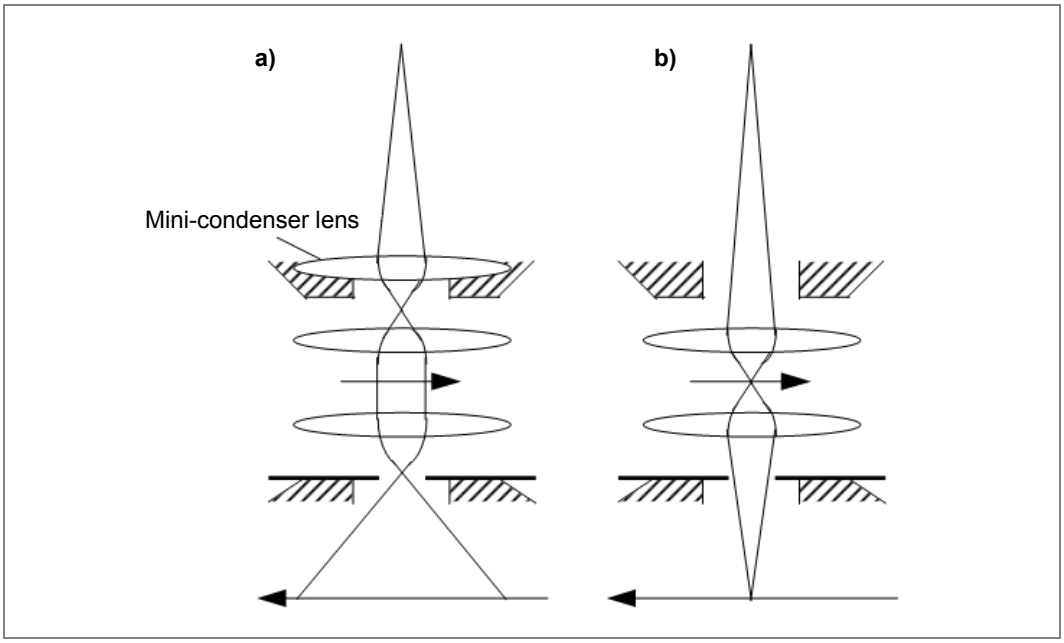


Imaging proceeds as follows: At the back focal plane of the lens, a diffraction pattern is formed; further down the column, the first intermediate image is at the image plane. This image plane is also called the selected area plane, where the selected area (or diffraction) aperture may be present. The objective aperture is positioned in/near the back focal plane of the objective lens.

In the conventional objective lens (*Figure 39*a), the specimen is positioned at a third of the lens gap from the upper pole piece, while the objective aperture is located at the two thirds. This lens has a number of disadvantages, such as the unusable space below the aperture for specimen tilting, an impossibility for small spot size, restriction for high angle scattering, and asymmetry of the magnetic lens field around the specimen. The symmetrical design by Riecke and Ruska overcomes the above drawbacks, but at the same time the beam cannot be spread very far, which severely restricts the field of view at lower magnifications (*Figure 39*b).

Based on the symmetrical design, the TWIN-lens has a mini-condenser lens (sometimes confusingly called Twin lens), which can be optically switched *on* or *off*. When the mini-condenser is on, the TWIN lens is in *microprobe* mode, providing a wide field of view and coherent illumination on the specimen, *Figure 40*a.

Figure 40 Mini-condenser in (a) Microprobe (on) & (b) Nanoprobe (off) Modes



This mode is used for normal TEM imaging. When the mini-condenser is off, the lens is in *nanoprobe* mode, creating a small illumination spot on the specimen. This mode is particularly useful for scanning transmission electron microscopy (STEM), microanalysis and convergent beam electron diffraction (CBED). To avoid thermal drift, the mini-condenser in fact is not physically switched off/on, but the direction of the lens current is reversed. In the case of the *microprobe* mode, the mini-condenser lens field is reinforced by the field of the objective lens field, while in the *nanoprobe* mode the fields cancel out. Keeping the mini-condenser lens running at the same current ensures that its temperature remains the same, an essential condition to reduce specimen drift.

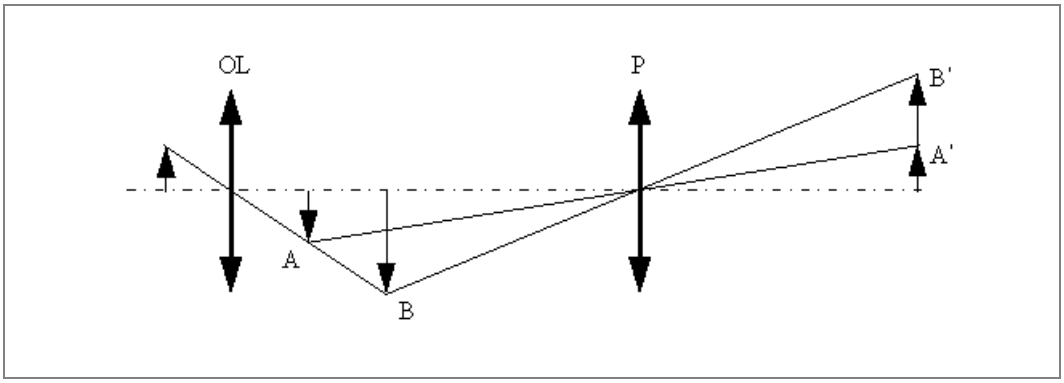
Talos introduces constant power technology coming from Titan platform into its objective lens. The focal length of an electromagnetic lens is adjusted by changing its current. This change on lens current produces a change on the lens temperature what, in the case of objective lens, will modify the temperature around the specimen inducing thermal drift. To avoid that, two windings are used on the objective lens, which permits changes in the electron optical power of the lens without changing its power dissipation.

Imaging Lens System

In an electron microscope, the positions of both the object (specimen) and the viewing screen are fixed. If no imaging lens exists below the objective lens (OL), one encounters the sole magnification problem because it is determined by the distance from an object to its image. Varying the objective lens current will only change the focusing condition.

One way to allow adjustable magnification with a focussed image is to make use of a projector lens P (one-lens imaging system, *Figure 41*).

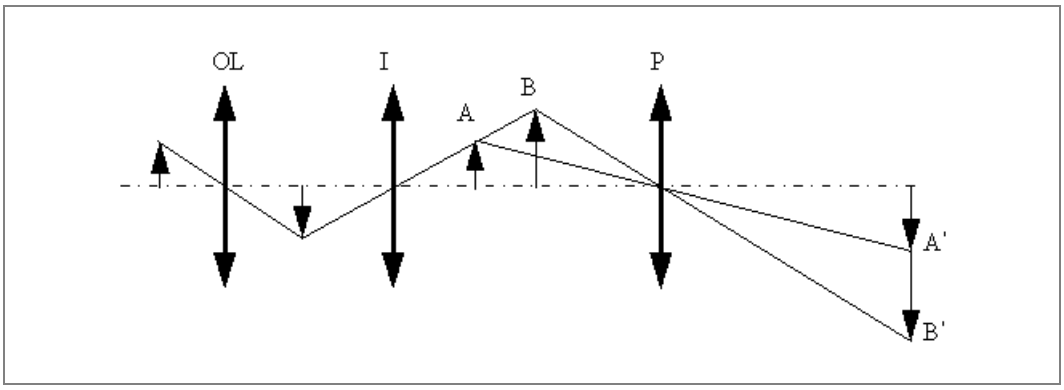
Figure 41 One-Lens Imaging System



The ray paths of A-A’ and B-B’ lead to two different magnified images. One needs to change the objective lens current so that the first intermediate image lies on either A or B, and, simultaneously, to change the projector current so that its objective plane changes accordingly. However, changing the objective lens current is a drawback since the temperature around the specimen also varies, causing instabilities. Besides, keeping the first intermediate image on a fixed plane will give us a chance to use a selected area aperture. The electrons passing the selected area aperture are coming from the parts of the specimen which can be seen through the aperture.

A two-lens imaging system can cope with the demands (*Figure 42*), for which the object plane, selected area plane and the viewing screen are all fixed. Different magnifications are achieved by manipulating imaging lenses, an intermediate lens I and a projector P.

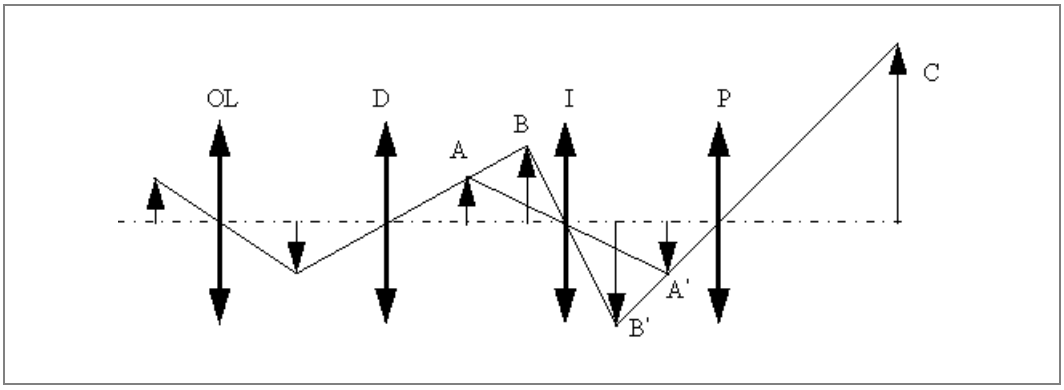
Figure 42 Two-Lens Imaging System



The additional information (diffraction pattern) at the back focal plane of the objective lens can be imaged by turning down the current through the intermediate lens, enlarging its focal length, and focussing the imaging system to that plane. When the imaging system focuses on the selected area plane, the M/SA mode, on the viewing screen we see the enlarged image; when it focuses on the back focal plane, the D mode, we see a diffraction pattern.

For further improvement of an imaging lens system, the third lens is needed (*Figure 43*). The system contains an extra diffraction lens, has larger possible magnification range and gives a freedom for optical aberration correction.

Figure 43 Three-Lens Imaging System



The last point is based on the fact that the same magnification image can be produced by the different lens current settings, which enable us to use the ray path with the lowest distortion. The Talos microscopes have an additional projector in the imaging lens system for extra flexibility. The two-projector combination ensures that an image crossover is located at the differential aperture, no matter what the microscope magnification is. The combination also helps for correcting the chromatic aberration and the image distortion.

The User Interface

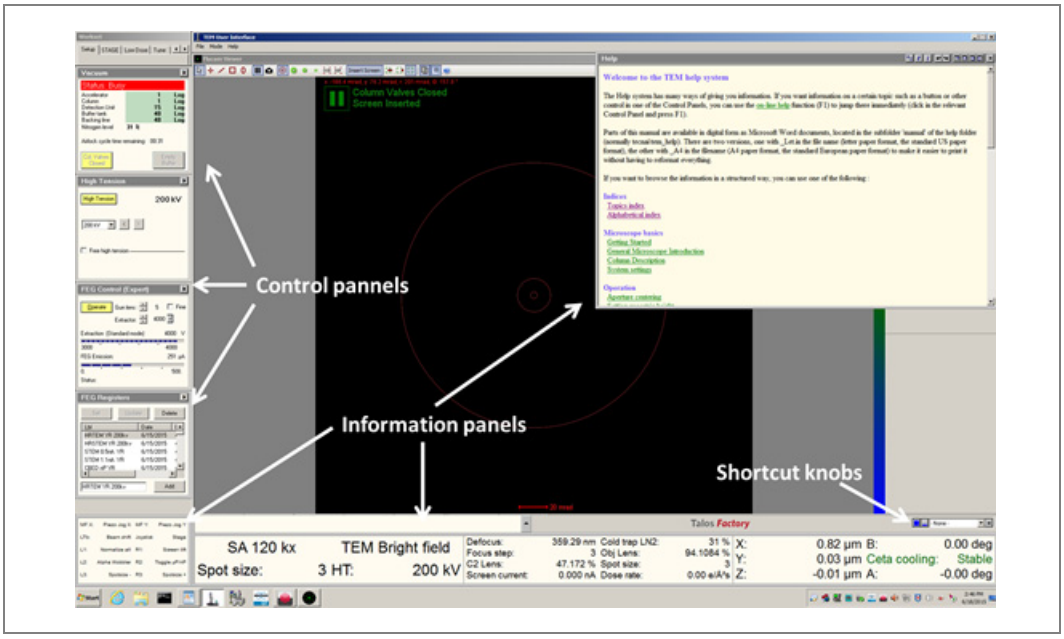
The basic rule for the Talos user interface (UI) is “what you do not need, you do not see.” So, Talos distinguishes several user levels with differences in the available functionality and access to system settings (see also Talos Help Navigator: User Interface or click **F1**).

At the start of a microscope session, simply log in. Personal settings like microscope alignments and preferences such as the selection of control panels are automatically restored and the microscope is ready for use. Personal preferences that can be changed include:

- Arrangement of the microscope and detector control panels in setups
- Function of the user buttons on the control pads
- Selection of microscope status information to be displayed

The UI consists of a number of separate elements (*Figure 44*), which can be divided into different categories.

Figure 44 User Interface



- **Main program**: This consists of the program title, menu bar and toolbar. It basically is a shell that allows you to define the “where, what and how” of the other user interface elements. The UI provides a number of fixed layouts (views) with rapid switching between them.
- **Control panels**: These are sets of controls that belong together and that are displayed in a fixed window (normally one of three displayed on the left-hand side of the screen). Control panels are grouped in work-sets which can be selected via a tab at the top of the area with the control panels.

- **Information panels**: These consist of a set of windows displayed near the bottom of the screen. They contain binding (i.e., assigning functionality to user-definable knobs and buttons on the control pads of the microscope), microscope status information, and messages or questions from the microscope to the operator.

Views

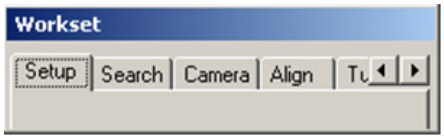
The microscope user interface supports a number of different viewing modes. These modes can be accessed with the aid of the blue shortcut knobs (*Figure 44*). The modes are

- Full screen application
- Full screen with open frame; data area left open
- Minimum size information area; status display limited to column with control panels and data area filled
- Minimum information area open frame; status display limited to column with control panels and data left open
- Taskbar only

In practice, these different views make it possible via the blue shortcut knob to switch quickly between normal Talos operation mode and maximum space available for data display and/or analysis (e.g., SmartCam, TIA).

Workset Tabs

At the top left of the user interface is a small window containing a user-defined tabs. Each tab controls access to a number of Control panels (typically a set of three). You can set by default or define a workset configuration (contents, name, order, color selection).



Worksets are intended to be arranged in sets that reflect a certain stage of operation of the microscope (but of course you are free to arrange them in any manner that you find suitable for the work you do). Thus, the Setup workset could contain those controls needed when starting a microscope session (vacuum, high tension and filament control). The Search workset provides controls that are useful when searching around the specimen for areas suitable for further investigation (the Stage control panel, for example, allows storing and recall of specimen-stage positions). The Camera workset provides access to digital cameras functions that allow recording of data like images and diffraction pattern during the more detailed investigations. The worksets are defined using the Workspace control panel.

Control Panels

Control panels are small windows, typically arranged in sets of three above one another at the left-hand side of the screen. Each control panel contains a coherent set of microscope controls (like vacuum system, electron gun or stigmators).

The combination of up to three control Panels forms a workset, defined by name and accessible through a tab in the workset selection window above the Control panels themselves. The rationale behind the control panels is very simple. They are meant to give rapid access to elementary (often- used) microscope functionality. Selection of microscope settings (used much less often) is not accessible through the control panels themselves, but is hidden away conveniently in flyouts: additional panels that appear to the right of the main panel. Panels with flyouts are recognizable by the flyout arrow button at their top right. When the flyout button is selected, the flyout panel appears. Flyouts remain visible until they are closed again (with the flyout button which has reversed its pointing direction). They disappear from view when another workset is chosen, but will reappear when the flyout workset is selected again.

Each control panel has its own online help, accessible by clicking somewhere inside the control panel, and then pressing **F1**. The Alignments control panel additionally has online help pages for each of the alignment subprocedures, activated (automatically) in a subprocedure.

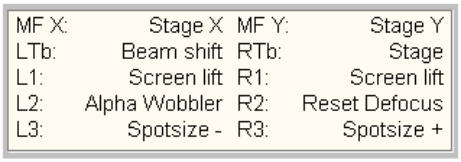
NOTE	Not all control panels may be available to all users, as some panels differ according to the user level (User, Expert, or Supervisor), or as some panels are related to specific microscope accessories hardware (STEM, HAADF, EFTEM).

Display

The microscope UI provides a series of panels containing microscope status information. These cover the binding display, message area, and microscope status display.

Binding Display

The binding display panel shows how the user-assignable knobs and buttons on the left-hand and right-hand Control pads are linked to microscope functions.



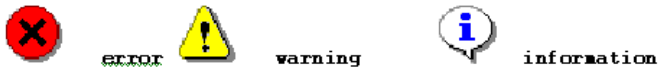
These knobs and buttons are:

- Left-hand track ball (typically assigned to beam shift)
- Right-hand track ball (typically assigned to CompuStage X and Y)
- Multifunction X
- Multifunction Y
- User button L1, user button L2, user button L3
- User button R1, user button R2, user button R3

You can choose the selection of the assignments displayed. A typical selection could be displaying two columns. The list of displayed assignment can be modified by clicking and dragging. Also, the function assignment can be easily changed using the mouse.

Messages

Messages by the microscope are shown in a dedicated part of the information area (above the status panel that typically contains the magnification and operating mode). Messages can have three different levels. Since only one message can be displayed at a time, important (higher level) messages will displace simple information messages. The different levels are indicated by different icons:



If the message requires confirmation, it will stay displayed until **Enter** is pressed. Some other messages automatically disappear after a minute. All messages are kept in a list that becomes visible when **Up** on the right-hand side of the message area is clicked.

Status Displays

Up to three status display panels (in the minimum-size info area view this number is reduced to one) can display a wide range of user-selectable microscope settings.

The left-most typically will display the microscope magnification and mode. This panel is also the one displayed in the minimum-size info area view. The two others can be configured by you (by default they will contain the settings shown at the right of the picture above, while the third panel will display the specimen-stage position). You can change all the settings.

Popup Panels

At the bottom right-hand side of the screen (right next to the microscope name) is a dropdown list box with a small **x** button next to it (in other views than the standard frame it may be located elsewhere on the screen).

The dropdown list gives access to Control panels that will be displayed in the corner just above the list box itself. The selection of these popups includes a number of Control panels that cannot be assigned to worksets (because of their size). The selection also includes those control panels that are not currently visible on the screen. This means, for example, that you can have the microscope display the CCD/TV Control panel to have quick access to the digital camera settings without changing from the current workset tab. As soon as the workset tab is changed to one containing the panel visible as a popup, the popup panel will disappear. The popup thus provides a method for rapid access to settings that are not found in the current workset. It also makes it possible to simplify the user interface worksets by only including the Control panels that are often used and using the remainder from the popup when they are needed.

Two popup control panels are worth mentioning here since they can only be accessed in the popup because they are too large to fit inside the space normally reserved in the worksets for Control panels: the Vacuum Overview and the Workspace Layout. The online help for these panels is accessible by clicking in them and pressing **F1**.

Help Window

The Help window, used for displaying the online help, is displayed in the area reserved for data (the empty area in the UI). Three views of different sizes are selectable through small buttons at the top of the Help window. In order to minimize the number of pages involved, many topics are arranged together with related topics on a single page. To allow rapid selection of relevant topics on such pages, they have been equipped with bookmarks (hyperlinks to the topics further down the page). The hyperlinks are clearly recognizable as such in the case of text (the unused hyperlinks are green, the used ones take the default color of the browser). It may also happen that hyperlinks are present on images, for

example on control panels. Move the cursor over the image and hyperlinks will show up by the changing of the cursor to a hand. Where relevant, the Help system will display information in different stages. At first, access shows basic information such as step-by-step procedures. Further down will be a short explanation of what is actually done on the microscope. And furthest is a detailed electron-optical description. The level of complexity thus increases with each level.

Windows Controls

The user interface of the Talos microscope contain a number of Windows control elements. These controls are listed below.

Table 6 Windows Controls (1 of 2)

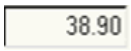
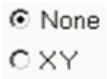
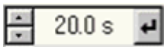
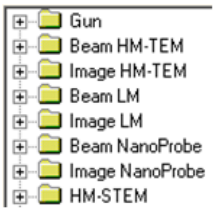
Appearance	Type	Description
	Button	Click to activate. If function cannot be done, the button is grayed.
	3-state button	In addition to normal and grayed states, this button has a yellow state that indicates that the function is on.
	4-state button	In addition to normal and grayed states, this button has a white state that indicates that the selection is on and a yellow state that indicates that the selection is on and function is active.
	Label	Text in a window; display only.
	Edit control	Allows insertion of text or numbers. Only used if there is some other way of having the program act on the change.
	Check box	Selects (cross is displayed in the square) or deselects a particular option. It also works when you click on the caption of the check box (so not only when you click inside the box itself).
	Radio button	Makes a selection from a series of mutually exclusive options. Clicking an unchecked option checks that option and “unchecks” all others. Also works when you click on the caption of a radio button (so not only when you click on the circle itself).
	Dropdown list	A list of items from which a selection can be made. In order to save space, the whole list only becomes visible when the arrow on the right is clicked. If text or a value can be entered at the top line, it is called a dropdown combo box.
	Spin button	Change a number up or down by clicking on the up or down buttons. The numbers will spin faster if you keep one of the buttons pressed instead of giving single clicks.

Table 6 Windows Controls (2 of 2)

Appearance	Type	Description
	Enter button	Confirm changes by clicking **Enter** (pressing the **Enter** key on the keyboard does the same).
	Spin-enter-edit	An edit control with spin buttons to change the value and an **Enter** button to confirm the change (after which the program will act on it).
	Track bar	Drag the gray handle to another setting to change a value. You can also click to the left or right of the handle to make it jump one step.
	Progress bar	Displays progress of a process or the current status as a fraction of the total range.
	Tab	Allows selection of one of a series of displays.
	Bitmap button	A button with a picture on it. These are used for the flyout buttons, but also in toolbars, etc.
	Tree view	Display a list of items, some of which (marked by a + sign) can be expanded to show their sub-items, and so on. Expansion happens when you click on the + (it will contract again when you click on the - sign that will take the place of the +).

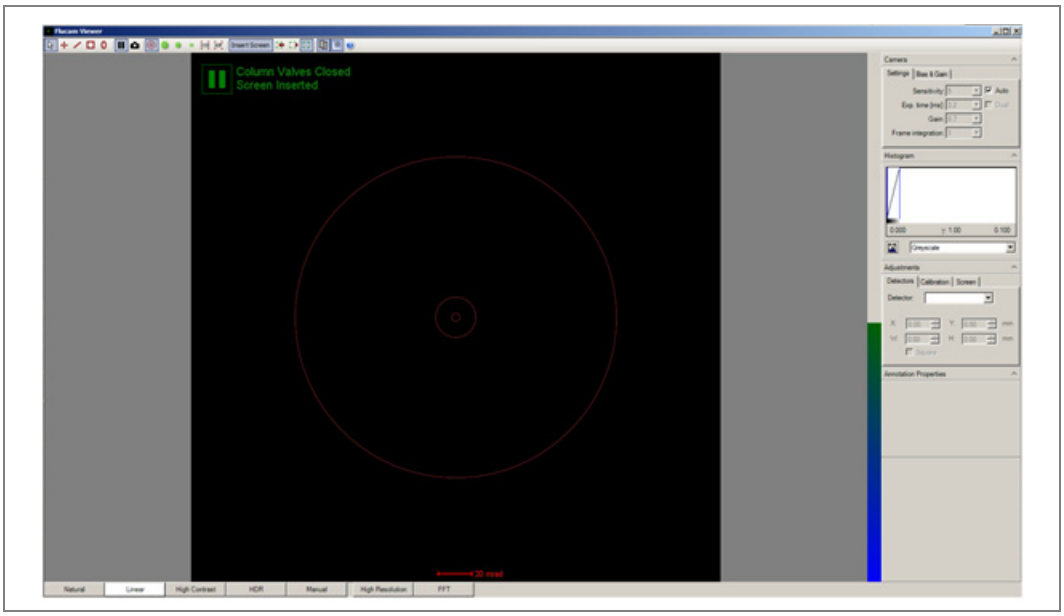
SmartCam

SmartCam Viewer displays images from the SmartCam, a camera that observes a fluorescent screen. Using SmartCam Viewer the microscopy tasks that were previously done using the fluorescent screen can now be done using the PC. A CCD camera observes a retractable fluorescent screen and the image is displayed on the computer monitor. The image distortion caused by tilting of the fluorescent screen is corrected in the computer.

Start SmartCam Viewer from the start menu, the application will adapt itself to the Microscope User Interface. It can be displayed on the second monitor by changing the application preferences in the TEM User interface. The camera starts automatically when the column valves are open and the screen is inserted. The camera has been designed to withstand extreme imaging conditions, during operation of the microscope there is no need to worry about damaging the camera, even with focused intense beams. However, the same precautions are valid when using the classic fluorescent screen: it is wise not to leave focused intense beams for long periods on the screen.

In summary, a microscope with the SmartCam needs the same attention as a conventional TEM: close the column valves when leaving the instrument and don't leave very intense focused beams on the screen for prolonged times.

Figure 45 SmartCam Viewer

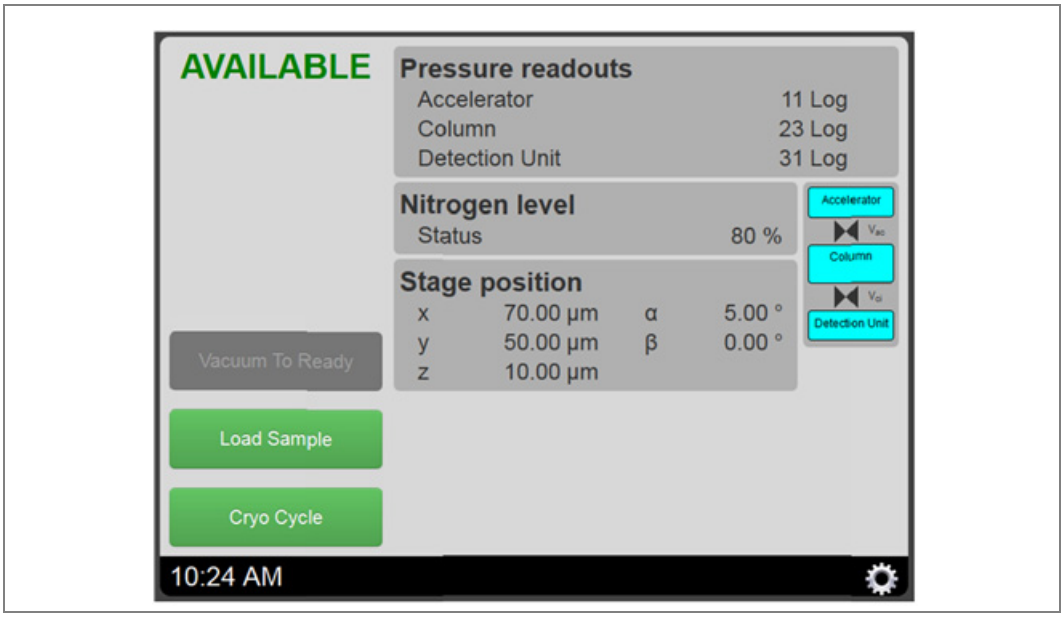


On-System Display

In order to improve the usability of the microscope, a separate touch-screen is mounted on the front side of the microscope. This display shows the most important status information such as vacuum status, nitrogen level and system status.

Furthermore, in a typical setup, the microscope and the microscope pc are physically far apart. In most cases, the microscope PC is located in another room. The On-System Display (see *Figure 46*) allows the operator to control the basic functionality of the machine such as loading a sample and starting/stopping Cryo cycle without having to walk back from the microscope pc to the microscope itself.

Figure 46 Main Screen of the On-System Display on Talos

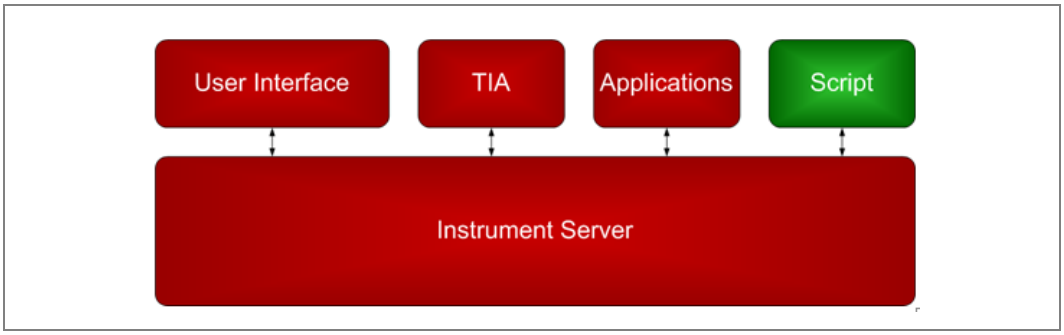


Software Architecture

Although knowledge of the software architecture is not a prerequisite for operating the microscope, it may help you in understanding how the software on the microscope works and why things have been placed as they are.

The Thermo Fisher microscope software basically consists of the instrument server and one or more programs running on top of it; see *Figure 47*.

Figure 47 Microscope Software



The instrument server is the program that controls basic functions of the microscope like vacuum, the electron source, high voltage, optics, stage, cameras, STEM detectors, etc. Some of these functions must be controlled even when no one is logged in and, therefore, the instrument server is running independently of the user and is automatically started when the microscope PC is powered up. If this is done differently, every user has to start up the microscope after log in and wait for the vacuum to get ready for operation and the lenses and high tension to stabilize.

Typically after logging in, start the User Interface in order to control the microscope. Depending on the experiments to be executed, you can start additional programs like TEM Imaging Analysis (TIA) and/or other applications such as TrueImage or Tomography. When the scripting package is available, it is even possible to run a script you programmed on the microscope. This can be done with or without running the user interface, TIA, or other applications.

3 TEM Modes & Contrast Mechanisms

Overview

This section deals with the most general and most often used operation modes of TEM. The operating principles of these modes are illustrated using ray diagrams, which are drawn for the TWIN-type objective lens system, but the basic principles are identical to the conventional objective lenses.

Bright-Field Imaging

Bright-field (BF) imaging is the standard method for TEM. It simply means making an image with the transmitted beam only. Two ranges can be distinguished, a high and a low magnification range. Except in the low magnification mode (LM), the objective lens is always the strongest lens in the microscope, magnifying between about 20 and 50 times, depending on the type of objective lens. The individual lenses of the magnification (or projector) system are not controlled directly by the operator, but instead the microscope contains a number of magnifications for image and diffraction mode, each with its own settings of the magnifying lenses.

The only imaging lenses that are controlled directly by the operator are:

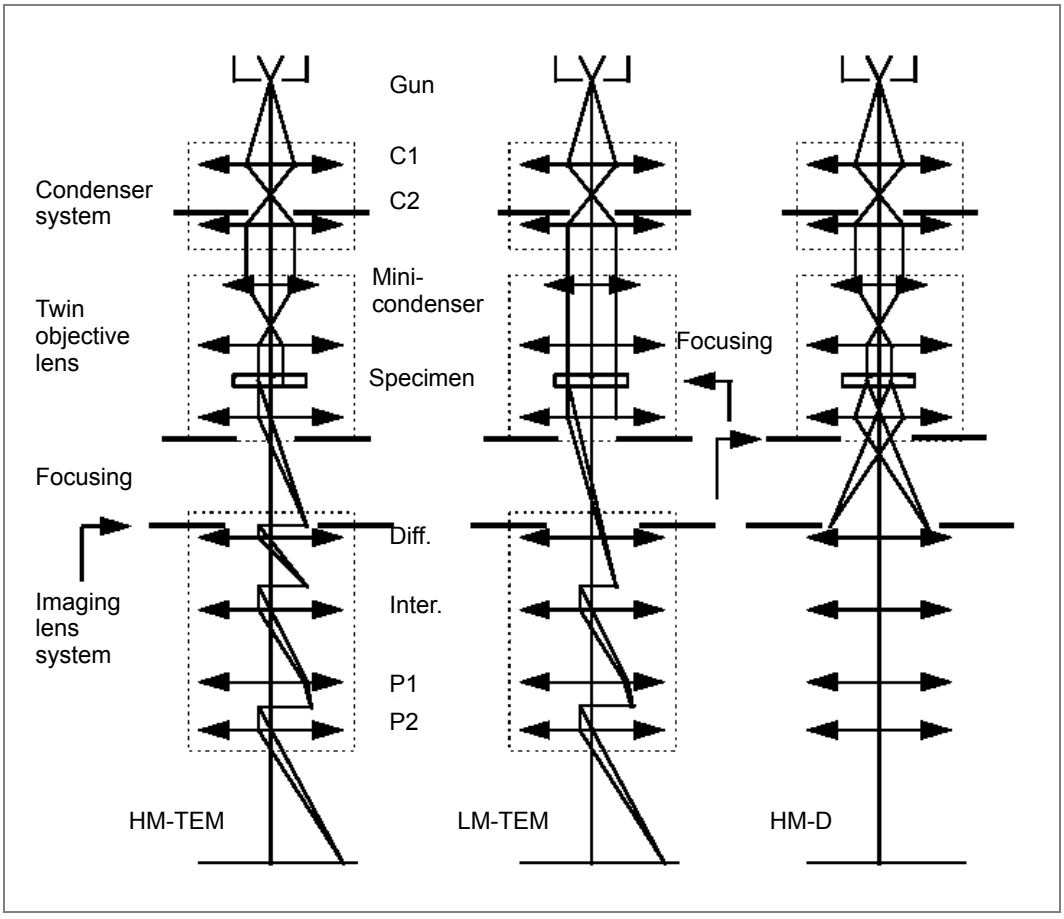
- The objective lens (for focusing the image)
- The diffraction lens (for focusing the diffraction pattern)

Table 7 Imaging Lens and Aperture Function for HM and LM Modes

	HM (High Mag) Mode	LM (Low Mag) Mode
Objective lens	Image Focus	Diffraction Focus (LAD)
Diffraction lens	Diffraction Focus	Image Focus
Objective Aperture	Contrast Forming	Area Selection
SA Aperture	Area Selection	Contrast Forming
Objective Stigmator	Image Stigmation	Diffraction Stigmation
Diffraction Stigmator	Diffraction Stigmation	Image Stigmation

In LM mode, the objective lens is switched (nearly) off in order to achieve the smallest magnifications. With the objective lens off, the diffraction lens is used for focusing the image. So, the electron optical configuration in LM mode is reversed with respect to the HM-mode: the functions of the objective and diffraction lenses and stigmators switch as do the functions of the objective and selected-area apertures, see *Table 7*.

Figure 48 Lens Configuration for HM-TEM, LM-TEM, and HM-D Modes



Nanoprobe and Microprobe

The symmetrical design of the TWIN-type lens allows operation in Nanoprobe and Microprobe mode. The Nanoprobe mode provides a very small probe with high beam intensity, which can be utilized for X-ray analysis, because it gives a better spatial resolution and a stronger signal. However, it is not an ideal mode for imaging since the area illuminated is small and, normally, the beam is convergent. Nanoprobe at 120 kX is good for SPA, since it can focus a parallel beam, whereas Microprobe can be convergent for small illuminated focus.

Talos microscopes equipped with thermionic guns allow probe sizes down to 2 nm, whereas field emission (FEG) type microscopes allow probe sizes even smaller than 0.2 nm.

Talos (S)TEM can resolve Si doublets, so the probe size is < 0.14 µm.

High Magnification (Mi, SA and Mh Range)

This is the normal TEM *Microprobe* mode, in which a parallel or nearly parallel beam illuminates the specimen. The mini-condenser lens focuses the beam at a point in the front-focal plane of the TWIN-type lens, producing a parallel beam onto the specimen (*Figure 48*). The diffraction pattern of the illuminated area of the specimen appears in the back-focal plane of the objective lens. The pattern may be of regular spots for a crystalline specimen, rings for a poly-crystalline specimen, or a diffuse distribution about the central spot for a noncrystalline one. An area containing both crystalline and non-crystalline parts will show spots or rings superimposed on a diffuse distribution.

An objective aperture positioned around the central spot, which is formed by transmitted (undiffracted) electrons, leads to a bright-field image. Only those electrons within the aperture contribute to the image, thus the thin, light parts of the specimen look brighter in the image than the strongly diffracting parts. This image contrast mechanism is called *mass-thickness contrast*.

The image of the objective lens is formed in the selected area plane (SA range), which is the object plane of the imaging system. By adjusting the objective lens current, you can position the objective-lens image accurately in the selected area plane, so that the image on the fluorescent screen is focused. The imaging system is pre-programmed in such a way that a change of magnification is automatically performed by simultaneously changing the current of the four imaging lenses.

Low Magnification (LM Range)

Lower magnifications of a broadly illuminated area are necessary to obtain a good overview of a specimen. This can be accomplished by almost switching off the objective lens and focusing the imaging lens system directly at the specimen. The focusing function is now performed by the diffraction lens. The diffraction pattern in the back-focal plane of the weakly activated objective lens is now situated in the selected area plane, which means that the image contrast can be enhanced by introducing a selected area aperture, if it exists. At the lowest magnification, a broad and bright illumination can be attained by extracting the C2 condenser aperture.

Low Dose

Specimens such as polymers, certain resin-embedded specimens, negatively stained materials, organic crystals, and frozen (hydrated) sections are often very sensitive to beam damage.

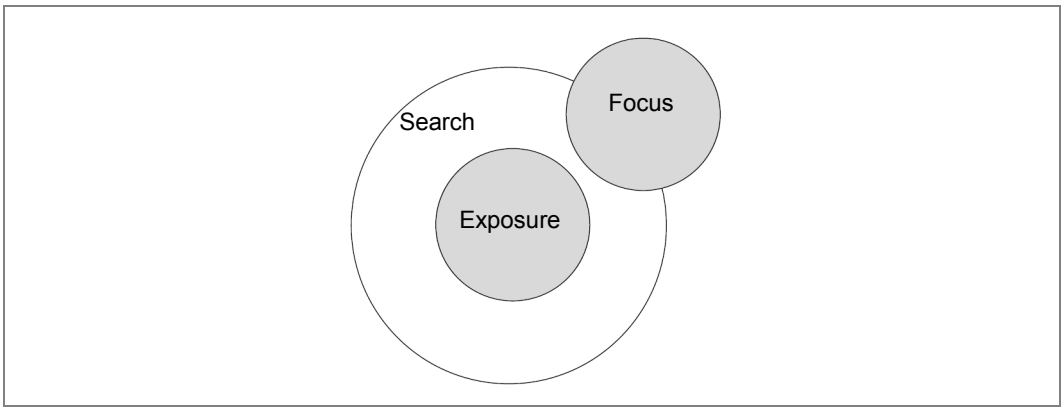
To reduce beam damage:

- Lower the beam intensity by over-focusing C2 (**Intensity**), using smaller spot sizes, and using smaller condenser apertures
- Scan a small probe over the specimen (STEM Imaging)
- Lower specimen temperature by cryogenic techniques
- Use the Low Dose mode

The Low Dose mode of the Talos microscopes provides a method for minimizing the amount of beam damage (minimum dose of illumination) to the specimen prior to recording. The mode or function has three states: Search, Focus, and Exposure.

Each of the Low Dose states can have its own setting for magnification/camera length, spot size, and intensity.

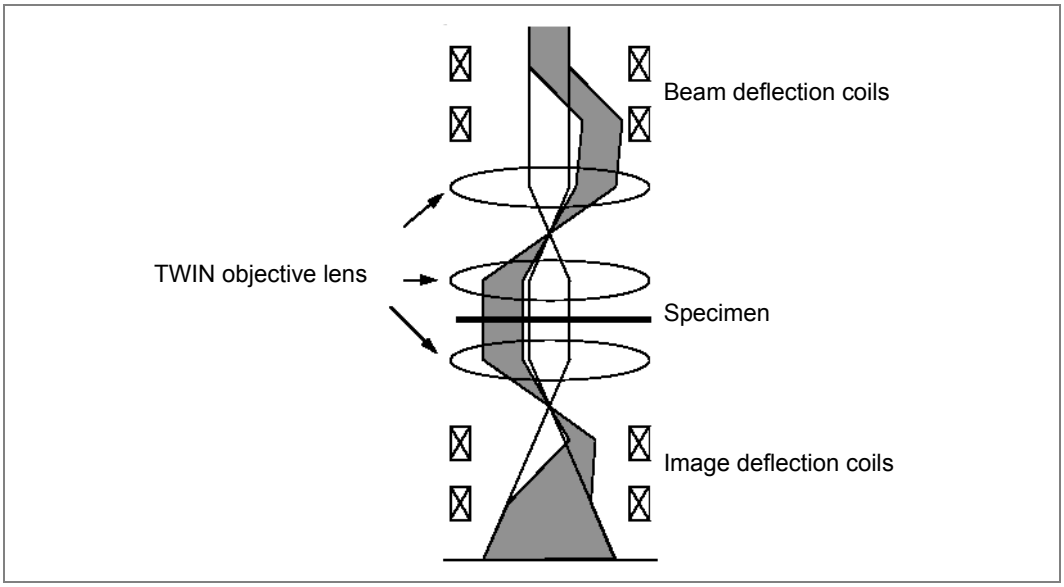
Figure 49 An Example of the Areas of the Three Low Dose States



- The *Search* state is set up for searching for suitable areas, usually at a low magnification (or defocused diffraction) with a small spot size to reduce the beam intensity. The Search state has an image alignment to allow accurate alignment with the Exposure state (area of interest).
- The *Focus* state is set up to allow image optimization (focusing, correction of astigmatism). In this state, optimization is performed at high magnification and high intensity at an off-axis part of the specimen, typically (but not necessarily) on one or two areas that are close to the exposure area (area of interest). The off-axis shift is defined and set with a distance and an angle. When the image is shifted off-axis, the beam will stay centered on the screen (a correction is made automatically to the beam shift, otherwise it would follow the image off-screen).
- The *Exposure* state is set up to the conditions that will be used for the actual exposure (and also defines the exposure time).

The switch between the areas of Search, Focus, and Exposure is achieved by a correct combination of the beam and image deflection coils as shown in *Figure 50*.

Figure 50 Low Dose Image Shift



For example, in the Focus state (see shaded ray-path) an off-axis area in a specimen is illuminated by the beam deflection coils and its image is shifted to the center of the viewing screen by the image deflection coils. In the Exposure state, neither the beam nor image deflection coils are activated, so the area around the optic axis is imaged.

Dark-Field Imaging

As seen before, the bright-field image (BF) is formed by beams including the central transmitted one, which has passed the specimen without having been significantly deflected. It is often more informative to form an image using only the deflected (diffracted) electrons, since these electrons have interacted more severely with the specimen. In other words, *dark-field* (DF) imaging is made by allowing one (or more) diffracted beam(s) through the objective aperture and blocking the central beam. A DF image has an inherent high and diffraction-selective contrast.

The DF image technique has the following applications:

- Simplifying the image by selecting a specific diffraction spot
- Improving image contrast and obtaining additional contrast information (e.g., determining nature of stacking faults)
- Aiding to the interpretation of diffraction patterns, especially in multiphase specimens having satellite diffraction spots

In principle, two different methods can be used for DF imaging (see *Figure 51*):

- *Off-axis imaging* by aligning the objective aperture around the diffraction spot of interest
- *Axial dark-field imaging* by tilting the incident beam so that the diffracted beam passes through the objective aperture along the microscope axis, while the central beam is blocked

Figure 51 Dark-Field Imaging

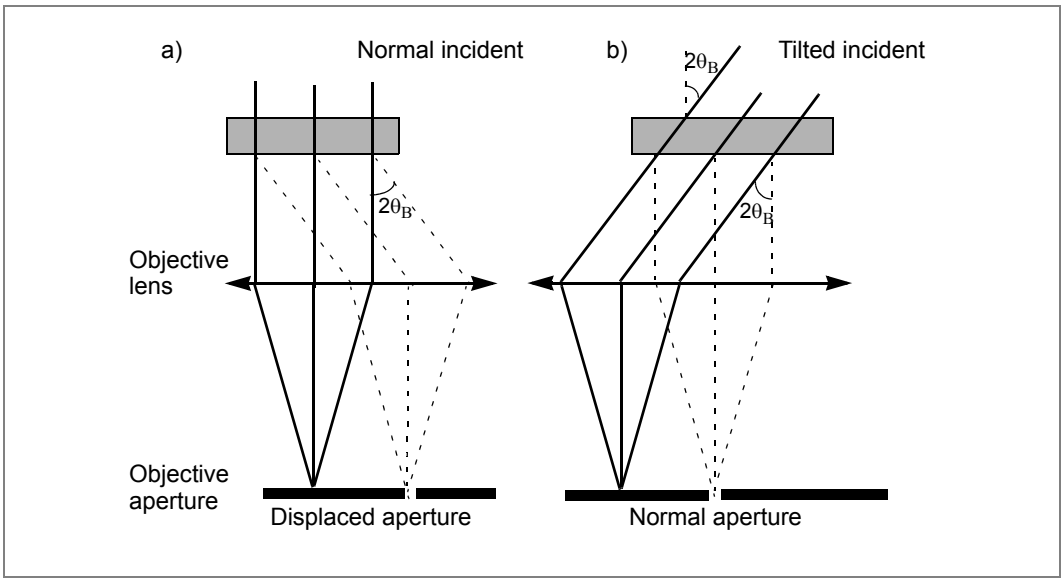


Figure 51 shows dark-field imaging by (a) displacing the objective aperture and (b) tilting the incident beam. Only one diffracted beam is shown in the figure.

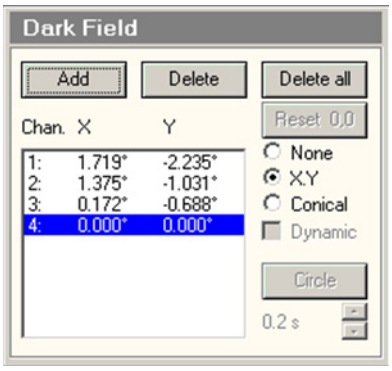
The axial dark-field imaging method is preferred because of the higher image quality obtained with on-axis imaging (least aberrations) and ease of use through simple change-over between BF and DF modes.

Off-Axis Imaging

The diaphragm is displaced from around the central spot in the diffraction pattern to around another spot or at the peripheral area of a diffraction pattern. Thus only those electrons that are scattered with a certain angle will contribute to the image (*Figure 51*a). The disadvantage of this technique is a loss of resolution due to the spherical aberration because those beams are nonparaxial. The radius of the circle of least confusion is $\frac{1}{4}C_s\alpha^3$, with C_s the spherical aberration constant and α the opening angle of the objective aperture. Displacing the aperture is equivalent to increasing a leading to a bigger circle of least confusion and lower resolution.

Axial Imaging, Tilted Beam Method

In this method, sometimes referred to as *high resolution dark-field*, the objective aperture is kept on the optical axis and the incident beam is tilted on the specimen, hence the diffraction pattern appears to shift across the objective aperture (*Figure 51*b). This operation is called axial or *centered dark-field imaging* (CDF). Because the electrons forming this dark-field image travel along the optical axis, no loss of resolution will occur.



There are two ways for beam tilting: X-Y and Conical (Dynamic Conical Dark-Field is available to Talos systems with STEM functionality). The beam tilts are set with the **Multifunction-X**, and **Multifunction-Y** knobs on the left and right-hand control pads.

The Conical mode is used to avoid asymmetries in the dark-field image by swivelling the direction of the tilt around a cone. This mode is a special submode of the tilted beam method, which allows high resolution dark-field imaging by all the electrons with a specific scattering angle of θ_B. This is achieved by automatically rotating the tilted beam about the optical axis. This technique is especially useful in imaging polycrystalline or amorphous specimens.

Switch between DF and BF images by successively pressing the **Dark Field** button on the right-hand control pad. When dark-field is active, the LED is lit.

Multiple settings for tilts can be stored in the dark-field channels.

Diffraction

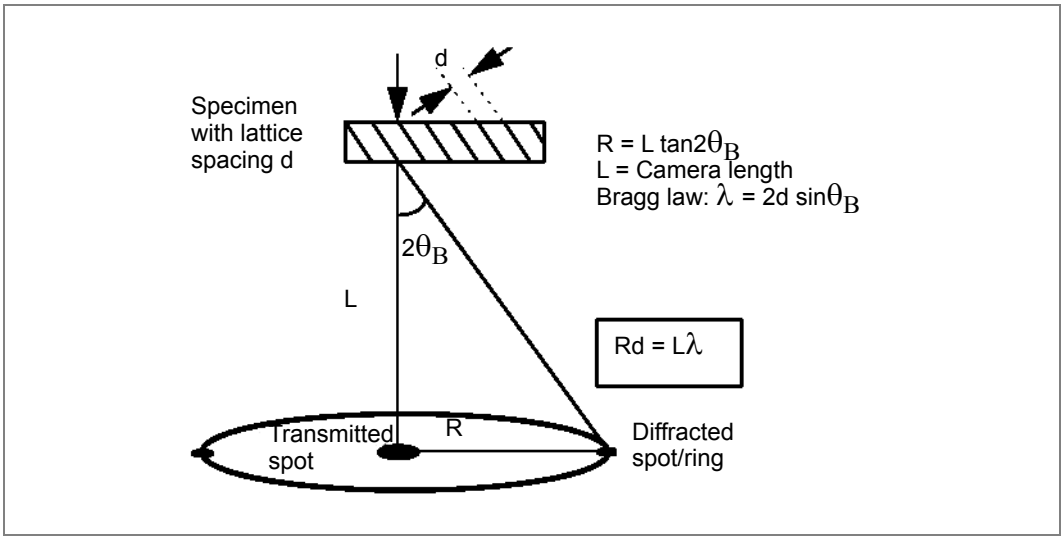
Press the **Diffraction** button on the right-hand control pad to switch to Diffraction mode. Instead of the selected area plane, the imaging lens system is focusing at the back-focal plane of the objective lens (see *Figure 48*).

A specimen with a *crystalline structure* (regular arrangement of atoms), will deflect incoming electrons in specific directions (Bragg angles θ_B). All electrons that are diffracted over the same angle and in the same direction will be focused at the same spot in the back- focal plane of the objective lens, which is further magnified by the imaging lens system.

A regular pattern of spots is formed for a single-crystal specimen or a specimen having a single crystal with a selected area. If the electron beam does not illuminate one single crystal but a cluster of randomly oriented crystals, the diffraction pattern will consist of concentric circles (which can also appear as a number of discrete spots lying on the concentric circles).

The distance from the central spot to a certain spot or the radius R of the circles is related to a characteristic spacing d between lattice planes in the crystal. *Figure 52* shows the relation between a diffraction pattern with a camera length of the electron microscope and the specimen lattice distance.

Figure 52 Specimen with Lattice Spacing



Selected Area Diffraction

If the illumination is parallel and the diffraction lens focuses at the back-focal plane of the objective lens, the diffraction pattern seen on the fluorescent screen will be sharp with small spots for a single-crystal specimen.

To obtain a pattern from a small area, a small illuminated area is needed, which can be partly achieved by appropriate settings of the condenser lenses. However, in normal diffraction work in order to have a parallel illumination, the beam cannot be extremely focused on the specimen to form a small illuminated area. The *selected area diffraction* (SAD) technique offers a solution to obtain crystallographic information of a small area by introducing an aperture in the first intermediate image (the image plane of the objective lens) in the selected area (SA) plane.

The observed diffraction pattern on the screen is now only formed by those electrons originating from the chosen small area of the specimen. Since the objective lens magnification is about 20 to 40 times, a 10 µm SA aperture will allow electrons from a specimen area of 0.50 to 0.25 µm to form a diffraction pattern. In other words, if an aperture is inserted in the objective image plane, it is equivalent to inserting an aperture in the plane of the specimen, but smaller by a factor of the objective lens magnification. At SA range of magnification, the first intermediate image is exactly in the SA plane.

Diffraction without SA Aperture

Instead of using a selected-area aperture to define the area from which a diffraction pattern is obtained, the beam can be located on an area of interest, either focused (convergent-beam diffraction, CBED) or defocused. There are several advantages to using the beam itself as area selection rather than the SA aperture:

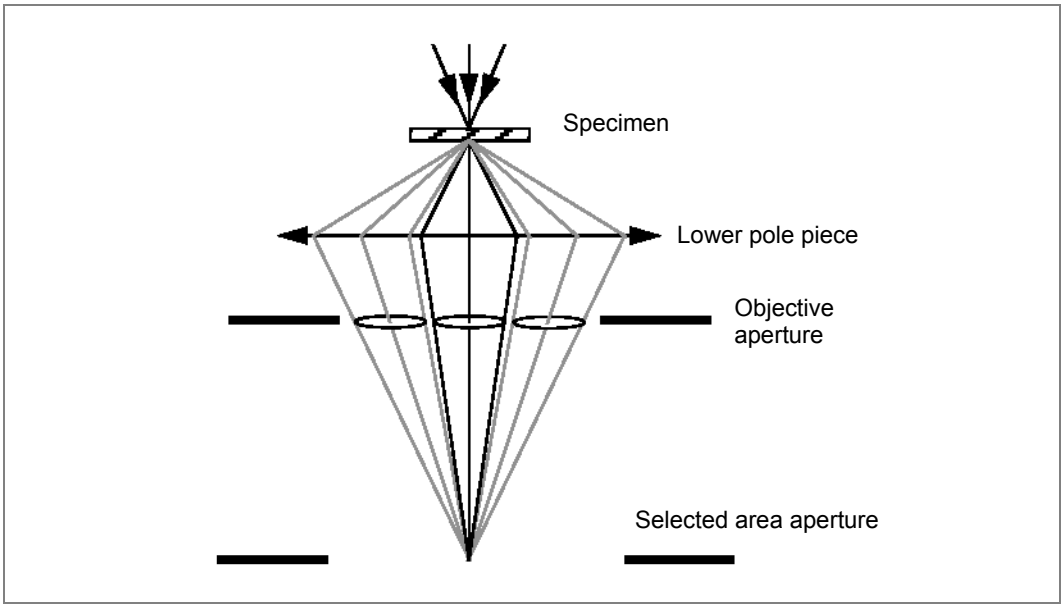
- The optical conditions are closer to those in imaging (which is especially an advantage in orienting a crystal for high-resolution imaging).
- There is often detail visible in the diffracted beams that makes orienting crystals easier (and more accurate) than with SA diffraction.
- Diffraction patterns can be obtained from small areas without the diffraction error of SA diffraction.
- The technique can be applied in Microprobe and Nanoprobe mode (SA diffraction is often difficult in nanoprobe).

The disadvantages of diffraction without an SA aperture are:

- Unless the beam is truly parallel the diffraction pattern contains disks in stead of spots (unless the diffraction pattern is defocused as well).
- With a strongly convergent beam, specimen damage by electron beam may occur.
- Especially with FEG, the beam is not homogeneous near its edge. Interference fringes and, especially the bright fringe near the edge, can give rise to a misleading ED pattern.

The procedure for aperture-less diffraction is simple: locate the beam on the area of interest and focus or defocus the beam (**Intensity**), then go to diffraction.

Figure 53 Focused Microprobe TEM Mode



In Microprobe mode, a micro-diffraction pattern is obtained using a condensing beam. The pattern consists of broad disc-like spots rather than sharp points due to the large convergence of the incident beam. The disc shape of a diffraction spot actually represents a range of the simultaneous illumination angles (*Figure 53*).

In addition to the improved spatial resolution, convergent beam diffraction (CBED) gives us more information than SAD, e.g., information on sample thickness, unit cell and precise lattice parameters, and the crystal system.

In Nanoprobe mode, the illumination can be focused to an even smaller spot size (Talos F200X: < 0.3 nm). The obtained pattern in diffraction mode is called the micro-micro-diffraction (μμD) or nano-diffraction pattern. This technique can be used for characterizing crystalline materials at the lower end of the submicron scale.

STEM

In the STEM (scanning TEM) mode, images are acquired by moving a focused beam across the specimen and by simultaneously collecting a signal at each scanning co-ordinate (x, y-pixels). The signals for all pixels make the image.

A wide range of detectors is possible for STEM. Some of these detectors produce a signal in a similar way to TEM imaging. For example, the bright field (BF) detector collects the same signal as the TEM BF image, i.e., the transmitted beam.

Other detectors such as the secondary-electron (SE) or energy-dispersive X-ray (EDX) detector can only be used with a focused beam because these detectors lack the optics (electron or X-ray) to separate signals from different areas of the specimen when a defocused (broad) beam is used.

Dark-field (DF), high-angle annular dark-field (HAADF), back-scattered electron (BSE) and EELS detectors are among the common STEM detectors. In principle, all detectors can be used to acquire STEM images.

Analysis

The strength of a TEM is that not only can it provide high-resolution images that can contain information below to 0.2 nm (Talos F200X), but it can also operate in various analytical modes (EDX and EELS). What is more, the use of small nanoprobes allows these analyses with a very high spatial resolution. These methods are referred to as microanalysis.

EDX Analysis

X-ray energy-dispersive spectrometry (EDX) analyzes the X-rays that are generated when the electron beam hits the specimen. EDX produces spectra that are plots of X-ray counts versus X-ray energy. When electrons ionize an atom, the emitted X-ray energy is unique to the ionized atom (element). So, EDX allows analysis of elemental composition of the specimen. However, EDX is sensitive to spurious signals, i.e., signals that have been generated outside the focused beam and thus outside the area of interest (signals from grid, holder, objective aperture, etc.). Some of these effects can be partially avoided by paying attention to the analysis conditions, but some spurious signals will always exist.

EELS Analysis

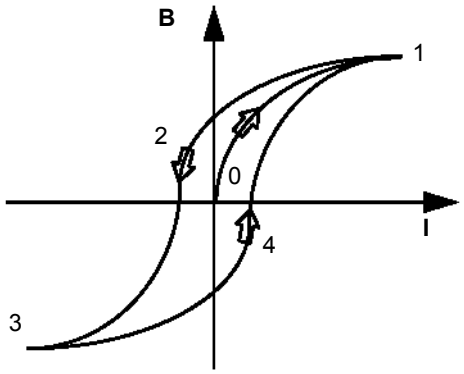
Electron energy-loss spectrometry (EELS) is the analysis of the energy distribution of electrons that have interacted inelastically with the specimen. Inelastic interactions highly depend on the electronic structure of the atoms in the specimen. This structure, in its turn, is governed by the nature of these atoms (elemental), their dielectric response, nearest-neighbor distributions, and bonding. With EELS, it is much easier to keep spurious signals from the detector.

Measuring and Reproducibility

Measuring dimensions in objects/diffraction-patterns is a very important and an often-used application in electron microscopy. Principally, measurements can easily be performed by laying a ruler across the features of interest in the micrograph and dividing the measured distances by a total magnification of the micrograph. The precision of this method is mainly determined by the accuracy of the magnification/camera length, which depends on the reproducibility and the accuracy of its calibration.

Objects of known size or lattice spacing can be incorporated into the specimen to provide calibration for images or diffraction patterns, respectively. An example of the latter consists of evaporating a thin layer of gold onto a crystalline material and comparing the rings of the diffraction from the gold with the separation of the spots from the crystal.

Figure 54 Magnetic Field Example



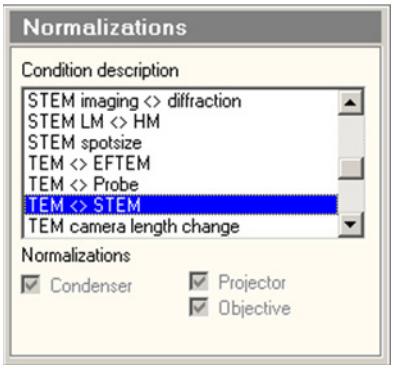
This shows magnetic field B at the optical center of a magnetic lens as a function of the current I through the lens coil.

Lens hysteresis and specimen height affect the reproducibility. Specimen height error can be minimized by keeping the specimen at the eucentric height, so it does not introduce any noticeable irreproducibility. Unfortunately, the lens magnetic field is not uniquely determined by the electric current in the lens coil because of the hysteresis effect, a physical property of lens materials. As shown in *Figure 54*, when the current I increases starting from zero (0), the magnetic field B also increases along the route of 0-1. The minus value of I or B in the figure means the change of direction. Once magnetized, the magnetic field of the lens follows the route of 1-2-3, when the current reduces and changes its direction, or the route 3-4-1 in the opposite condition.

NOTE	For a coil in a vacuum, there is no hysteresis.

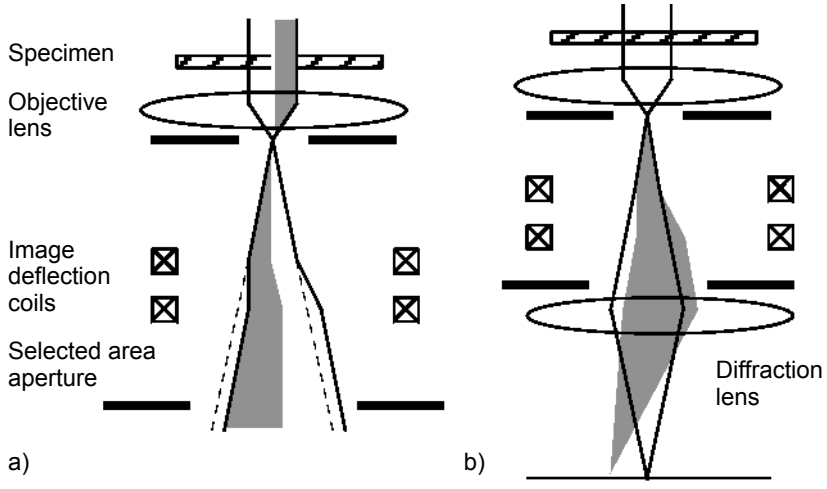
Normalization means that the current of all the lenses is momentarily brought to the maximum value (point 1 in *Figure 54*) before the original current is restored. So, the magnetic field then always follows the route 1-2-3, thus giving a high reproducibility of magnification and camera length (< 1.5%).

Normalization on Talos microscopes can be executed by hand (by assigning projector normalization to one of the control pad user buttons) and/or automatically. The automatic normalizations are determined by the settings selected in the Normalizations control panel.



The total accuracy attainable for measuring dimensions is equal to magnification/camera length reproducibility plus its calibration (inaccuracy > 2%). In the Talos microscopes, this drawback has been overcome by incorporation of an online measurement function. The operation of this system is based on the image deflection coils, which shift the first intermediate image in the SA plane or the diffraction pattern with extreme accuracy and reproducibility (*Figure 55*).

Figure 55 Online Measurement Facility



The post-specimen a) image and b) diffraction pattern deflection system forms the basis for the online measurement facility. The required currents are converted to distance in the image or diffraction pattern.

The measurement function is carried out by determining the image/diffraction pattern shift (given by the currents in the deflection coils) that is needed to adjust the points of interest onto a reference point. You must perform a calibration using a specimen with known dimensions in advance.

Apart from its higher accuracy, the advantage of the method is that it does not require time and money spent on photographs. The higher accuracy is due to the fact that the reproducibility (1.5% after normalization) is not a limiting factor anymore because this inaccuracy is related only to the imaging lens system below the objective aperture or the selected area plane.

Since the objective lens current is always adjusted so the first intermediate image is exactly in the SA plane or the diffraction pattern in the objective aperture plane, this lens introduces no irreproducibility error. The online measurement can be performed with an accuracy of approximately 0.5% or less, mainly determined by the measurement calibration.

Understanding Image Contrast

For an electron microscope, variation in image contrast (not the image intensity) determines the quality of, and the retrievable information from, an image.

There are three contrast mechanisms in electron microscopy:

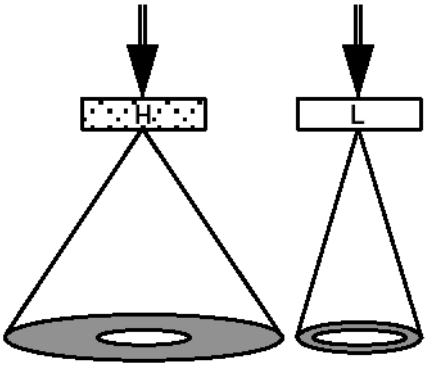
- Mass-thickness contrast, see below
- Diffraction contrast, see *page 84*
- Phase contrast, see *page 85*

In this section, we will begin with a brief introduction of the first two and relatively simple mechanisms. Then we will describe the phase contrast in a simple way and explain a very important criterion in applications for focusing the specimen: the minimum contrast.

Mass-Thickness Contrast

Mass-thickness contrast bears a close resemblance to optical microscopy, in which contrast is produced by variation in optical absorption from point to point at the specimen. However, the electrons are not absorbed by the specimen but scattered outside the objective aperture and do not contribute to the image. Thus, the region with higher scattering power (heavier constituent elements or greater foil thickness) appears darker in intensity.

Figure 56 Mass-thickness Contrast



The difference in scattering power and an objective aperture produce the mass-thickness contrast.

Figure 56 is a schematic representation of this contrast mechanism, where the specimen region with higher scattering power is labelled H and the lower one marked L. More electrons are scattered to higher angles by H than by L, though the electron distribution declines quickly with the angles. The total number of electrons reaching the base plane of the illumination cone in *Figure 56* is the same for both regions, as long as both are equally illuminated by the incident beam.

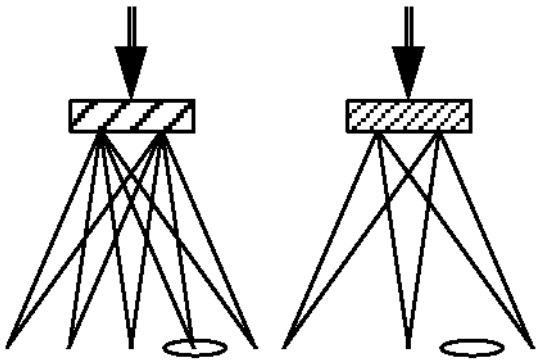
When an objective aperture (the bright circle in the figure) is used, the number of electrons contributing to the image will be less from H than from L, so in the photograph H appears darker than L. Even without physically inserting an aperture, the limited size of the lens-bore always acts as one and some mass-thickness contrast may be seen.

The contrast mechanism is applicable to most biological studies, to the conventional applications at low and moderate magnification. The mass-thickness contrast can be enhanced by the use of smaller objective aperture and of lower accelerating voltage. The former is because of the very high concentration of electrons at small scattering angle, and the latter causes electrons to be scattered to higher angles so that a particular objective aperture size is more effective in removing scattered electrons. Also, the total scattering cross-section at lower kV is greater than at higher kV, so more of the electrons are scattered at all angles.

Diffraction Contrast

Diffraction contrast is a term often mentioned by the material scientists in study of crystalline specimens. A crystal has a few preferred directions (Bragg angles/reflections) for scattered electrons, which results in bright spots in a diffraction pattern. Crystals with different structures, as well as the same crystals with different orientations with respect to the incident beam, create different patterns of Bragg reflections. That difference causes image contrast, as shown in *Figure 57* schematically, where two crystalline specimens have different preferred scattering angles. Within the objective aperture shown in the figure, there are no electrons scattered by the crystal on the right, so its image appears darker than that of the left.

Figure 57 Diffraction Contrast in Dark-Field Imaging



The difference in intensity of electrons scattered into Bragg-reflections from various parts of a specimen produces diffraction contrast.

Diffraction contrast is widely used to study defects in crystals, because the regions around the defects have Bragg angles differing from the perfect crystalline matrices and thus produce contrast to be observed.

Such features as bend contours and thickness fringes are the result of diffraction contrast. Diffraction contrast is strongly dependent on orientation; whereas mass-thickness and phase contrast are not.

Phase Contrast

Phase contrast is essential to understand imaging of unstained biological cryo specimens. Such a specimen consists of a suspension of biological objects embedded in a slab of amorphous ice, so there is not much difference in scattering between the objects of interest and the background. In the ideal case, this specimen is illuminated by a perfectly parallel beam of mono-energetic electrons, and in the Krios with a FEG source, the real situation is not far from the ideal. Such illumination can be understood as a plane wave, where the frequency and phase of the wave is the same for every position at the top of the specimen. As the wave propagates through the vacuum above and below the specimen, its form is unaltered, but as it passes through the specimen, it is changed.

On one level the specimen consists of a collection of nuclei and electrons, each of which generates a Coulomb potential, and the potential at any given point is the sum of the potentials of all the constituents. Since the potential from each nucleus or electron falls off as 1/r, the potential at any point depends on what kinds of atoms are near that point and how distant each of them is. As the wave propagates through the varying potential, a fraction of it undergoes changes in its phase and direction of propagation. If the potential is positive, the electron speeds up (because its total energy is conserved, and due to its negative charge, its potential energy is decreased), so its phase advances, and the opposite is true if the potential is negative.

At the bottom of the specimen, that fraction of the wave that has interacted with the potential (the scattered wave) emerges in a form where the phase at each point has shifted by the integral of the shift at each point along its path, and there is a distribution of directions of motion, so the wave is not completely parallel. The part of the wave that has not interacted (the unscattered wave)—most of the wave in the case of practical interest—has not changed. As the wave continues to propagate through the imaging lenses, the scattered and unscattered waves are recombined to form the image, and the intensity at any point depends on how the two waves add. If the phase and direction have not changed, the waves add to their initial intensity, but if the phase has changed, the intensity of the combined waves is less, and this decrease in intensity is greater for greater phase changes. (The last part is true for the small changes seen in biological cryo specimens, but for large phase changes, the intensity can oscillate, which, for example, causes thickness fringes in the images of crystalline wedges.)

Wave and Coherence

To understand phase contrast, we need to define a *wave*. Any radiation is wave-like, whether it is visible light, X-ray, or electron radiation. The typical behavior of a wave is the oscillation, a kind of periodic vibration. Two physical quantities are employed to characterize a wave: amplitude and phase. The *amplitude* is the maximum elongation for such an oscillation and the *phase* determines how a wave oscillates spatially and temporally.

Figure 58 Wave

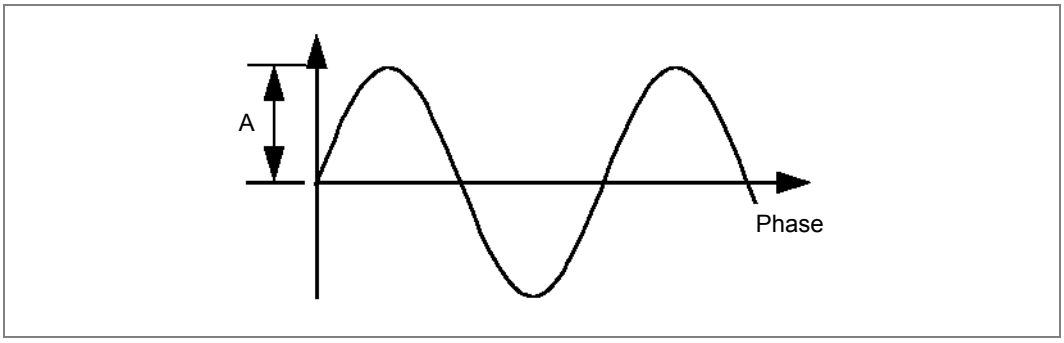


Figure 58 is a diagram showing a wave graphically, where the horizontal axis is the phase and the maximum oscillation vertically represents the amplitude. Mathematically, a wave is represented by amplitude A and phase in a form of a complex number, with i representing the imaginary part: $Ae^{i\theta} = A(\cos\theta + i\sin\theta)$.

The physically detectable quantity of a wave is its *intensity*, the square of its amplitude. In normal photography, only intensity is recorded and the phase information is lost. When two waves meet, they add up in different ways, depending on their coherence.

Coherence can be understood as a measurement of independence of the two waves. If they are completely incoherent (independent), the resulting wave intensity is a sum of the intensities of the two individual waves. The typical example is two normal light bulbs, which are absolutely independent of each other. Mathematically, if I represents the resulting wave intensity, $I=I_1+I_2$. (This is the result of adding many waves with uncorrelated phases.) If waves are coherent, the resulting wave is a sum of the two waves, and the resulting intensity is not necessarily higher than any individual wave intensity but depends on the phase difference of the two waves. With the above wave expression, the new wave has a form of: $Ae^{i\theta} = A_1e^{i\theta 1} + A_2e^{i\theta 2}$.

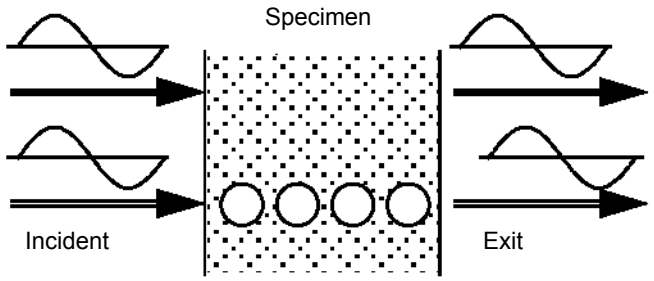
The electron source of a microscope is close to complete coherence. To make it better, which is necessary for high resolution electron microscopy, a parallel (better spatial coherence) and stable (better temporal coherence) beam is ideal.

A point source is equivalent to perfectly parallel illumination since one can be transferred into the other by a perfect lens. The smaller the volume in phase space (position + momentum coordinates) occupied by the electron beam, the higher the coherence.

Phase-Object

A *phase-object* refers to a specimen so thin that when a wave passes through it, only the phase changes but the amplitude keeps its original value. Many medium-thin biological specimens and thin material specimens belong to this category.

Figure 59 Phase-Object



When an incident beam passes a phase-object, the phase of the exit wave has been modified but not the amplitude.

Figure 59 schematically shows that two waves transiting a phase-object, one goes through the atomic column and another through the region between atomic columns. They gain a phase difference through the different interaction between the waves and the atoms. The two waves have equal amplitude and equal phase initially, but only equal amplitude after passing the phase-object. Due to this, there is no intensity/amplitude variation in the exit wave, and a minimum contrast image is observed when the exit wave is properly imaged at the in-focus condition.

When doing the column alignment procedure of a Talos microscope, operators are frequently asked to focus the specimen. The operation brings the object in focus. The criterion is that the thin specimen should have the minimum contrast, because the object can be treated as a phase-object. Normally, an edge of thin carbon film is ideal for such minimum contrast focusing. However, if a small objective aperture is used at the same time, the minimum contrast is less obvious because the mass-thickness contrast also exists. In practice, the minimum contrast is less sensitive to the focus changing at lower magnification.

In addition to minimum contrast, there are several other ways to determine focus:

- At high magnification, the FFT will have the greatest extent of the central disk at focus.
- If the image does not shift when the beam is tilted, the specimen is in focus.
- When the interference fringe at the edge of an object disappears, the image is in focus.
- Often crystals appear dark with bright shadows along side them; when the image is in focus, the bright shadows overlap the dark crystal (which provides minimum contrast).

At LM mode, for which the objective lens is almost switched off for small magnification, the minimum contrast is even more difficult to recognize. In case of doubt, the wobbler can be used to assist focusing the specimen.

Phase Contrast

If two exit waves in *Figure 59* are incoherent, there will be no image contrast because the amplitudes of both exit waves are equal. On the other hand, if the illumination is coherent, it is possible to reveal the specimen structure information by analyzing the phase changes after a specimen. In this case, the recorded image intensity will depend on their phase difference, which bears the structure information we need.

The image contrast, because of the phase change, is called *phase contrast*. For a reliable analysis, the phase change by the specimen should not be altered further by the optical system. Unfortunately, the electron optical system is not perfect. An intrinsic (and perhaps the most important) imperfection comes from the spherical aberration of the objective lens.

The phase shift by the spherical aberration is not homogenous, but scattering-angle dependent, so the exit wave phase is distorted. To compensate the distortion, an underfocus is employed. The optimum underfocus, at which all the phase distortions by the optical system are approximately equal, is called *Scherzer focus*. It is frequently referred to in high resolution electron microscopy applications.

To increase the phase contrast of noncrystalline specimens, defocus away from the in-focus or the minimum contrast condition. By doing so, you artificially introduce imperfections to the electron optical system. The image no longer properly represents the true structure, however, and the image intensity is no longer nearly constant across the specimen.

Quantitative Imaging

For the first 50 years or so, electron microscopy was used to produce images that were examined visually to see what particular structures looked like and often how these were located in relation to other structures. As long as an image showed the structures of interest clearly, it was satisfactory. These can be called “qualitative images.” Recently, however, images have had not only to show particular structures, but they have been subjected to processing to improve the information that can be obtained. Such processing requires that the values of the intensity at each point of the image be known as precisely as possible, thus they can be called “quantitative images.”

Processing is used to produce so-called “structure images” from images of materials by removing the effects of aberrations of the lenses and obtaining information beyond the point resolution out to the information limit, and it is essential in biological imaging for both single-particle analysis and tomography. In each of these cases the information in many images is combined and often subjected to procedures such as inversion of the contrast transfer function, determination of the differences in defocus for different parts of an object, modeling of an average object from a large number of images, and removal of images that are not sufficiently close to the model, indicating that they have been damaged or are otherwise unsuitable. Quantitative imaging has stricter requirements, both in specimen preparation and in setting up the parameters of the microscope, than does qualitative imaging.

Understanding Diffraction

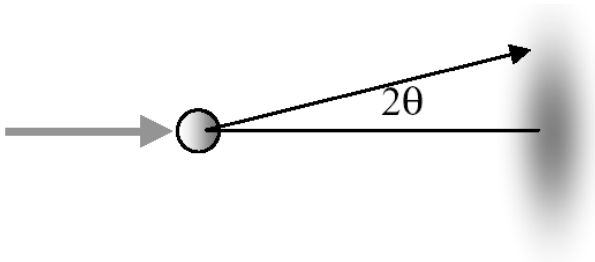
Elastic Electron Scattering

Although the word “diffraction” is more frequently mentioned in materials science, it is not restricted to that field. In fact, one may observe diffraction patterns from any kind of specimen, whether it is single crystalline silicon or a thin kidney section. Probably, “scattering” is a plain word equivalent to “diffraction” in describing the phenomenon within the electron microscope. A diffraction pattern is actually the angular distribution of the incident electrons being scattered by the specimen.

Electrons are scattered by atoms in a specimen when they pass through it. *Elastic scattering* refers no energy exchange when this happens, so the electron does not lose its energy. Contrary to that is *inelastic scattering*, where energy exchange is involved during the scattering process. A simple model to describe the elastic scattering is by either a single atom, or a group of independent atoms.

The elastic electron scattering power depends on the specimen density, Z number and its thickness. That is the principle of mass-thickness contrast. The phenomenon is called *Rutherford scattering*, after the name of a physicist. The Rutherford scattering strongly peaks in the forward direction and rapidly damps with the scattering angle 2θ (*Figure 60*). Even for a heavy atom like gold, at a scattering angle of only 3º the scattering power reduces to < 10% from its peak value at $2\theta=0$.

Figure 60 Rutherford Scattering



Rutherford electron scattering power rapidly reduces with the increase of the scattering angle.

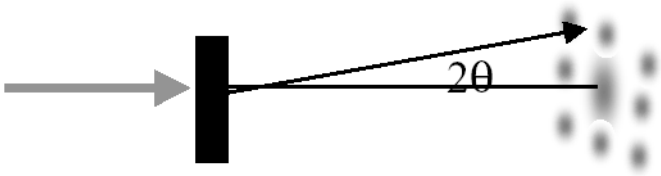
In fact, the independent atomic group means the atoms are irregularly arranged, such as most biological samples. When atoms are regularly arranged, as in the case of crystalline structure, another kind of scattering happens. This second type of scattering is termed *Bragg scattering* or *Bragg diffraction*.

If the atoms are regularly arranged, the strong Bragg scattering occurs only at certain scattering angles. Bragg associated the angles of strong scattering with the distance between atoms (or rather atomic planes) within the crystal structure. If the atomic plane distance is d and the wavelength is λ, then the strong scattering should appear at an angle of 2θ which satisfies:

$2d \sin(\theta) = n\lambda$ (n=1,2,3..)

The formula is called *Bragg's law* and it is very basic for crystallography and diffraction. According to Bragg's law, apart from the strongest forward direction, electrons are also peaked at a few Bragg angles. The chance of electrons going to the other angles is extremely low. In a language of wave physics, this is due to the anti-phase interactions, namely the counteraction of waves meeting at the spots. A regular pattern is formed, as shown in *Figure 61*.

Figure 61 Bragg Scattering



Bragg scattering creates sharp spot at Bragg angles.

Bragg scattering only happens at a small scattering angle, however. Once 2θ is greater than, for example 3º, the Bragg scattering is almost negligible. If the regular arrangement of atoms does not exist, the Bragg diffraction does not occur. The corresponding diffraction pattern should be similar to that in *Figure 60*.

It looks like Rutherford scattered electrons are independent of the others, while Bragg scattered electrons are strongly related. For this reason, Rutherford scattering is also referred to as *incoherent scattering* while Bragg scattering is also referred to as *coherent scattering*. The same words are used to describe the nature of wave and the quality of the electron source. Please note the difference.

What is commonly called a diffraction pattern is nothing other than Bragg diffraction. If the sample is not crystalline, the Bragg pattern is not so apparent. In the case of amorphous (materials with atomic arrangement semi-regularly between the crystalline and the irregular) no sharp pattern is observed but the broad rings. This is frequently seen on carbon supporting film of TEM samples. In other words, the pattern is created by partial Bragg diffraction.

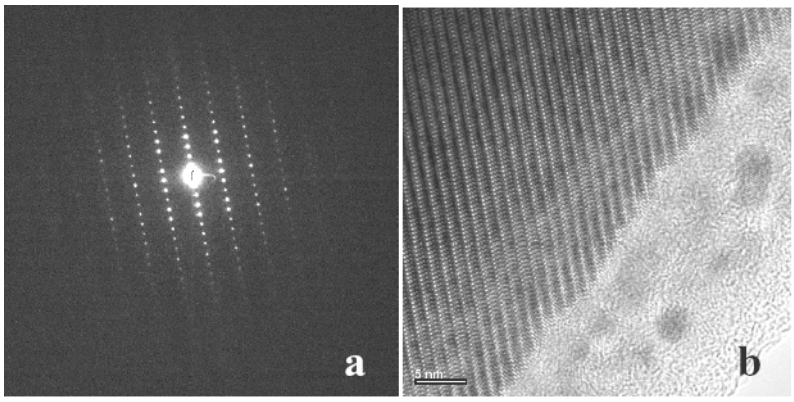
In the materials referred to, there are many identical, or nearly identical, interatomic distances but with random orientations. Waves scattered from different pairs of atoms with the same distances and orientations will interfere constructively, and those having the same distance but a different orientation will contribute intensity at a different point with the same scattering angle. This results in a ring where all orientations are present. Furthermore, since inter-atomic distances vary, the ring is broadened.

In the other extreme case, the totally irregularly arranged atoms only result in Rutherford scattering patterns. It is almost true if your sample is, for example, a thin section of kidney. Now, you may understand why we said before any sample may give a diffraction pattern if you accept that *diffraction* is the angular redistribution of incident electrons being scattered by the sample.

Typical Diffraction Patterns

For a crystal, the Bragg diffraction is more prominent and meaningful, while Rutherford scattered electrons only contribute as a background.

Figure 62 Diffraction Patterns

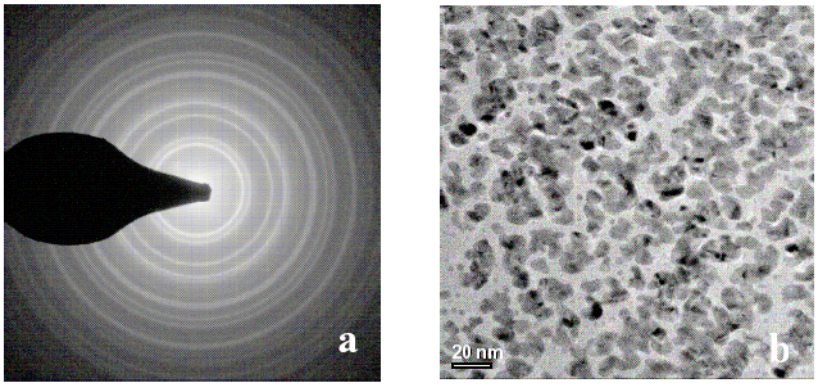


Diffraction pattern of YBaCuO (a) and the corresponding high resolution image (b).

In *Figure 62*, “a” is a typical diffraction pattern obtained from superconductor YBaCuO recorded with a CCD camera, and “b” is a corresponding phase contrast TEM image showing the crystalline fringes. In the diffraction pattern, the central spot (the forward direction or $2\theta = 0$) is so strong compared to the other spots that the CCD pixels were actually saturated and artifacts may be observed. The largest Bragg angle with recognizable intensity in *Figure 62*a is < 2º.

If there are many small crystal particles within the illuminated area, the structure is called *polycrystalline*.

Figure 63 Poly-crystal Ring-like Diffraction Pattern

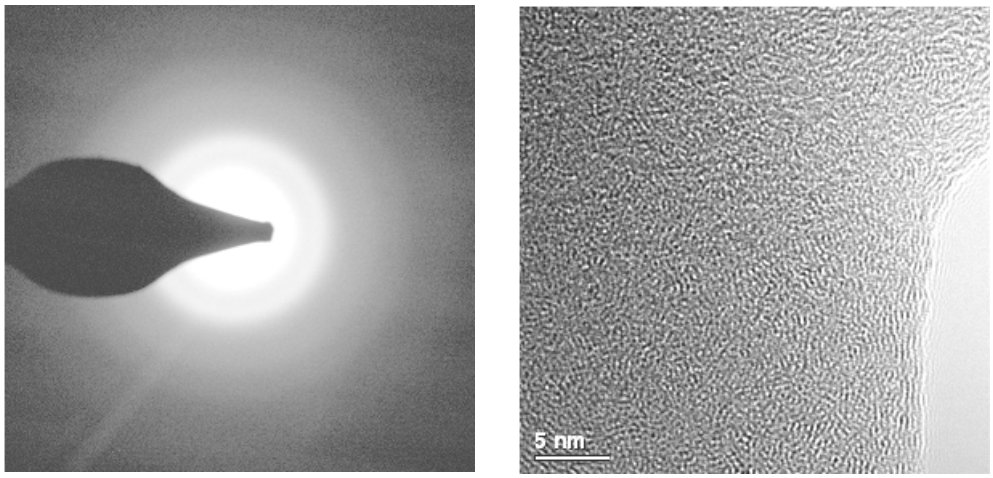


The poly-crystal ring-like diffraction pattern (a) obtained from many gold particles (b).

In (b) the darker gold crystals are in a zone-axis orientation, whereas the lighter crystals are not.

As shown in *Figure 63*b, the gold particles appear darker than the carbon support film due to the mass-thickness contrast. The diffraction pattern consists of concentric rings around the central transmitted beam. The beam stop was used during the acquisition to block the strong central beam so that diffraction rings may be seen clearly. You may think that the rings are generated by the rotational average of the spots similar to those in *Figure 62*a. Because there is no preferred orientation, the particles are sitting differently in azimuth. The results will be a rotation average. More accurately, the average is over a three-dimensional assortment of crystal orientations rather than a two-dimensional one, and this will lead to more rings than would be produced from a rotational average of any particular two-dimensional pattern of spots. *Figure 62*a is a typical ring pattern obtained from many gold particles in random orientations.

Figure 64 Diffraction Pattern from an Amorphous Carbon Film



The diffraction (a, left) from an amorphous carbon film (b, right).

Figure 64 shows the diffraction pattern (a) from an amorphous carbon film (b). A diffuse ring can be observed, which reflects its amorphous nature. In fact, it relates to the nearest C-C atomic distance of 0.34 nm. Due to the amorphous nature, no clear crystalline fringes, like those in *Figure 62*b, may be observed in *Figure 64*b.

For most biological specimens, the Bragg diffraction does not exist. In principle, the diffraction is mainly Rutherford scattering as shown in *Figure 60*. Due to the poor scattering power of the light elements, the damping with the increase of the scattering angle is much faster than, for example, that of gold. In most cases, there is a thin amorphous carbon film to support the biological sample. Therefore, a weak amorphous diffraction pattern, similar to *Figure 64*a may be observed.

It is worth mentioning that in this section, only elastic scattering has been discussed. Inelastic scattering, which involves the energy exchange between the incident electron and the sample, leads to the diffusion of diffraction spots/rings and the formation of Kikuchi patterns. The topic will not be addressed further here.

Applications of Diffraction Pattern

We know now what a diffraction pattern is and what they should look like for different specimens. It is easy to accept it as a measure of crystallinity. When pressing the **Diffraction** button on the control pad, you can easily tell if the sample is single crystal or noncrystal, without the need to go to high magnification to study the sample. Actually, this is a very powerful and fast application since a pattern may be obtained in seconds, while the other diffraction techniques normally need hours to collect a pattern.

Since the back focal plane of the objective lens is the plane of diffraction and also the plane (or close) of objective aperture, a diffraction pattern is employed to align the objective aperture. In diffraction mode, when the aperture is inserted, one may see the pattern is cut-off by a shadow, resulting from the aperture. To align the aperture means to align the shadow centering to the transmitted beam (bright field) or a diffracted beam (dark-field).

A diffraction pattern contains the structural information, which is more attractive to us. Based on the Bragg's law, a more practical expression of diffraction is written as:

$Rd=L\lambda$

Here R is the distance of a diffraction spot (or a ring) from the central spot measured on a recorded diffraction pattern, d is the atomic plane distance that might be unknown and to be determined, L is called camera length that is equivalent to the magnification of diffraction pattern and readable from microscopes, and λ is the electron beam wavelength. With the help of this, it is possible to determine the atomic plane distance, and so the structure. Of course to do so you need not only one, but a few patterns to construct a 3-D view.

In dealing with a single crystal, you may recognize the orientation of the crystal by reading the diffraction pattern according to the plane distance and the angles between the planes. In high resolution work, where crystal orientation is crucial for high quality images, we use this for tilting crystal to a needed orientation.

4 TEM Optimization

Overview

This chapter deals with the optimization of the Talos microscope performance and operation. It covers how to diagnose a particular misalignment and what action to take and it includes tips not normally found in user guides.

Introduction

TEM optimization involves the alignment of the electron optical elements, the various apertures and correction of lens astigmatism. The major reasons for aligning a microscope are convenience and improved resolution. For instance, misalignment of the illumination and imaging system will lead to movement of illumination and image, respectively, when changing the magnification. Further, a shift of beam should only result in a pure shift and no tilting should occur, and vice versa (pure tilt). It is important, especially in high resolution work, to operate with a circular spot of illumination and with maximum brightness. Also, alignment of image or diffraction pattern with detectors is essential. Alignment settings are dependent on the actual mode of operation (e.g., LM, HM, STEM, Nanoprobe), as different lens excitations, lens configurations, and deflection coils are used.

When to Optimize the Microscope

Before microscopes are shipped, they are mechanically and electronically aligned thoroughly within the factory to satisfy Thermo Fisher's high quality standards. After installation at the customer's site, electronic alignment is still needed now and then. Notably, after exchanging a filament, the gun alignment needs to be done to ensure the best illumination. Some alignments, e.g. rotation center alignment, need to be done for individual experiments. The whole column alignment should be checked once a month and be performed once in every six months to ensure the ultimate performance of the microscope.

In other words, good alignment of a microscope is the prerequisite for a high quality outcome, even though a non-optimal microscope can also provide an image. A quick check of the alignment can be done by focusing an image at LM and keeping the illumination centered. It should then be possible to increase the magnification to a maximum with the image remaining centered. The ability to remain centered on the viewing screen is a measure of the accuracy of the alignment of the imaging lenses (diffraction, intermediate and projector lenses). If the objective lens is not correctly aligned, the image will sweep about a point other than the center of the screen. If properly aligned, the image at the center will not be displaced. See also *Table 9 on page 106* for misalignment diagnosis and alignment actions.

Correction of the objective lens astigmatism is crucial to obtain high quality photos, especially at high resolution. However, it changes with the local magnetic field, which can be due to the specimen itself, its position, and contamination of the objective aperture. The best astigmatism correction must be done under the particular microscope conditions with the specimen close to the area where the photos are going to be taken.

What To Do for Optimization

In the Talos microscopes, control panels equipped with online help and action instructions are devoted to alignment actions. Alignments can be performed using the control panel for Alignment procedures or the control panel for Direct Alignments (see *"Alignment Procedures" on page 98* and *"Direct Alignments" on page 106*, respectively). Correction of astigmatism of condenser, objective and diffraction lenses can be done via the Stigmator control panel. This panel can also be activated by pressing the **Stigmator** button on the left control pad (*"Stigmators" on page 108*).

Talos Alignments

When a user logs into the microscope (by starting the Talos user interface), the microscope will recall the necessary alignments. Alignments will exist for a particular user if the user has ever executed (part of) an alignment procedure or used Talos Alignments to save an alignment.

Alignments are always saved as a single block (so even if a user changes only one parameter, the whole alignment will be saved for that user). There is only one alignment present for any particular user at a time. If more than one alignment is necessary (for example, for different conditions), use Talos Alignments to save and reload alignments. User alignments are located in the Windows registry. A user can thus make multiple alignments. Users can also copy alignments from other users (provided those users saved their alignments using Alignments as well). All alignments saved by Alignments are together and will be visible in the Alignments list.

Aperture Centering and Eucentric Height

It is of importance to operate the microscope with correctly positioned apertures. For example, if the condenser aperture (C2) is misaligned, the expanding or contracting beam will not stay centered on the screen. Also, it will lead to unreproducible illuminating conditions, as it results in a beam tilt and thus a change in rotation center. Correct positioning of the objective aperture and the diffraction aperture is essential. An off-center of the objective aperture can cause astigmatism in the image. For a correct centering of these apertures, see Talos Help hyperlink: *Aperture Centering*.

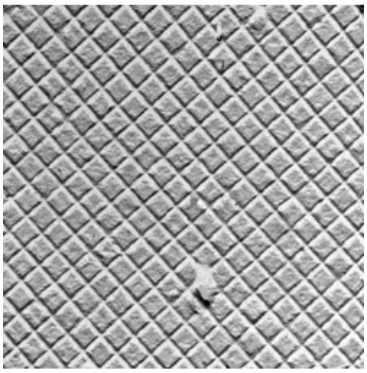
Eucentric height is important in microscope operation. It is not only convenient that the area of interest at this column height stays centered when tilting around the tilt axis, but it also defines a reference value for the objective-lens current. The normal changes made to the objective lens for focussing have little effect, but stronger changes (changes in focus by several tens of micrometers or more) can have considerable effect on proper alignments and on the effective magnifications and camera lengths. The objective lens not only focuses the image but also contributes the largest magnification of any lens in the system; strong changes in objective lens current change this magnification and thereby also the final magnification and camera length.

The eucentric height can be found by activating the wobbler of the CompuStage, using the maximum available tilt angle. Sideways motion of the image (details) are minimal at eucentric height. The specimen can be put at this height using the **Z-Axis** height control button on the left control pad.

Alignment Specimens

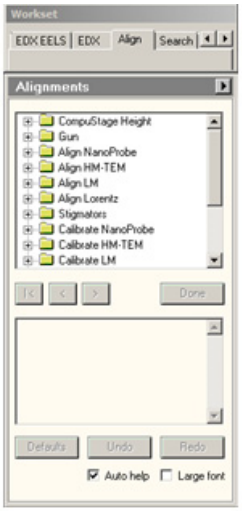
In principle, microscope optimization can be performed using any specimen, but a few types of specimens are particularly useful if microscope alignment is to be done completely.

The cross-grating specimen is one of the best specimen. It consists of a thin carbon foil on which a square pattern of gold particles has been deposited. Because of the crystalline nature of gold, the specimen is easy to focus in all modes. Each square has a fixed dimension (2160 lines/mm, hence 463 nm per square) that is useful for measurement calibrations. Also, the diffraction pattern shows a series of concentric rings, which can be used for calibrating diffraction measurements. However, alignment of objective stigmator and image coils pivot point are difficult to do. In practice, it is normally possible to find identifiable imperfections on the cross-grating that can be used for these alignments.



Alignment Procedures

The alignment procedures of the Talos microscopes are accessible through the Alignment control panel. Alignment procedures may differ depending on the level of user, with Expert and Supervisor levels having more accessible alignments. The procedures, split into logical units as Gun, HM-TEM, HM-STEM, etc., are displayed in a tree view, as shown below.



Each procedure contains subprocedures. Procedure selection is done by clicking on the + or double-clicking the name. The tree view branch discloses its subprocedures, which are accessed also by clicking. Navigate through a particular (sub) procedure by using the buttons on the control panel (|<, < or >) or the user- assignable buttons on the microscope control pads. Sub-procedures that follow the standard alignment sequence (they are not skipped) are designated by . Less often used subprocedures (), i.e., alignments that are not sensitive to changes in operating conditions (e.g., pivot points) are skipped automatically, but can nevertheless be accessed by simple clicking.

All subprocedures have online help pages that describe the purpose and operation of the particular alignment. Help pages can be called by pressing **F1** while the subprocedure is active. Also, it is possible to select the Auto Help function: the online help is displayed and updated automatically.

The Alignment control panel displays the instructions for the active alignment step. The convenience of performing a procedure is that an operator can follow the instruction step by step until the end. Note, however, that specific alignments are not changed by simply navigating through a procedure; only by actually changing the settings (multifunction knobs, or sometimes even **Focus** and **Intensity**).

The changes are stored after exiting the (sub)procedure (after last step or by clicking **Done**). The changes within a certain subprocedure can be undone by clicking **Reset**, which resets the active alignment to the setting it had when the step was entered. **Restore** allows an undo of the **Reset** button.

Talos microscopes incorporate, depending on the specific hardware configuration of the instrument, the following alignment procedures:

- Gun
- Align NanoProbe
- Align HM-TEM
- Align LM
- Align Lorentz
- Stigmators
- Calibrate Nanoprobe
- Calibrate HM-TEM
- Calibrate LM
- Calibrate Lorentz
- Dyn. Conical DF MicroProbe
- Dyn. Conical DF NanoProbe
- HM-STEM
- STEM MicroProbe
- LM-STEM

For most alignment procedures, it is of great importance to operate under the correct conditions, i.e., centered C2 aperture, and a focused specimen (image) at eucentric height.

To successfully execute the alignment procedures, the following tips will prove very helpful:

- Use a specimen that is relatively irradiation-damage resistant with good contrast. You can always change back to your desired specimen when the procedures have been finished.
- Set demanded high tension (High Tension) and emission (FEG Control or Filament) and adjust the specimen eucentric height before starting a procedure.
- Whenever the beam spot is out of the viewing screen or too weak to be seen, lower magnification until the spot is found or spread the beam by defocusing it. Readjust the illumination (**Intensity** and LTb beam shift) while restoring the magnification back to the original one.
- In the online instructions, "Focus spot" means making the illumination spot smaller by turning the **Intensity** knob.
- "Focus specimen" means minimizing the image contrast by turning the **Focus** knob, which is most obvious without the objective aperture.

Table 8 summarizes the essential alignments that need to be performed for optimized microscope operation for different modes. Note that eucentric height is needed for all operations.

Table 8 Optimization Techniques

20-50,000x	>50,000x	HREM	Dark Field	Diffraction	Measuring	Nanoprobe	STEM
Check gun C2 aperture Objective aperture	All at left Rotation center Objective astigmatism	All at left Astigmatism Pivot point Coma free	Beam tilt pivot point	Diffraction astigmatism	Calibration	Spot size Rot center C2 astigmatism C2 aperture	All at left Beam shift pivot point Detector alignment

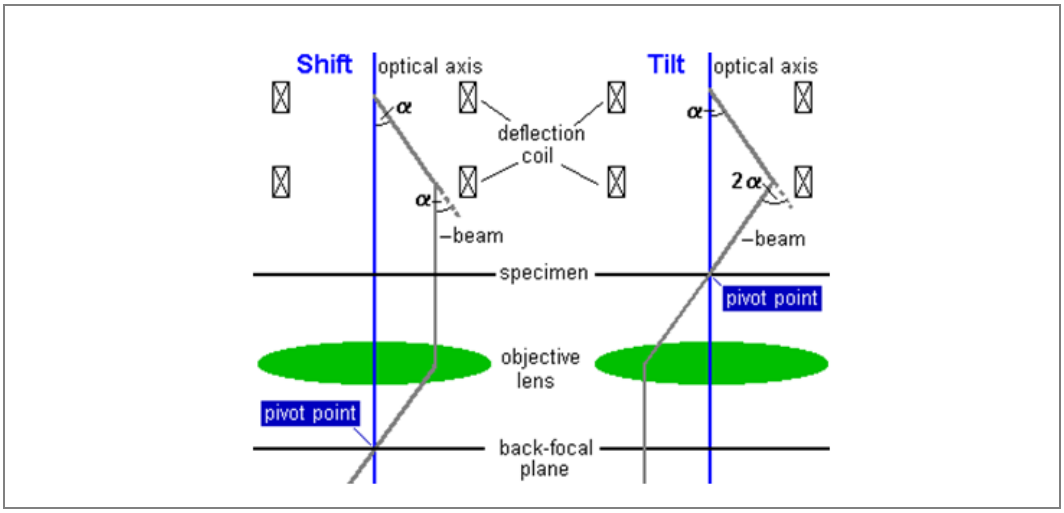
As mentioned before, details concerning the theoretical background of a specific alignment procedure and its instructions can be found in Talos Help. Nevertheless, in the following sections attention is paid to the basic principles of aligning the optical elements of the microscope.

Deflection Coils and Pivot Points

Deflection coils play an essential role in the alignment of the microscope and are used for aligning the gun, beam, objective lens, magnification system (image and diffraction shifts to the center of the screen), and detector. Most of the steps in the procedures either align the deflection coils themselves or use the deflection coils to align another optical element.

Double deflection coils are capable of two completely independent actions: tilting and shifting. In a correctly operating microscope, these actions should be uncoupled. Considerable effort is spent in correctly aligning these deflection coils (see *"Deflection Coil" on page 30*). This is done by setting the pivot points, a point around which the beam will pivot. The alignment makes sure that this point is correctly set.

Figure 65 Pivot Points for Deflection Coils



The concept of pivot points is schematically depicted in *Figure 65*. A beam shift occurs by deflecting the beam through an angle α by the upper coil and then doing the reverse with the lower coil. In a perfect system the beam would come out parallel to its initial direction but displaced sideways. Since all beams that are parallel at the image plane must go through a single point in the back-focal plane, shifting the beam should have no effect on the location of the beam in the back-focal plane.

A beam tilt occurs by deflecting the beam through an angle α with the upper deflection coil and then deflecting by -2α by the lower coil. A beam tilt will result in a beam shift in the back-focal plane, but should cause no shift in the image plane.

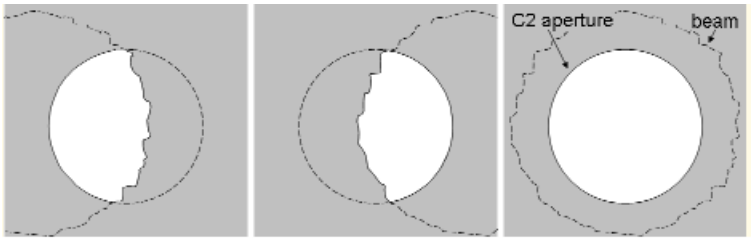
Setting the pivot points is done by deflecting the beam with a wobbler and minimizing any movement, either of the beam in the diffraction plane in the case of beam shift (no tilt should occur) and of the beam in the image in the case of beam tilt (no shift should occur). A wobbler is a mechanism for rapidly switching a microscope element or function from a negative value to an identical but positive value; it can thus be on beam shift or beam tilt, image shift, a stigmator, objective-lens current, high tension, even though the traditional meaning is the beam-tilt aid for focussing the TEM image.

Gun Coils

The gun coils perform two functions. They make sure that the electron beam enters the C1 lens parallel to the optical axis by means of a gun tilt, and make sure that the beam goes through the center of this lens by means of a shift. Alignment is done using the Gun Alignment Procedure. Gun alignment usually requires iterating between gun tilt and gun pivot points.

The gun tilt is simply aligned by maximizing the intensity. For a S-FEG, the correct gun alignment will give a small, very intense spot in the center of a nearly focused beam. In thermionic guns, the maximum intensity should be achieved if the undersaturated filament image is symmetrical. For a X-FEG the gun tilt is aligned simply by aligning the electron beam with the C2 aperture. Note that, depending on extraction voltage and gun lens setting, the beam (limited by the gun aperture) can even be smaller than the C2 aperture. Gun tilt is optimized by shifting the beam relative to the C2 aperture with gun tilt, finding the edges of the beam on either side and placing it in the centered with the C2 aperture (see *Figure 66*).

Figure 66 X-FEG Gun Tilt Alignment

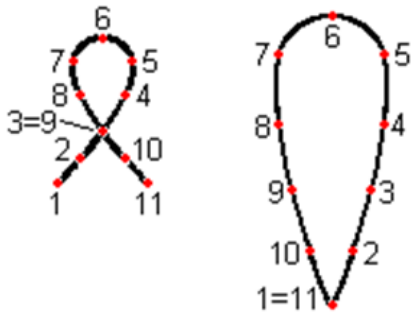


The gun shift alignment consists of two steps:

- The overall gun shift is optimized
- A fine adjustment is made for the individual spot sizes.

For this second step, spot sizes 3 and 9 are selected because this minimizes the overall spot shift as a function of spot size (*Figure 67*).

Figure 67 Pivot Points for Deflection Coils



The overall shift of the spot when the spot size is changed is smaller when the gun alignment is done for spots 3 and 9 than when it is done for any other combination.

In this way, the electron beam is aligned along the optical axis, and a spot displacement when changing spot sizes is minimized. In an additional step, this displacement can be further minimized by aligning all other spot sizes as well (spot size-dependent gun shift).

Beam Coils

The beam deflection coils (situated above the objective lens) serve many purposes. Beam coils:

- Shift and tilt the beam, both static and dynamic (e.g., scanning modes)
- Are used for aligning the objective lens
- Correct beam movement caused by the condenser stigmator.

As such, they play a major role in many alignment steps.

Image Coils

The image deflection coils, situated below the objective lens, have many uses. Image coils:

- Shift the image and the diffraction pattern to align various magnifications, camera lengths, and modes (such as TEM and STEM)
- Correct image or diffraction-pattern movement caused by the objective and diffraction stigmators, respectively
- Set the Detector alignments that move the image or diffraction pattern to a detector that is situated off the microscope axis (STEM BF/DF, Off/Near axis CCD, TV).
- In addition, the image deflection coils can be used coupled to the beam deflection coils in a number of instances, e.g., for image shift in HR-TEM, Low Dose, or descanning (making the image of the beam remain stationary below the image coils in scanning modes).

Stigmators Coils

Talos microscopes have a set of three stigmators (see *"Stigmator" on page 31*):

- Condenser stigmator to make the focused beam circular
- Objective stigmator to correct astigmatism in the high magnification and the low-angle diffraction pattern
- Diffraction stigmator to correct astigmatism in the diffraction pattern and in the low magnification image.

Condenser Stigmator Calibration

The Talos microscopes contain a condenser stigmator to correct for beam astigmatism. When this stigmator is misaligned, adjustment of the stigmator setting will lead to an apparent beam shift. When the stigmator is aligned correctly, these shifts are compensated by an identical but opposite beam shift—this time using the beam deflection coils. In order to align the stigmator, it is necessary to determine the relation between a change in stigmator setting and the beam shift. This relation is determined in the procedures for aligning the stigmator.

Objective Stigmator Calibration

When the objective stigmator is misaligned, adjustment of the stigmator setting will lead to an apparent HM image (or LM diffraction) shift. When the stigmator is aligned correctly, these shifts are compensated by an identical but opposite shift—this time using the image deflection coils. In order to align the stigmator, it is necessary to determine the relation between a change in stigmator setting and the HM image shift. This relation is determined in the procedures for aligning the stigmator.

Diffraction Stigmator Calibration

If in microscope operation the diffraction stigmator is misaligned, adjustment of the stigmator setting will lead to an apparent HM diffraction shift/LM image shift. When the stigmator is aligned correctly, these shifts are compensated by an identical but opposite shift—again using the image deflection coils. In order to align the stigmator, it is necessary to determine the relation between a change in stigmator setting and the LM image shift. This relation is determined in the procedures for aligning the stigmator.

Objective Lens

The alignment of the objective lens is the most important of all alignments because it is this lens that strongly affects the quality of the images. A misaligned objective lens may result in image distortions due to the presence of lens errors such as coma, which produces a streakiness in the TEM image and a one-sided tail on nanoprobe/scanning spots. Alignment procedures are available to align the mini-condenser and the objective lens (TWIN-objective lens configuration of the Talos microscopes) and to set the focal length of the mini-condenser lens at the front-focal plane of the objective lens.

Objective lens alignment is done with Rotation center as a first step, suitable for relatively low magnification work. When high resolution imaging is required, Coma-Free alignment should be used as the final alignment after Rotation center.

The objective lens alignment is the one that is done most often, especially in high-resolution imaging. The reason for its frequent use is the fact that is depends on the centering of the C2 aperture. Often, this aperture is displaced several times during a microscope session, which may lead to slight changes in the objective lens alignment. The most commonly used method for aligning is the Rotation Center method. This method is based on a beam tilt, which explains why this alignment can be found as a subprocedure in the Beam alignment procedure: the microscope wobbles the current of the lens, making the image go through focus. In case of a misalignment, this will result in large sideways movements of the image, whereas these movements are minimized in case of an aligned beam and optical axis of the lens (upon tilting the beam to this axis).

The Rotation Center alignment on the Talos microscopes is based on wobbling the objective lens current; the wobbling of the high tension is not implemented on these system. In Coma Free alignment, the beam is wobbled between a certain ±X and ±Y tilt. Alignment is achieved by adjusting this tilt to make the defocus of the two wobble directions identical. Note that both Coma Free and Rotation Center alignments affect the same parameter (beam tilt); therefore, there is no point in iterating these alignments: they differ in method, not in result (though the former method allows a finer alignment).

Direct Alignments

Once fully aligned, the Talos microscopes require minimal adjustment, varying from nothing for routine imaging, to a combination of objective lens alignment plus coma-free pivot points for high resolution imaging, or rotation center and diffraction-pattern centering for scanning. For this, the microscopes can be aligned using direct alignments.

Table 9 summarizes possible remedies for the effects of most frequently occurring misalignments and may prove helpful in troubleshooting.

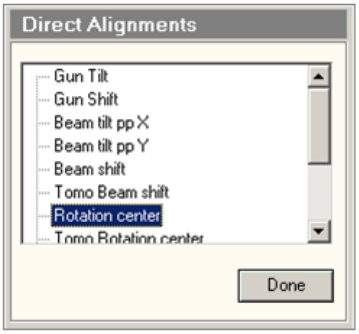
Table 9 Alignment Troubleshooting (1 of 2)

Changing or Doing...	Observation	Alignment Needed	Action
Intensity and Filament	Filament image not centered	Gun Tilt	Maximize brightness (MF-x,y).
Spot size	Beam shifts	Gun Tilt	Center spot 9 (LTb) and center spot 3 (MF-x,y).
Magnification	Image shifts	Image & Focus	Center image detail (MF-x,y) and focus at different magnification range.
Focus	Image moves	Rotation Center	Minimize image movement (MF-x,y).
Beam Tilt (Dark Field)	Focuses spot moves	Beam Tilt Pivot Point	Overlap two spots (MF-x,y).
Astigmatism Correction	Image is blurred and moves	Stigmator	Condenser: make bars cross at center of screen (**Intensity**, MF-x,y). Objective and Diffraction: overlap images (MF-x,y).
Camera Length	Diffraction shifts from center	Diffraction	Center diffraction pattern at all camera lengths (MF-x,y).
Rotation Center	Beam moves	Beam Tilt Pivot Point	Overlap two spots (MF-x,y).
Spot size	Spot defocuses	Spot Size Intensity Calibration	Focus all spot sizes starting with spot 3.
Intensity	Illumination not concentric	Condenser Aperture	Check centering of aperture by through-focusing beam (**Intensity**). Beam needs to expand/contract symmetrically at screen center.
Diffraction	Main beam is off aperture center	Objective Aperture	Switch to Diffraction and center aperture around bright central spot.
Spot size > 10	Spot disappears	Spot Size-Dependent Gun Shift	Align all spots relative to spot 10.

Table 9 Alignment Troubleshooting (2 of 2)

Changing or Doing...	Observation	Alignment Needed	Action
Nanoprobe Mode	Large elliptical tail of beam	Rotation Center	Minimize beam movement eccentricity (MF-x,y).

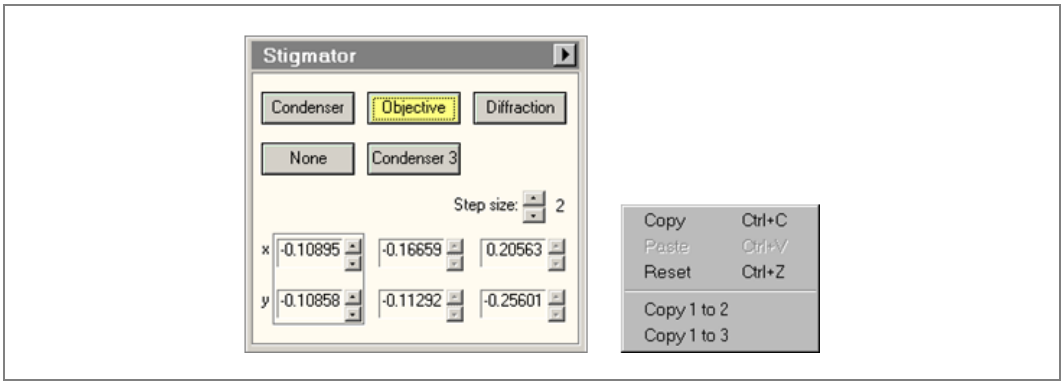
The direct alignments are quick, efficient, and straightforward actions. They may be carried out when an experienced operator diagnoses a specific misalignment, or for special applications, e.g., high resolution work. Some actions are not essential in one mode but will be critical in another, e.g., beam tilt pivot point for normal imaging and for dark field imaging, respectively. These direct alignments can be executed by clicking the particular subprocedure on the Direct Alignment control panel (*left*).



Stigmators

Astigmatism correction can only be achieved with the Stigmator Control Panel, or by pressing the **Stigmator** knob on the left control pad of the microscope. On the control panel (*Figure 68*), the operator can select one of three stigmators (sometimes more, depending on hardware configuration), choose the required stigmator and knob sensitivity (track bar) and channel.

Figure 68 Stigmator Control Panel & Popup



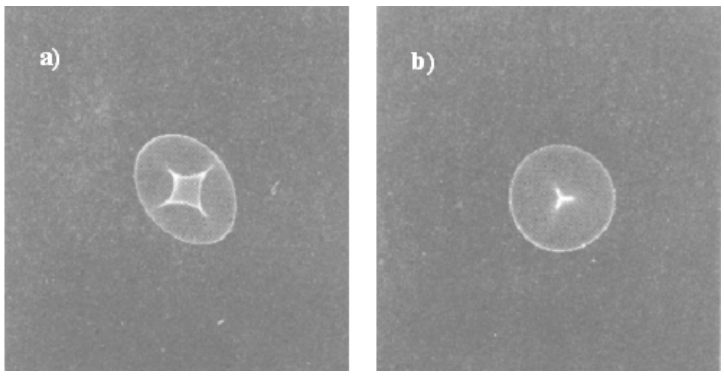
Stigmator settings can be stored in three channels, copied, and reset (zero settings). The active channel is outlined by a frame. The criteria for astigmatism correction are summarized in *Table 10* (see also Talos online help *Astigmatism Correction)*.

Table 10 Criteria for Astigmatism Correction

Lens	Criteria for Correct Setting Using MF-x,y
Condenser	Focused beam circular or filament image sharp
Objective (M/SA)	Symmetric Fresnel fringe or, better, minimum contrast at high magnification
Diffraction (LM)	Symmetric Fresnel fringe or smallest focused image movement with wobbler
Diffraction (D)	Symmetric 3-pointed diffraction crossover image (Mercedes star)

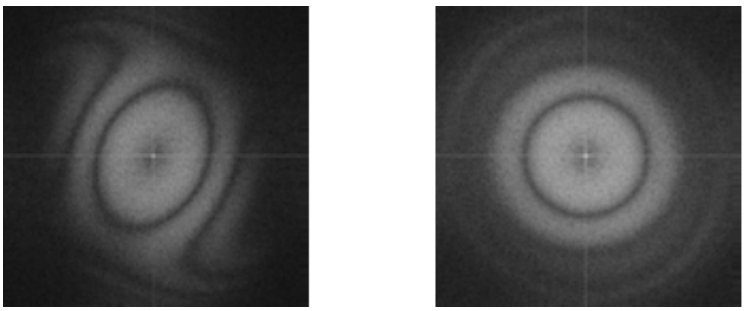
Diffraction lens astigmatism can be corrected according to the “Mercedes star” symbol. By turning the **Intensity** and **Focus** knobs in Diffraction mode without specimen, you may see a pattern as shown in *Figure 69*a. On the other hand, the astigmatism corrected pattern should be like a Mercedes star in *Figure 69*b.

Figure 69 Diffraction Lens Astigmatism



The objective lens astigmatism is an important factor for image quality. For high resolution work, it may even cover the feature of atomic structure. To minimize objective lens astigmatism acquire a life image of an amorphous area on digital camera at enough high magnification (> 100 kx) with live (reduced) FFT. Stigmate to get round features at approximately 100 nm defocus; 1-2 rings visible (see *Figure 70*).

Figure 70 Objective Lens Stigmatism in FFT (Uncorrected and Corrected)



Stigmators are aligned by small procedures as mentioned above. If they are misaligned, the astigmatism correcting operation will create a shift in image/spot. To correct astigmatism, you need a well aligned stigmator; to align a stigmator, you also need small astigmatism. Therefore, you should occasionally perform the following sequence: correct astigmatism, align stigmator, and correct astigmatism again.

5 Talos Applications

Overview

This chapter provides information on the following:

- General handling, startup, and user maintenance
- Gun alignment
- CETA 16M
- STEM imaging
- Calibrations
- High resolution TEM imaging
- High resolution STEM imaging

Handling the Talos

General

- Close the column valves when doing anything related to the vacuum system.
- A red light on the CompuStage means do not remove or insert a specimen holder.
- Never apply excessive force on the mechanics.

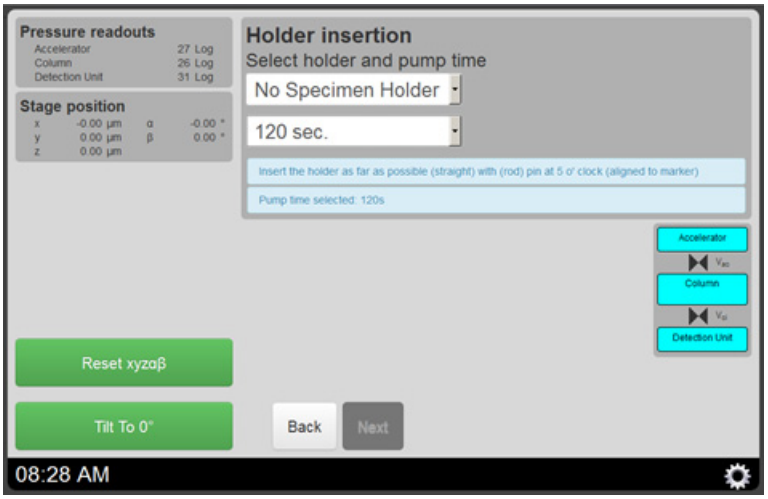
Starting a Microscope Session

1. Log on to the computer
2. Start the programs in the following order (if present):
   - Talos User Interface
   - Digital Micrograph
   - TIA
   - SmartCam Viewer
   - Esprit
3. Check the vacuum status. All pressure indicators should be green.
4. Check that the HT is switched on.
5. In systems equipped with SuperX (and hence, big dewar), check that the liquid nitrogen level is enough for your session (around 20% or more for 8 hours of work). If only a small dewar is present, fill it with liquid nitrogen and wait about 30 minutes before starting.

6. Click the **Filament** button (W/LaB6 systems) or the **FEG Operate** button to get an electron beam.

Inserting a Specimen

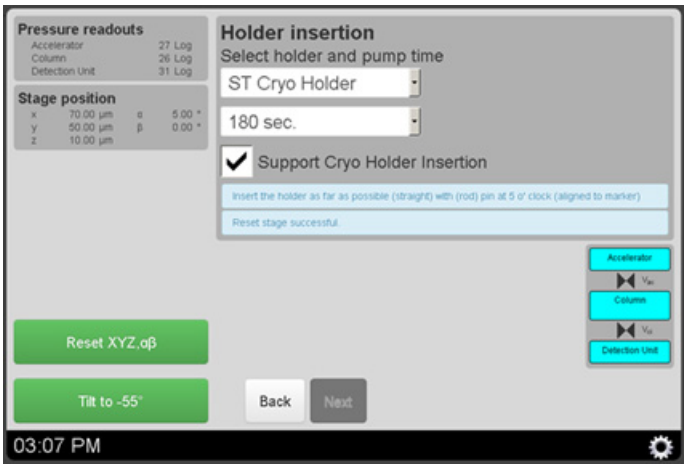
1. After clicking the **Load sample** button on the main screen, the screen below is shown:



- The upper left corner shows the pressure readouts as well as the stage position. On the right side, the simplified graphical vacuum overview is shown.
- When the column valves are open, the column valves are closed automatically at this point.
- When 'Auto reset stage' is enabled in the settings screen, the stage is reset automatically to 0,0,0,0,0.
- Before inserting the holder, the pump time should be selected using a dropdown list. The previously selected pump time is remembered and already pre-selected.

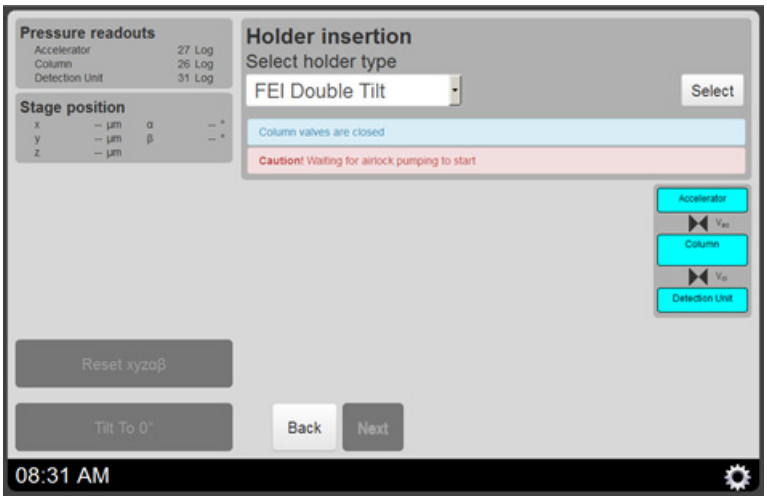
- On systems with Cryo Holder, a special **Support Cryo Holder Insertion** check box is shown because it needs a different inserting approach than single or double

tilt holder. This check box provides same functionality as the check box in Vacuum OCX, settings tab.

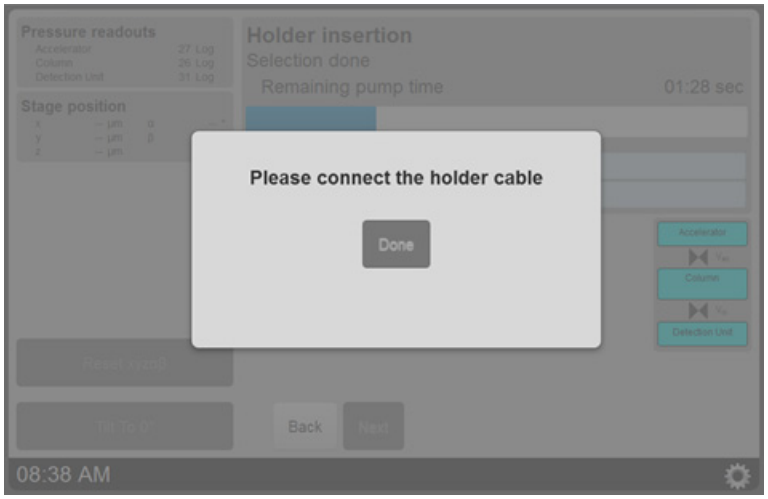


- When the holder is detected, the **Next** button is made available.
- On the left side, a button to explicitly reset the stage is available as well as a button to reset the alpha tilt to -55. This button toggles between rotating the alpha axis to -55 and 0 degrees in order to aid with inserting a cryo holder.

2. When the holder is detected, the type of holder must be selected using the dropdown list.

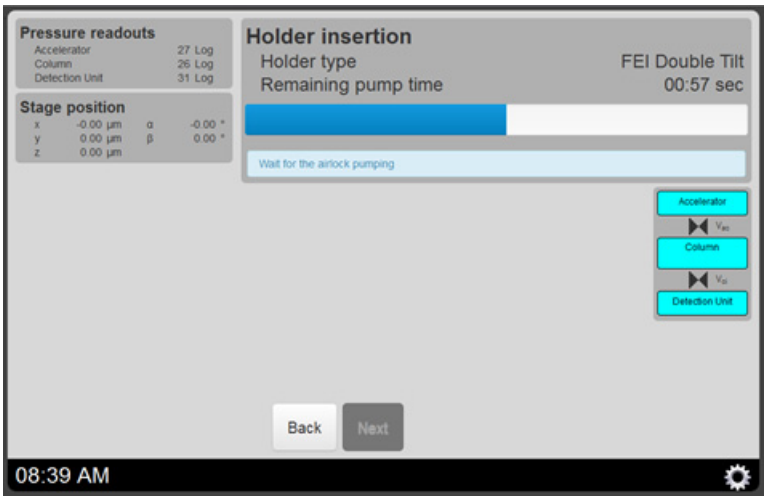


- For a double-tilt holder, a message is displayed indicating that the cable must be connected. Once the cable is connected, the **Next** button is available:



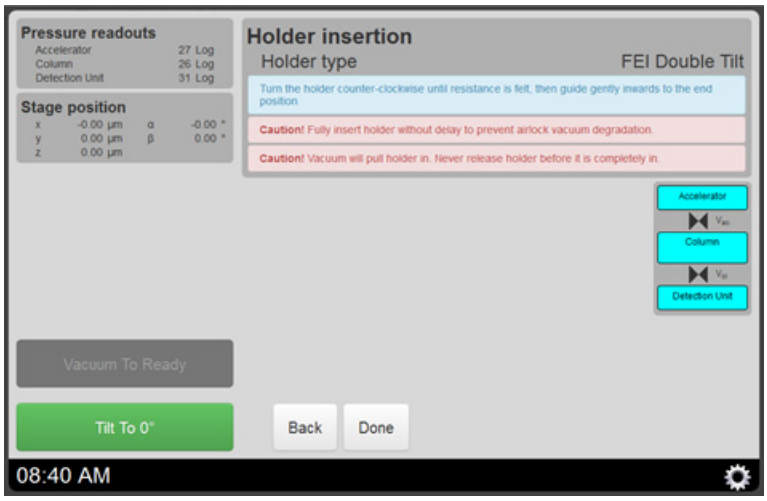
- When loadlock pumping starts, a progress bar is shown that indicates the progress of the pumping.

3. After selecting a holder type and connecting the cable in case of a double tilt holder, an overview screen is shown with the progress indicator for the loadlock pumping:



- When pumping is finished, the **Next** button becomes available.

4. After loadlock pumping has finished, the last screen in the loading procedure is shown, indicating that the operator should push the holder from the loadlock into the column. Cautionary messages are displayed:

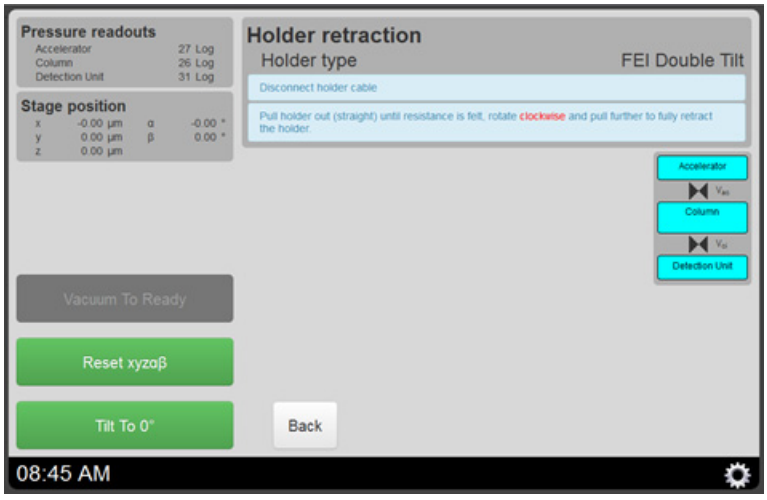


Clicking the **Done** button redirects to the main screen again.

- In systems with small dewar, keep the LN_2 level always at least 10 cm under the rim (typically check and fill every 2-3 hours).
- During breaks and when you leave the microscope, close the column valves. Also if during a session the column vacuum goes above 20 log (for FEG) close the column valves.
- Try to keep the room temperature as constant as possible. Keep doors, windows and curtains shut.

Removing the Specimen Holder

1. When clicking the **Remove sample** button on the main screen, the screen below is shown with information on how to retract the holder.



2. When 'Auto reset stage' has been enabled in the settings screen, the stage is reset to 0,0,0,0,0 automatically. Additionally, buttons are available to reset the stage, or to rotate the stage to -55 degrees (this button toggles between -55 and 0 in order to aid with retracting a cryo holder).
3. When the holder is removed, the main screen is shown automatically.

Ending the Session

1. Close the column valves.
2. Check that the liquid nitrogen level is enough until next check if only small dewar is present and the microscope is not going to be used during the next few hours. Remove the LN_2 dewar and start cryo-cycle in the Cryo tab of the Vacuum flyout. Use the settings Start after 10 minutes and Duration 240 minutes.

User Maintenance

Some maintenance can be done by the user to spot problems in an early state. When in doubt, call your Thermo Fisher Service Representative.

Bi-Weekly

Top up the dewar with liquid nitrogen.

Monthly

- Check if the SF_6 gas pressures at the HT tank and the accelerator have changed. Note that the absolute minimum SF_6 pressure for the accelerator chamber and the HT tank is 4 bar.
- Check the flow rate of the cooling water; the indicators should be between the red markers. Ensure that the water color is not too dark, which means heavy algae growth.
- Visually check for water leaks.

Yearly

- Calibrate the magnifications.
- Check the apertures for contamination.
- Warm up the EDX detector and remove the water inside the dewar. Simply remove the cap and let the LN_2 + water evaporate for 1–2 weeks. This should not be done more than twice a year, and can be done during a holiday

FEGGun Alignment

Perform this procedure to obtain a well-aligned field emitter gun, which is paramount to successful and correct operation of the Talos.

1. Select the correct FEG settings according to *Table 11*.

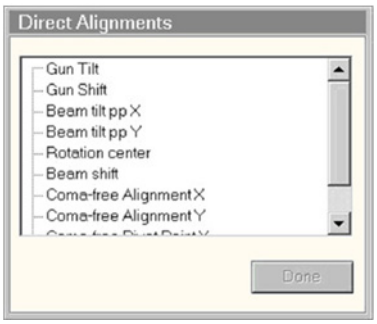
Table 11 FEG Settings

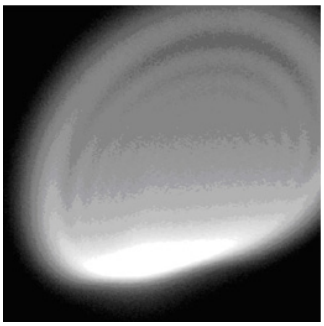
Application	C1 (µm)	C2 (µm)	Spot size	Extraction Voltage (kV)	Gun Lens[1]	Mode
(HR)TEM	2000	50–150	2–5	As set by FSE	1–5	µP
Analytical-STEM	2000	70–100	5–8	As set by FSE	4–6	µP
HR-STEM	2000	70	7–10	As set by FSE	5–6	nP
EFTEM	2000	150	1–3	As set by FSE	2–5	µP
Holography	2000	50–150	4–6	As set by FSE	4–5	µP
EELS	2000	50–100	1–11	As set by FSE[2]	1–8	µP/nP
EELS Minimized Side Lobes	30	150	1–6	As set by FSE	6–8	nP
Optimum EDX Analysis	30	150	1–6	As set by FSE	6–8	nP
LACBED	2000	150	5–8	As set by FSE	1–5	nP
CBED	2000	50	5–8	As set by FSE	1–5	nP
Nano Diffraction	30/2000	20	8–11	As set by FSE	1–8	µP
Lorentz	2000	50–150	1–3	As set by FSE	1–8	µP
Low Dose	2000	50–150	2–8	As set by FSE	1–5	µP/nP

[1]Fine gun lens control for highly accurate dose control.
[2]Extraction voltage can be lowered for a limited period of time for better energy resolution. For details, contact FSE or Applications support.

2. Insert a 100 or 150 µm condenser aperture and center the aperture by alternating the C2 current (**Intensity**) and making the beam open concentrically.
3. Click **Eucentric Focus** or focus (Objective lens) specimen at the eucentric height position of the stage.

4. Check the beam tilt pivot points in Direct Alignments (at least at 20 kX in magnification) and minimize beam movement (see misaligned pivot point below).



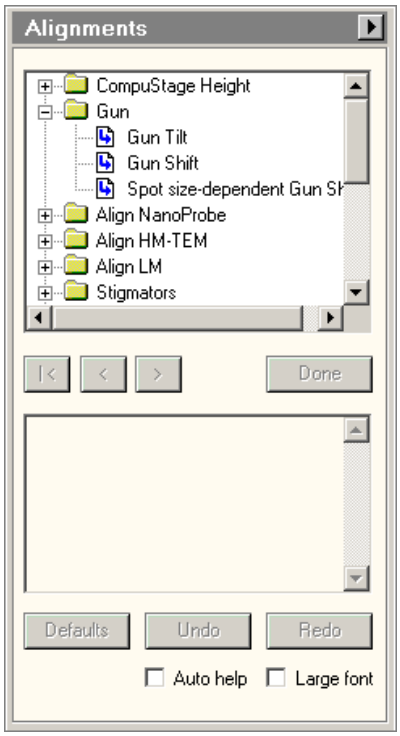


Misaligned pivot point

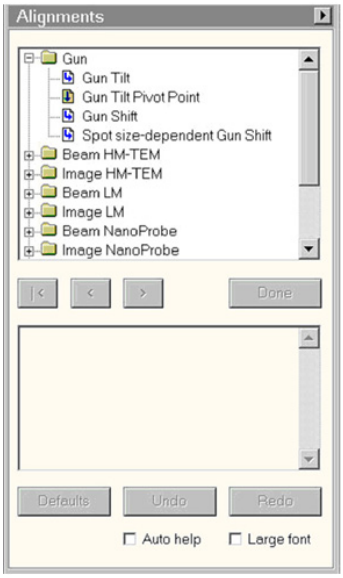
5. Check the rotation center of the objective lens in Direct Alignments by focusing the beam and minimizing the imagemovement.

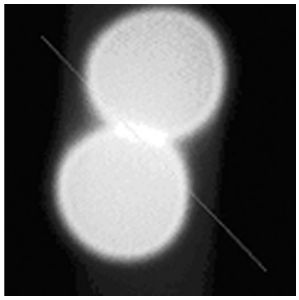
NOTE	These pivot points depend on the Extraction Voltage and Gun Lens settings and need to be checked/corrected when these conditions are altered. If using the gun tilt to maximize brightness, hardly any movement of the beam is a sign for a good gun tilt pivot point alignment.

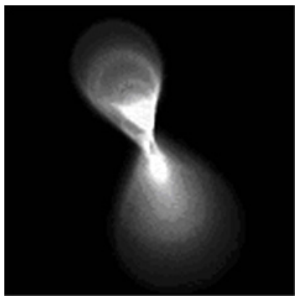
6. For an X-FEG gun, follow the gun tilt and gun shift on the Alignments page. To continue, go directly to the beam shift step on *page 120*.



7. For an S-FEG gun, check the Gun Tilt Pivot Points on the Alignments page.




Misaligned beam tilt pivot

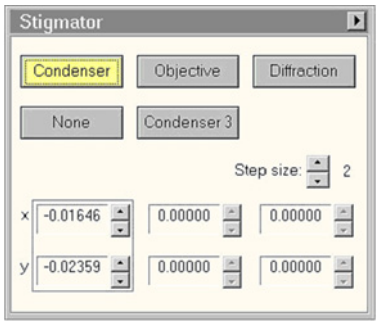

Misaligned rotation

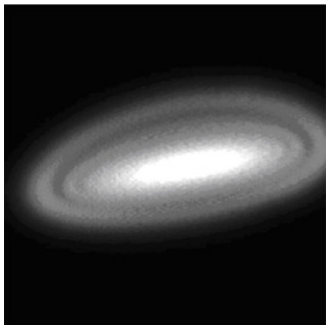
8. Maximize brightness of the spot by using the Gun Tilt on the Alignments page. To do so, look at the measured exposure time and minimize this value.

9. Focus spot (with **Intensity**) in such a way that you can see a bright spot within the disk of the probe.

10. Make sure the beam expands concentrically by using the condenser stigmator (select **Condenser** in the Stigmator panel).

11. Use Gun Shift in the Alignments page to bring the spot into the middle of the disk. If the gun shift needs to be changed a lot, also recheck the C2 condenser aperture centering. The brightness might decrease again (measured exposure time is increased again) after doing so. Due to this fact, and if gun tilt and/or gun shift changed a lot, redo steps 6 to 10 iteratively until the measured exposure time is at its minimum and the spot is in the middle of the disk.

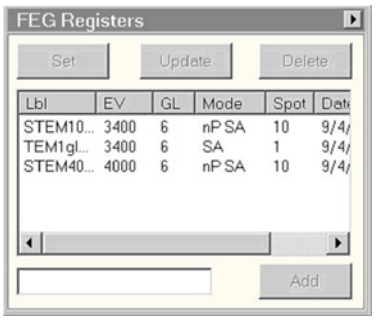


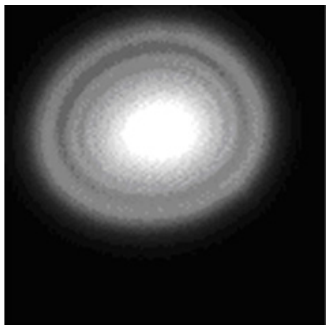

Optimized gun shift/tilt

NOTE	Sometimes this iteration can be done until the gun tilt is at a maximum (no convergence of the iteration). Then return to minimum gun tilt/shift values and try to find a compromise between brightness and extreme high excitation of the gun tilt unit (look at the system status window to find out the excitation of the gun tilt/shift unit). This means normally a 10-20% higher measured exposure time compared to the minimum value.

12. Use beam shift (Direct Alignments) to position the beam at the center of the phosphor screen.

13. Add the probe to the FEG Registers control panel by giving it a name in which the mode, spot size, and gun lens are mentioned (all options in the flyout window should be activated) and click **Add**.





Optimized condenser stigmation

Ceta 16M

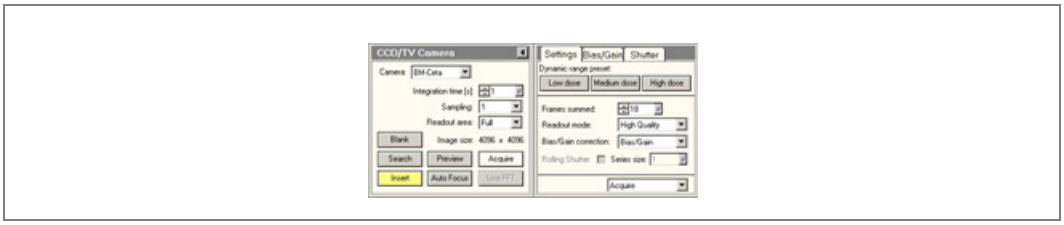
This section describes setting up the Ceta camera for acquisition in TIA.

Setting up Ceta Camera Conditions

Below, a stepped approach towards Ceta CMOS imaging is presented: setting up and acquiring.

1. Select the BM-Ceta in the CCD/TV camera control panel (*Figure 71*). If only one digital camera is embedded on the Talos/Titan, it is automatically selected.

Figure 71 Ceta Access and Settings



2. Define the acquisition parameters for the three different acquisition modes in the flyout Settings tab (*Figure 71*) according to the table below:

Table 12 Advised settings for Search, Preview and Acquire

	Readout Area	Integration Time[s]	Sampling
Search	Full	0.1	2 or 4
Preview	Full or Half	0.5	1
Acquire	Full	1.0	1
Choose "High Quality" for the best quality (*page 124*)			

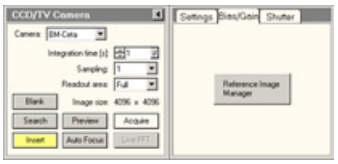
3. Define the Frames summed. Summing of multiple frames can be used to increase the *dynamic range* of the Ceta. The Integration time will be divided into sub-frames, so that the effective exposure time remains independent of the Frames summed setting. To achieve an optimal Signal-to-Noise-Ratio, the number of Frames summed should be chosen as low as possible while not over-exposing individual frames (the exposure time is leading, frame sum decreases if it does not fit). Three default settings of Frames summed can be toggled by clicking the button in the Settings tab (*Figure 71*).

Table 13 Default Settings

	Frames Summed	Maximum counts (with Sampling 1)	Description
Low dose	1	8000	Beam-sensitive samples. This means low count rate on camera or very short exposure times (fast frame rates). If needed also activate CDS (*page 124*).
Medium dose	4	32000	Medium dose TEM imaging.
High dose	18	144000	High intensity (HR)TEM, diffraction techniques (CBED, weak beam, SAED) and long exposure times (several seconds).

4. To reduce inhomogeneous intensities, it is necessary to record a gain reference image (without a sample: click the **Reference Image Manager** button in the Bias/Gain flap out tab (*Figure 72* and *Figure 73*)) and use the check boxes dropdown menu in the Settings tab to make sure that all newly acquired images are gain corrected (*Figure 71*). Bias references are acquired at each new acquisition automatically.

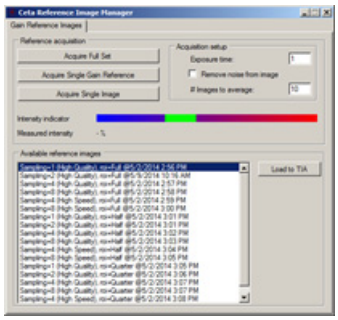
Figure 72 Gain Acquisition Access Tab



5. Make sure the beam intensity is never too high (this may damage the camera) or too low (this will lead to a noisy image due lack of information). As a reference: the SmartCam should be homogeneously illuminated at moderate intensities. Adjust the Frames summed accordingly (as described in step 3).

6. Select the desired shutter; post or pre-specimen (*Figure 74*). During continuous acquisition the *Rolling Shutter* can be activated, which enables the camera to read out the chip without shuttering (check box in *Figure 71*). The rolling shutter gives a higher frame rate for exposure times > 55 ms. For exposure times < 55 ms do not use the Rolling Shutter to avoid longer exposure times than entered.

Figure 73 Reference Manager

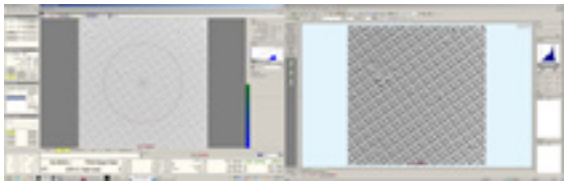


Choose either Acquire Full Set for acquiring all possible Sampling and FOV combinations, or select only one from the list and select Acquire Single Gain Reference.

Acquiring a Ceta Image

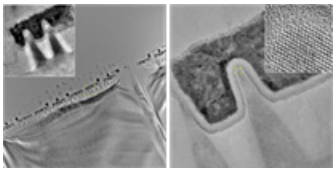
1. Find a region of interest. This can be done either on the SmartCam or on the Ceta by using the Search mode. For searching on Ceta, click **Insert** (*Figure 71*) and Search (the 'live' image appears on the monitor). Search for the feature of interest, focus, and freeze the image by clicking the **Search** button again.

Figure 74 Typical 2-monitor View



2. For accurate focusing: activate Preview and focus image with or without live FFT. Click **Preview** again to stop the image acquisition.

Figure 75 Imaging FinFET



Imaging of a FinFET transistor structure. The two images, acquired at 10kx (left) and 230kx magnification, capture a whole range of scales from the whole row of ~30 devices down to the atomic lattice of the silicon gate (inset).

3. The final high quality image can be taken by taking a snapshot in Acquire mode. The acquired image can be further processed, and saved in several file formats (*emi*, *tiff*, *bmp*, *jpeg*, etc.). It is advised to first save the images in the TIA format (*.emi*).

Hints

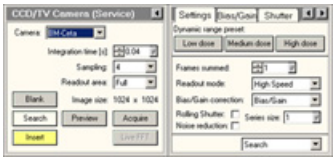
- Insert the camera and take the picture after at least 5-10 seconds for the best quality.
- Only acquire Gain references if the Ceta cooling has been on for at least 1 hour.
- If n > 1 is indicated in the Series size box of the Settings tab, the last n images will be displayed as a series in TIA (*Figure 71*).

Frame Size Ceta-16M

Resulting Down Sampling	Pixel Binning	Pixel Skipping	Full Readout Area	Half Readout Area	Readout Mode
1x1	1x1	1x1 (none)	4096 x 4096	2048 x 2048	both
2x2	2x2	1x1 (none)	2048 x 2048	1024 x 1024	both
4x4	2x2	2x2	1024 x 1024	512 x 512	High Speed
4x4	4x4	1x1 (none)	1024 x 1024	512 x 512	High Quality
8x8	4x4	2x2	512 x 512	256 x 256	High Speed
8x8	8x8	1x1 (none)	512 x 512	256 x 256	High Quality

CDS for Life Science Low Dose Applications

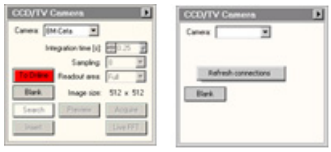
- To activate Correlated Double sampling for noise reduction in Low Dose, select the **Noise reduction** check box in the **Settings** tab. This only works for snapshots and for readout area “full” (4096 x 4096) with sampling 1.



Troubleshooting

- If the connection to the camera is lost, a red button **To Online**, located above the **Blank** button will become visible. Click the **To Online** button to attempt to restore the connection to the camera.

- If there is no connection to the CCD, you may be able to activate the CCD by right-clicking on the CCD/TV control panel and a **Refresh connections** button may be visible. Click the button to try and activate the CCD.



- If the above does not solve the problem: restart both the Talos/Titan User Interface and TIA. Otherwise call customer service.

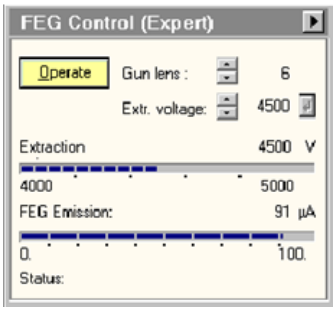
STEM Imaging

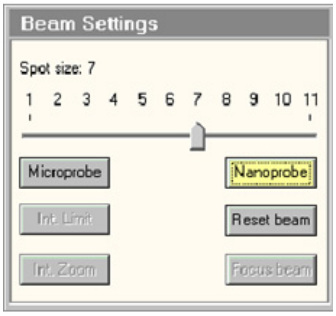
This section describes setting up and acquiring STEM images. Parameters are optimized for Talos F200X systems.

Set up STEM Conditions

1. Insert the sample and find a thin specimen area for tuning the microscope. Adjust the Z-height to the eucentric height with the alpha wobbler.
2. Select gun lens 5 and optimize gun shift and gun tilt or recall a FEG register.

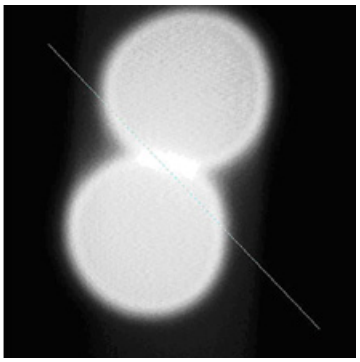
NOTE	You can also directly recall an alignment that is stored in STEM mode using the FEG registers. In that case, leave Diffraction mode by clicking **Diffraction** and then center the condenser aperture.





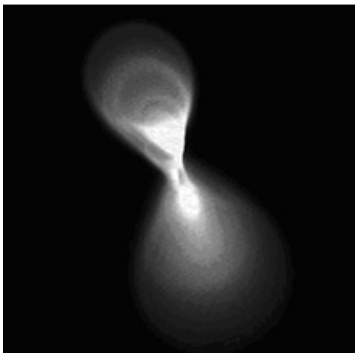
3. Insert and center the 150 µm C2 condenser aperture; remove the objective aperture.

4. Switch to STEM, select spot size 7 and leave Diffraction mode by clicking **Diffraction** on the Control pad.
5. Select a magnification of at least 50 kX and center the beam with beam shift (from the Direct Alignments panel) if necessary.
6. Press the **Eucentric focus** button, select Intensity list focus on Direct alignments panel and focus the beam using the focus knob.
7. Adjust the beam tilt pivot points (Direct Alignments), such that the beam does not move any more.



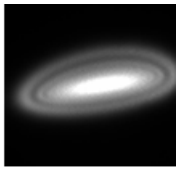
Misaligned beam tilt pivot points

8. Adjust the rotation center intensity (Direct Alignments) by minimizing the overall movement of the beam.

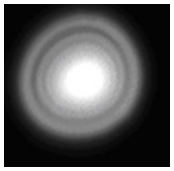


Misaligned rotation center

9. Carefully stigmate the condenser lens until the beam opens and closes concentrically with the focus knob.



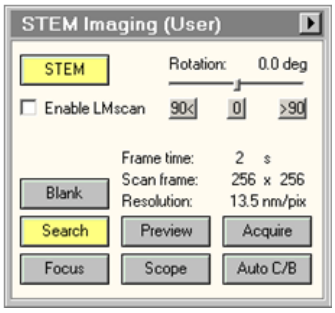
Misaligned condenser stigmator

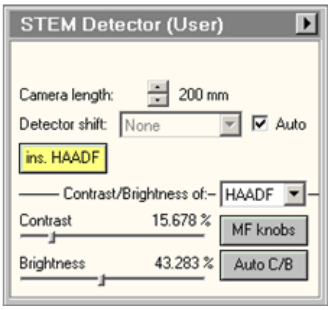


Optimized condenser stigmator

10. Click **Diffraction** to return to normal STEM mode.
11. Insert the 70 µm condenser aperture.

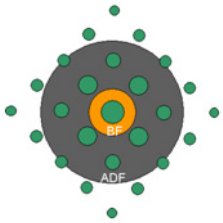
12. Click **Ins. HAADF** and select HAADF detector (if available) for Contrast/Brightness or lift the screen and select BF/DF2/DF4 detectors.



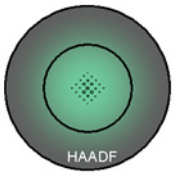


13. Select a camera length of 100-200 mm. A larger camera length results in stronger signal on the DF/HAADF detector, because the bright-field/diffraction contributions become stronger.

NOTE	If the position of the diffraction pattern changes when adjusting the camera length, click **Align Diffraction Pattern** in HM-STEM alignment.



Alignment of diffraction pattern on BF/DF

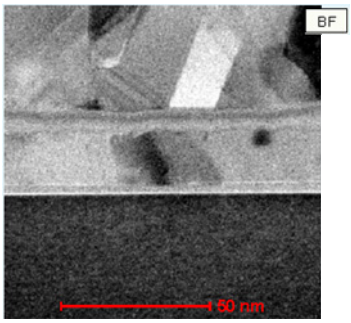


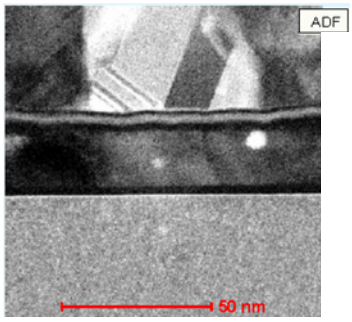
Alignment of diffraction pattern on HAADF

14. Start continuous scanning using Search mode at 15-30 kX magnification (512 x 512 pixels, 1-2 seconds per scan) [3].

NOTE	If the beam moves visibly on the fluorescent screen while scanning, the AC beam tilt pivot points in Beam Nanoprobe (slow scanning direction) or the DC beam tilt pivot points in HM-STEM (fast scan direction) need to be aligned.

15. Check that the beam is centered in/at the detector and adjust using diffraction alignment.
    - **HAADF detector:** Visually check on fluorescent screen that the beam is in the center of the shadow image of the HAADF detector.
    - **BF/DF2/DF4 detectors:** Lift screen and roughly position beam at detector position. Use diffraction alignment to maximize the signal on the BF detector (watching the scope is easier than watching the actual image) and adjust detector contrast/brightness if necessary. At optimum detector alignment (and short camera length), the BF and the DF2 images should be the inverse of each other.
16. Bring the image in focus using the focus knob.



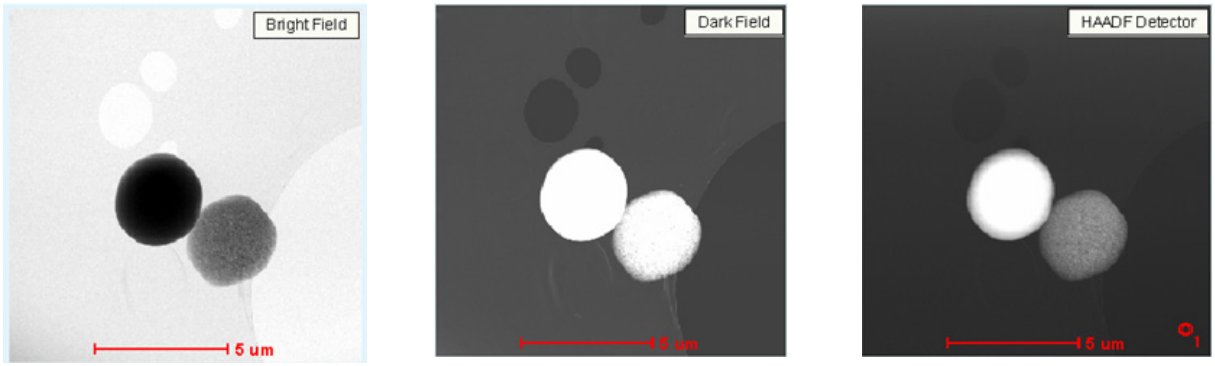


Acquire a STEM Image

1. Search for the area of interest and adjust the magnification.
2. Click **Focus** (in STEM Imaging Control panel) and adjust carefully with the focus knob. (The focus window can be moved and/or enlarged.)
3. Select Preview mode (1k x 1k pixels, 30-60 sec. per scan) and check focus.
4. Automatically optimize detector amplifier by clicking **Auto C/B**.
5. Manually adjust the detector amplifier.
6. Stop preview.
7. Select a high contrast area using the line tool .
8. Click **Scope** and adjust the detector contrast and brightness so that all intensities are within the central 25% of the grey levels.

9. Click **Acquire** to acquire the final image.

Figure 76 STEM Imaging Examples



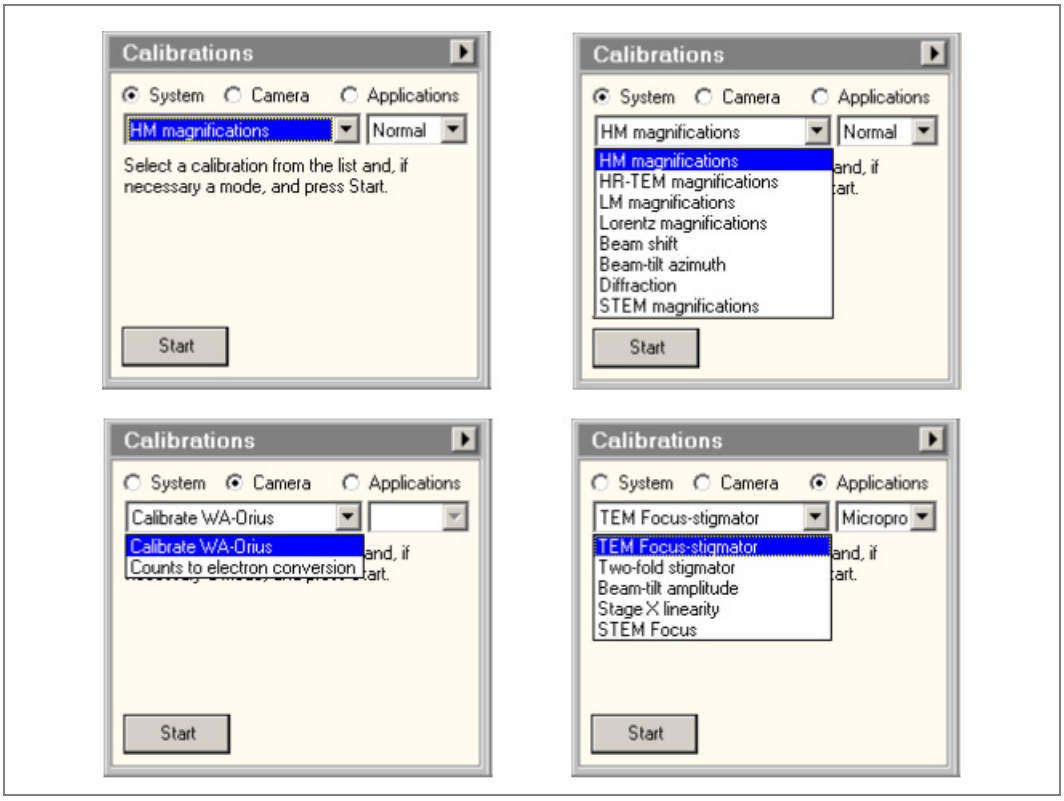
Examples of bright field (BF), dark field (DF), and high angle annular dark field (HAADF) STEM imaging of two particles on a holey carbon supporting film.

Calibrations

Background

The calibrations system of the TEM has grown “organically” over the years, leading to a proliferation of calibrations—some of those duplicating the calibrations of other programs. The main reason for the proliferation has been the increasing demands made on the calibrations, with sometimes newer demands incompatible with the design of the older calibrations. A new—centralized—calibrations design has been initiated, one that should not be limited in the foreseeable future.

Figure 77 Calibrations Control Panel



The calibration system has now migrated to a single system. Some (magnification) calibrations can be done through the Magnification calibration control panel, which also retains the functionality to create reports from calibrations done and load magnification tables from file. Other calibrations can only be accessed through the Calibrations control panel. Some of the latter were previously present in the Magnification calibration control panel but have been removed since they are not magnification-related.

NOTE	■ Calibrations access is limited to supervisor level or higher. ■ When instructed to check something on the digital camera, always use the Preview function there. The Preview settings are changed to the settings as used by the program itself.

Calibration Type Selection

Because of the large number of available calibration procedures (which would result in a very long selection list), the calibrations have been split into three categories, System, Camera and Applications. Each of these has its own calibration procedures. When you select one of the types, the contents of the Procedures list will change and be filled with the procedure specific to that type. Typical contents of the procedures list are:

- **System:** The basic (magnification, image shift, stage shift, camera lengths) calibrations.

- **Camera:** The calibrations of other cameras against the reference digital camera and counts to electrons calibrations for the individual cameras.
- **Applications:** Calibrations specific to applications software such as iCorr, AutoAdjust, etc.).

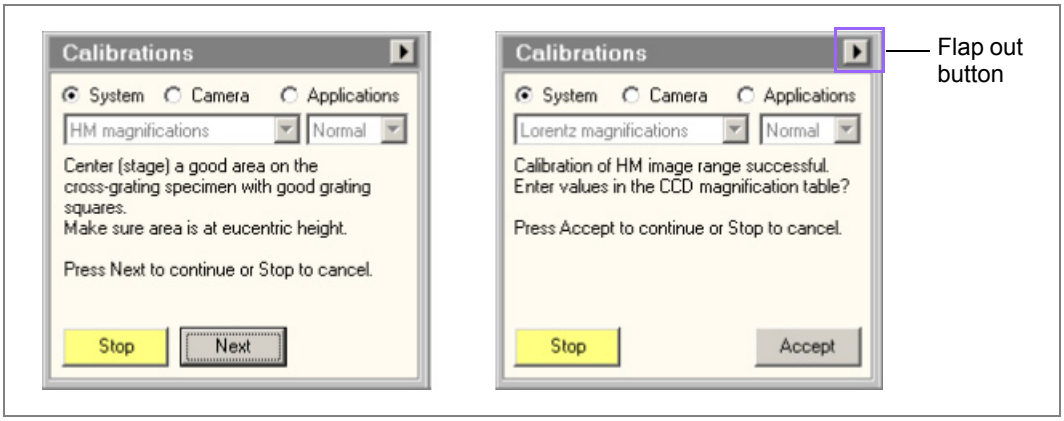
Instructions

The instructions field will display instructions on how to proceed or a status description of the function currently running or finished.

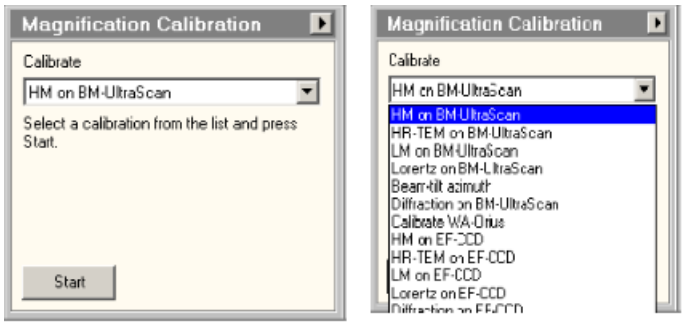
Table 14 Calibration Instructions

Item	Description
Start (stop) button	The **Start** button starts the calibration procedure. The **Start** button will change to a yellow color and its caption to Stop. Clicking the **Start** button when it is yellow will stop the calibration procedure. Starting the calibration procedure will open a log in a separate window. The log shows what is being done and what the results are. It can be saved in text format (under the File menu of the log window). The log can also be printed (if a printer is available to the system). Text files can also be opened in the log file window. The log is saved automatically (*c:\tecnai\log\calibration* or *c:\titan\log\calibration*).
Next button	During the calibration procedure the operator will be expected to do certain things such as setting up illumination, specimen area and focus or change specimen. Once these things have been done (as given by the instructions displayed), clicking the **Next** button will continue with the procedure. Once a calibration procedure is finished, the user will be asked to accept the results and the **General-purpose** button will change to Accept. If the results are not accepted no system calibrations are modified.
General purpose button	The **General purpose** button is a single button that performs a range of functions. Depending on the particular function needed, the button will show a different caption.
Flap out button	The **Flap out** button leads to the Options tabs of the Calibrations Control Panel.

Figure 78 Calibration Control Panel Buttons



Magnification Calibrations



The calibration system has migrated to a single system. Some (magnification) calibrations can be done through the Magnification calibration control panel, which also retains the functionality to create reports from calibrations done and load magnification tables from file. Other calibrations can only be accessed through the Calibrations control panel. Some of the latter were previously present in the Magnification calibration control panel but have been removed since they are not magnification-related.

TIA can now use the calibrations in the TEM server. Advantages in using this functionality are:

- Users no longer need to download magnification tables in TIA when the high tension is changed.
- TEM server calibrations follow the optics model so there is no possibility of duplicate magnifications (e.g. in differing optical modes) leading to errors in calibration (because TIA—and DigitalMicrograph™ as well—store magnifications by magnification value).

The functionality is activated by a check box in the Acquire menu of TIA. Note that only TIA Administrators can change the selection, for other users the setting is visible but disabled.

The Magnification Calibration Control Panel semi-automatically calibrates:

- The HM magnifications on an embedded digital camera
- The LM magnifications on an embedded digital camera
- The Diffraction camera lengths on an embedded digital camera
- Multiple cameras
- The HM-STEM magnification
- If installed, the calibration of the Microprobe STEM against the normal (HM-) STEM
- The calibration of the LM-STEM against the normal (HM-) STEM

The values measured are entered into the TIA CCD or STEM magnification/camera length tables as well as the calibration database. If the digital camera is a Gatan camera, the values are also entered into the magnification tables of DigitalMicrograph. The control panel is accessible only to Supervisor (if the software function was purchased), Service and Factory.

Instructions

Table 15 Magnification Calibration Instructions (1 of 2)

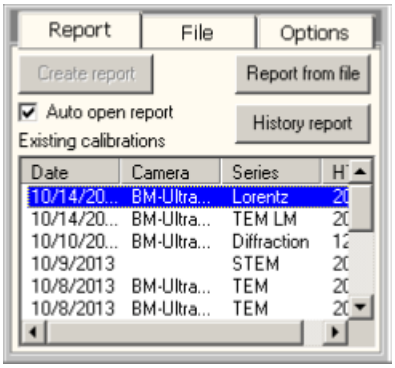
Item	Description
Start button	The **Start** button starts the calibration procedure. The software will link to TIA (and DigitalMicrograph if the camera is a Gatan CCD). The **Start** button will change to a yellow color and its caption to Stop. Clicking the **Start** button when it is yellow will stop the calibration procedure. Starting the calibration procedure will open a log in a separate window. The log shows what is being done and what the results are. It can be saved in text format (under the File menu of the log window). The log can also be printed (if a printer is available to the system). Text files can also be opened in the log file window. The log is saved automatically (*c:\tecnai\log\calibration* or *c:\titan\log\calibration*). 

Table 15 Magnification Calibration Instructions (2 of 2)

Item	Description
Next button	During the calibration procedure the operator will be expected to do certain things such as setting up illumination, specimen area and focus, as well as change specimen (later in the procedure). Once these things have been done (as given by the instructions displayed), clicking the **Next** button will continue with the procedure. If the calibration for a particular magnification fails, the user will have the selection of stopping, redoing the failed magnification or skipping the failed magnification. The **Next** button will be changed to **Redo** for this purpose.
	When a calibration is done for a series of magnifications or camera lengths, there is a point in the procedure (once the stage and image shift have been calibrated) where it is possible to stop the procedure and still have the results stored (but at that point the calibrations are of course not complete and finishing the calibrations requires going through the whole procedure). These points are (always once the procedure has gone to the next magnification after the required point): • HM magnifications on cross-grating, after the first SA magnification (Titan) or first Mi magnification (second magnification done). • HR-TEM magnifications, after the first. • Certified TEM magnifications, after the lowermost SA magnification (second magnification done). • LM magnifications generally after the 6th magnification. • Diffraction after the first camera length. • HM-STEM after the first STEM magnification. • Certified STEM after the first STEM magnification (second magnification done).
	Once a calibration procedure is finished or canceled (and at least one magnification has been calibrated), the user will be asked to accept the results and the Next button will change to Accept. If the results are not accepted: • No magnification table file is written. • No calibrations are downloaded into the database, TIA and DM magnification tables. • No system calibrations are modified.
Skip button	If the calibration for a particular magnification fails, the user will have the selection of stopping, redoing the failed magnification or skipping the failed magnification. The Skip button will be made visible to the right of the Next (Redo) button for this purpose.
Flyout button	The flyout button leads to the Report, File and Options tabs of the Magnification Calibration Control Panel.

Magnification Calibration Report Control Panel

The **Report** tab of the Magnification Calibration Control Panel contains the controls used for report generation. All reports are in Acrobat® Reader (*pdf*) file format.

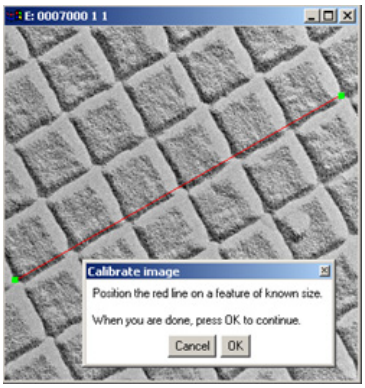


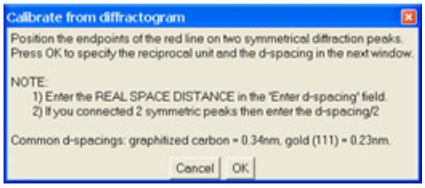
Two types of report can be created:

- A tabular overview of the results of a calibration sessions, displaying the TEM magnification, the measured value(s) according to the method used, which value is used for the magnification table, the associated consistency error, and the magnification factor from plate camera to digital camera or the ratio between nominal and measured STEM magnifications.
- A graphical overview of the results of (at most) the last twelve calibrations done.

NOTE	The STEM magnification is a rather arbitrary value, based on the approximation that a full STEM frame should correspond to a frame covering 100x100 mm at the same TEM magnification. Significant deviations from this arbitrary value can be found. However, such deviations are unimportant. The only really relevant value is the pixel size, which is what is calibrated.

High Resolution TEM Imaging with Digital Camera





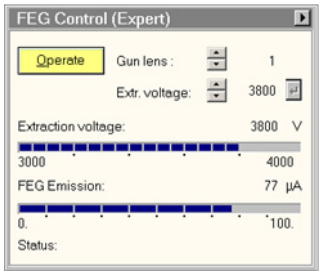
NOTE	■ The magnification table can be edited manually with Microscope/Magnification Table. You can also manually save a backup of the magnification table here. ■ When switching over to another camera, the corresponding magnification table is selected automatically.

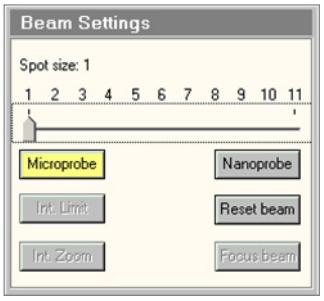
NOTE	Calibrations can only be changed by users with TIA administrator rights (e.g., Supervisor). EELS and EDX calibrations can only be performed by Thermo Fisher Customer Service.

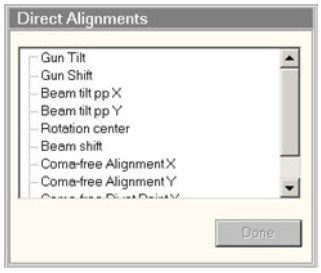
This section describes how to obtain high resolution TEM images with the help of a digital camera. Parameters are optimized for a Talos F200X system.

Setup

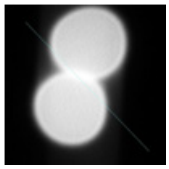
1. Find an amorphous specimen area (with recognizable image features) for tuning the TEM. Adjust to eucentric height.







2. Select gun lens 2-5, spot size 3 and Microprobe, or recall a FEG register. If gun lens was changed, do gun tilt and gun shift on direct alignments.
3. Insert the 100/150 mm condenser aperture and center the aperture such that the beam opens concentrically when changing the condenser 2 current (**Intensity** button) at low SA magnification.
4. Go up to ~125 kX magnification, click **Eucentric Focus**, and focus the specimen at eucentric height (objective lens focus).
5. Set the beam tilt pivot points X & Y in Direct Alignments. The focused beam should not move (see misaligned pivot point on the right). If the beam is not visible, lower the magnification.


Misaligned pivot points

6. Optimize the objective lens rotation center in Direct Alignments at 125 kX. The image feature in the center of the screen should exhibit no displacement.

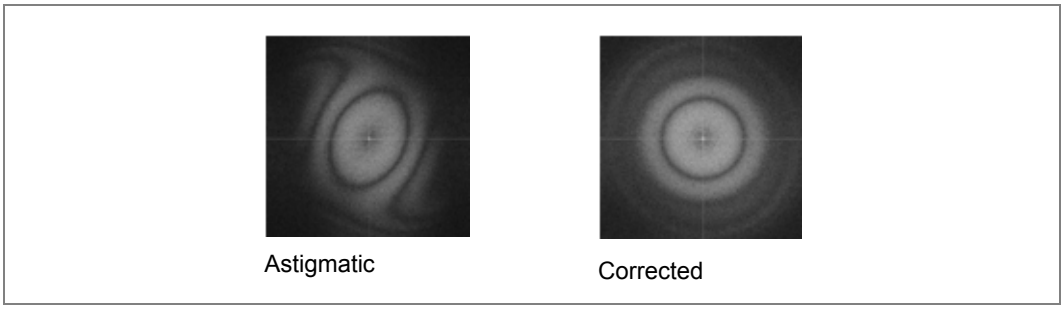
   If there is no suitable object to minimize the image displacement during the alignment of the rotation center, focus the beam (**Intensity** knob) and optimize the rotation center to get a symmetric opening and closing of the beam. This results in a rough alignment of the upper pole piece. High precision is achieved during coma-free alignment.

7. Minimize condenser lens astigmatism.

8. Minimize objective lens astigmatism (see *"Ceta 16M" on page 121*).

   - Acquire a life image of an amorphous area on digital camera at the highest SA magnifications (~400 kX) with live (reduced) FFT.
   - Stigmate to get round features at approximately 100 nm defocus; 1-2 rings visible (see upper right images).

Figure 79 Objective Lens Astigmatism in FFT

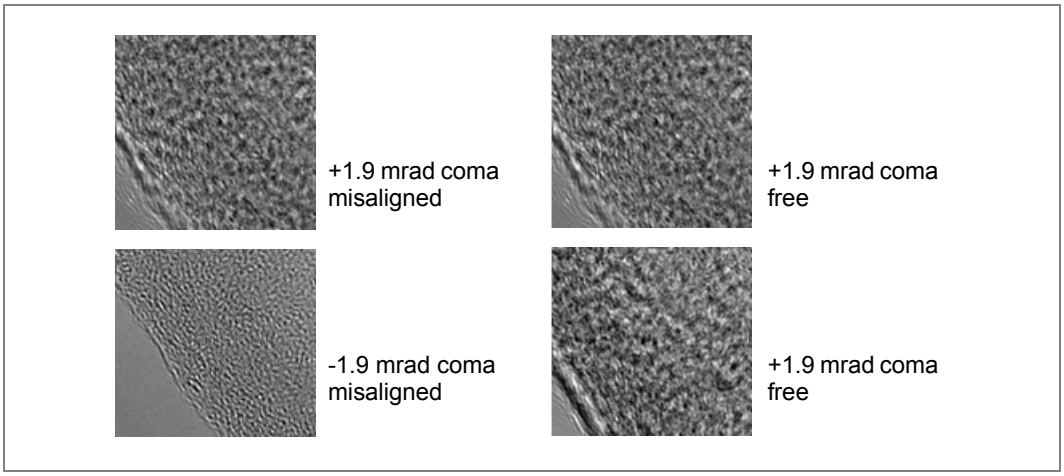


NOTE	Spread the beam (**Intensity** knob) to obtain (mostly) parallel illumination to have uniform incident beam angle and high coherence. Therefore, do not acquire HREM images in Nanoprobe mode.

9. Optimize coma-free alignment: focus the image at 350 kX or higher, select **Coma-free Alignment X & Y** in Direct Alignments, and make the contrast as similar as possible for both beam orientations.

NOTE	The angle for the beam tilt can be adjusted in Direct Alignment in Diffraction mode. The coma-free tilt pivot points must be aligned to prevent the beam from disappearing during coma-free alignment.

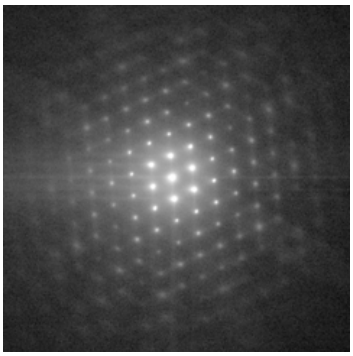
Figure 80 Coma-Free Alignment



If adjustments were strong, repeat the procedure starting at step 8.

Acquire an HR TEM Image

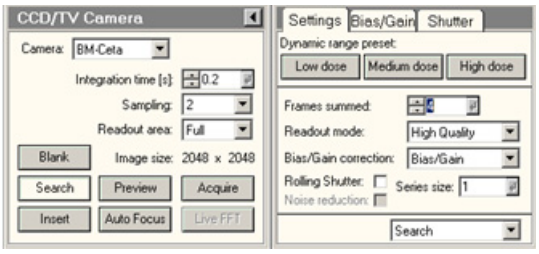
1. Find a thin region of interest, orient onto the zone axis using α- and β-tilt in Diffraction mode (see below). Adjust eucentric height.



Oriented SAED Si (110)

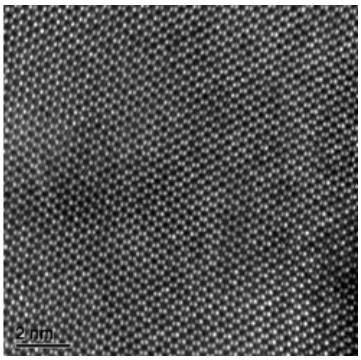
2. Focus roughly, e.g., minimize contrast in a thin amorphous area or minimize Fresnel fringes at an interface; reset defocus as a reference.

3. Increase magnification (typically to the highest SA magnification ~400 kX) in Search mode (0.2 s integration time, binning 2, Full readout area). Spread the beam to get a reasonable number of counts, i.e., do not over-saturate and ensure good signal-to-noise ratio.



NOTE	Spread the beam (**Intensity** knob) to obtain (mostly) parallel illumination to have uniform incident beam angle and high coherence. Therefore, do not acquire HREM images in Nanoprobe mode.

4. Focus the image accurately.
5. Minimize mechanical vibrations (e.g., air conditioner, conversations).
6. In Preview mode, focus precisely.
7. Acquire a full image via **Acquire** with acquisition time of approximately 1 s.



Si (110)

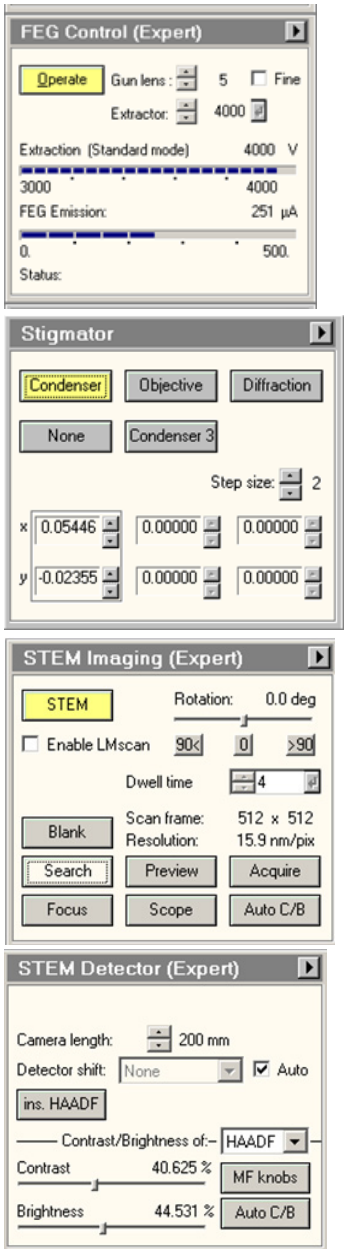
NOTE	■ Spread the beam (**Intensity** knob) to obtain (mostly) parallel illumination to have uniform incident beam angle and high coherence. Therefore, do not acquire HREM images in Nanoprobe mode. ■ The final image should have a good signal-to-noise level. The optimum signal-to-noise is obtained around ½ of the maximum allowed counts, bearing in mind the above bullet point.

High Resolution STEM Imaging

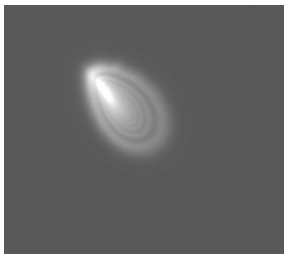
This section describes how to obtain high resolution STEM (HR-STEM) images, which are most sensitive to environmental imperfections. Parameters are optimized for a Talos F200X system.

Setup and Fine-Tuning

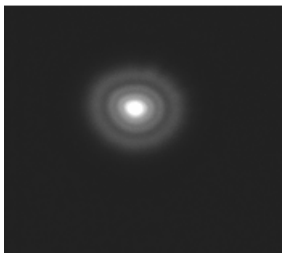
1. Find a suitable specimen area, e.g., on Si<110>, orient on the zone axis and adjust to eucentric height. Store the stage coordinates in the Stage Control panel.



2. If necessary, store the current TEM gun alignment in the FEG register for convenience.
3. Change to 150 µm C2 aperture and center it.
4. If a high-resolution STEM probe was stored, recall it from the FEG register, check C2 aperture centering and rotation center.
5. Set the extraction voltage to 4.5 kV and gun lens to 5-6.
6. Go to STEM mode and select spot size 8-10. Uncheck **Diffraction**.
7. Check intensity list focus, beam tilt pivot points and rotation center intensity on direct alignments. Optimize the probe with condenser stigmator.



Misaligned probe

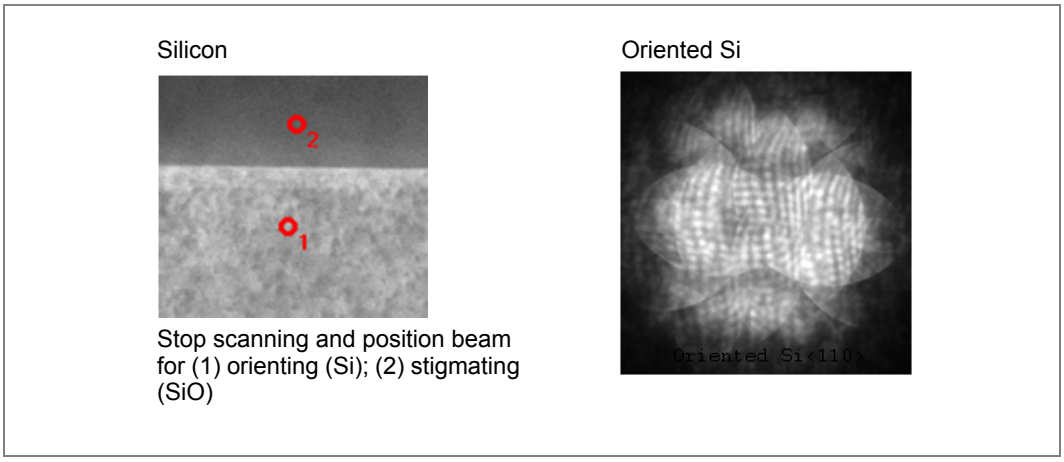


Aligned probe

8. Check **Diffraction** again, and select and center the 70 µm condenser aperture.
9. Click **STEM**. Make sure the spot size is a prealigned one and the HAADF detector is selected.
10. Set camera length to 200 mm, a typical value for silicon.
11. Recall the stored stage position. Start scanning in Search mode. Use a low magnification (< 50,000 kX) and a 512 x 512 pixel raster with a fast readout (typically 2 s frame time).
12. Focus specimen reasonably well with the focus knob.

13. Stop scanning by deselecting Search and move the beam to the region of interest by placing and dragging the Beam Position Marker Tool in TIA.

Figure 81 High Resolution STEM on Si



Stop scanning and position beam for (1) orienting (Si); (2) stigmating (SiO)

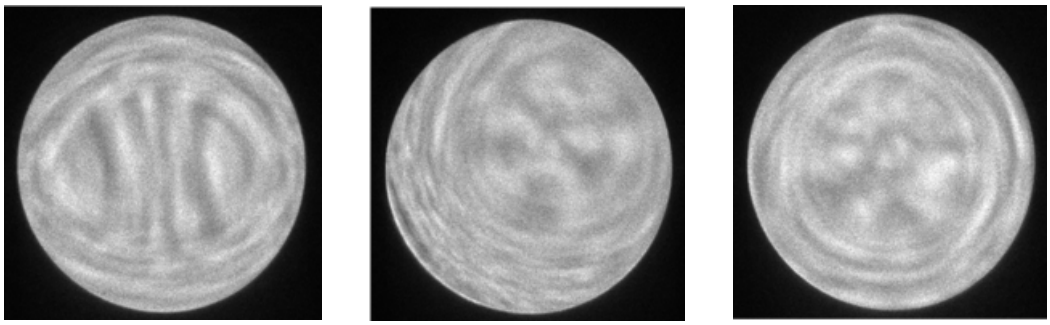
14. Orient the region of interest (position 1 in *Figure 81*) to be accurately on zone axis according to its diffraction pattern. Silicon pattern in <110> with slightly underfocus is shown.

15. If necessary, activate STEM Search mode and move the specimen close to an amorphous area, preferably near the region of interest. Then stop scanning by deselecting Search.

16. Move the spot to the amorphous area (position 2 in *Figure 81*) using the Beam Position Marker Tool in TIA.

17. Focus, stigmate, and precisely align the condenser aperture based on the Ronchigram (the shadow image within the aperture). Proceed to step 20 unless it is difficult to achieve a well-tuned Ronchigram due to the excessive astigmatism.

Figure 82 Ronchigrams—Large C2 Aperture



Large C2 aperture; astigmatic probe

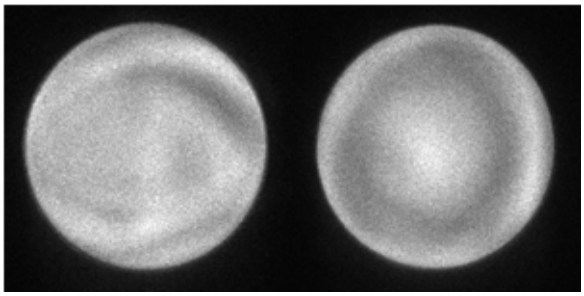
Large C2 aperture; misaligned C2

Large C2 aperture; optimized condition

18. Change the condenser C2 aperture to a larger one, e.g., 150 µm. A larger Ronchigram is now visible, which makes it easier to tune. Focus, stigmate, and align the aperture based on the Ronchigram.

19. Switch back to the 70 µm condenser C2 aperture and repeat Steps 17 and 18.

Figure 83 Ronchigrams—Small C2 Aperture

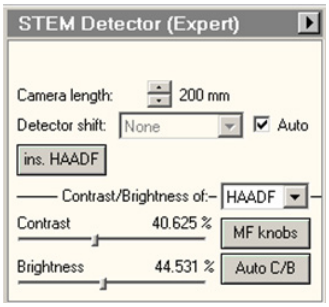


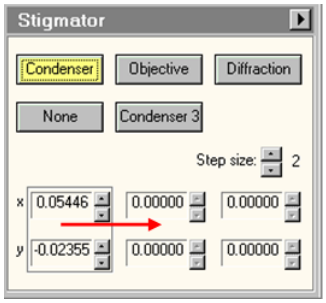
Astigmatic Optimized

20. Move the area of interest into the field of view manually or recall the stored stage position.

Acquire an HR-STEM Image

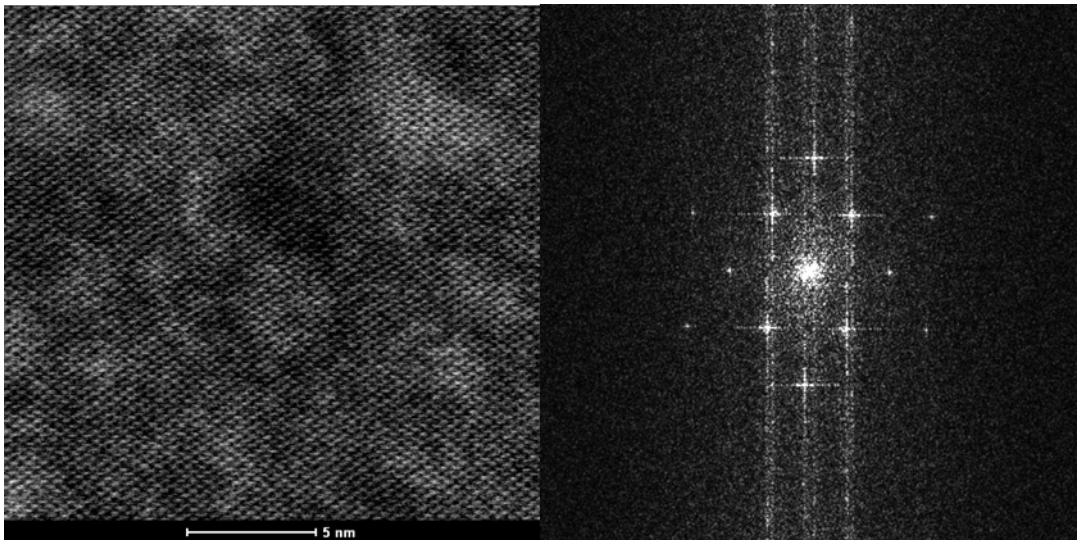
1. Start scanning in Search mode.





2. Increase magnification (typically minimum 5.1 MX for silicon), switch to Preview mode, and focus image slowly (focus step 1 or 2) to make the inter-atomic plane lines visible.

3. Bring the image into sharp focus with focus step 1 and reset defocus value to 0 as a reference via the binding knobs or in the Image Setting panel.
4. Copy the current condenser stigmator values to another memory. Vary stigmator (step size 1) and focus (step size 1) to further tune the image.
5. Acquire an image by clicking **Acquire** in the STEM panel.
6. Store the FEG register.
7. Deselect STEM to quit STEM mode. Recall the stored TEM gun alignment in the FEG register to resume TEM mode. The final HR STEM silicon image is shown below.



Glossary

A

amorphous specimens: having no definite or recognizable form.

anode: a plate at the positive high tension (HT)+ end of the microscope that accelerates and attracts the electrons from the hot surface of the filament. The terminal on a device that receives current.

astigmatism: a lens defect caused by magnetic field asymmetry, resulting in different lens strength in perpendicular planes. Causes only part of the image to be in focus at a time, i.e. a point will appear elliptical. Corrected with stigmators.

AutoLoader: a device mounted on the column opposite the CompuStage that makes it possible to transfer samples automatically into the microscope's vacuum space onto the CompuStage and back out.

azimuth: angular measurement in spherical coordinates. The vector from an observer (origin) to a point of interest is projected perpendicularly onto a reference plane; the angle between the projected vector and a reference vector on the reference plane is called the azimuth.

B

binding: assigning functionality to user-definable knobs and buttons on the control pads of the microscope.

binding display panel: shows how the user-assignable knobs and buttons on the left- and right-hand control pads are linked to microscope functions.

binning: the contents of adjacent pixels are added together before being read out. A binning factor of 2 means that 2 x 2 pixels are taken together, while binning 8 means 8 x 8 pixels together.

Boersch effect: in the focusing of low-energy electrons at a point, the electrons disadvantageously undergo energy broadening by virtue of the random interaction of the electrons with one another. This phenomenon, known as the Boersch effect, is caused by the fact that the electrons, when passing through focusing points are subject to scattering as a result of the high current densities prevailing in this region, which leads to the increase in energy breadth.

Bragg angle: the angle between an incident X-ray beam and a set of crystal planes for which the secondary radiation displays maximum intensity as a result of constructive interference.

Bragg scattering (Bragg-diffraction): the diffraction phenomenon exhibited by a crystal bombarded with X-rays in such a way that each plane of the crystal lattice acts as a reflector.

Bragg's law: when X-rays hit an atom, they make the electronic cloud move as does any electromagnetic wave. The movement of these charges re-radiates waves with the same frequency (blurred slightly due to a variety of effects). This is also known as *elastic scattering*. It can be expressed as $2d \sin(\theta) = n\lambda$ (n=1,2,3..), where n is an integer determined by the order given, λ is the wavelength of the X-rays (and moving electrons, protons and neutrons), d is the spacing between the planes in the atomic lattice, and θ is the angle between the incident ray and the scattering planes.

bright-field (BF) imaging: the standard method for TEM. It simply means making an image with the transmitted beam only.

C

cathode: the filament at the negative high tension (HT)- of the microscope. In vacuum electronic devices, electrons are emitted by the cathode and flow to the anode. It is from the cathode that electrons flow into these devices.

CCD camera: a charge-coupled device (CCD) camera that consists of a two-dimensional array of light-sensitive elements. When struck by light, the elements generate electrons that are stored inside the element until the camera is read-out. On a slow-scan CCD camera, an image is acquired by letting the electrons from the beam strike the camera for the full integration time (so an image is acquired in a single cycle.

chromatic aberration: a resolution limiting factor that results when waves of different energies converge at different focal planes. Chromatic aberration manifests itself as "fringes" of color along boundaries that separate dark and bright parts of the image, because each color in the optical spectrum cannot be focused at a single common point on the optical axis.

circle of confusion: an optical spot caused by a cone of light rays from a lens not coming to a perfect focus when imaging a point source. Also known as *disc of confusion, circle of indistinctness, blur circle,* or *blur spot*. Lenses do not focus all rays perfectly, so that a point is imaged as a spot rather than a point. The smallest such spot that a lens can produce is often referred to as the *circle of least confusion.*

coherence: the electron beam is considered coherent when the source of the electrons is a point (as small as possible) and when the energy of all the electrons is the same (as small an energy spread as possible). This condition produces maximum phase contrast.

coma-free: having a distortion-free beam due to beam tilt. A coma-free alignment tilts the beam and changes the FFT.

CompuStage: the compucentric stage used on the TEM.

condenser lens: one of the main components of the optical system of the microscope, it concentrates electrons into a projected beam that is in turn focused through the object and magnified by the objective lens.

Conical mode: used to avoid asymmetries in the dark-field image by swiveling the direction of the tilt around a cone.

contrast transfer function (CTF): defines the transfer of contrast from the sample onto the image. It is the function that modulates the amplitudes and phases of the electron diffraction pattern formed in the back focal plane of the objective lens. It allows the evaluation and performance (point-to-point resolution and information limit) comparison of different microscopes.

control pads: right- and left-hand control pads sit on either side of the microscope keyboard. They contain a trackball or joystick and a number of buttons and knobs for controlling the microscope.

control panels: small windows, typically arranged in sets of three above one another on the left side of the screen, that contain a coherent set of microscope controls for frequently used microscope functionality. The combination of up to three control panels forms a workset.

convergent-beam diffraction (CBED): a technique that is capable of furnishing remarkably accurate crystallographic information: sample thickness, unit cell and precise lattice parameters, and the crystal system.

crystalline structure: a structure with a regular arrangement of atoms.

D

dark field imaging: an illumination technique used to enhance the contrast in unstained samples. It works by illuminating the sample with light that will not be collected by the objective lens, and thus will not form part of the image. This produces the classic appearance of a dark, almost black, background with bright objects on it.

deflection coils: in principle, a more or less uniform magnetic field B, perpendicular to the incident electron beam and generated by the current through the coils, acts on the electrons and deflects them. The combination of two such coil pairs perpendicular to each other can deflect a beam in any direction.

dewar: the vacuum flask invented by Scottish physicist and chemist Sir James Dewar in 1892. Also known as a “thermos,” it is a storage vessel that provides thermal insulation by interposing a partial vacuum between the contents and the ambient environment.

diffraction: the angular redistribution of incident electrons being scattered by the sample.

Diffraction mode: the mode in which the imaging lens system is focusing at the back-focal plane of the objective lens.

diffractogram: the end product resulting from the interaction between monochromatic X-rays and crystalline material. Also known as a *diffraction pattern*. A diffractogram is generated using a single wavelength.

DigitalMicrograph: *see Gatan DigitalMicrograph.*

E

EDX: energy dispersive X-ray analyzes the X-rays that are generated when the electron beam hits the specimen. EDX produces spectra that are plots of X-ray counts versus X-ray energy. When electrons ionize an atom, the emitted X-ray energy is unique to the ionized atom (element). So, EDX allows analysis of elemental composition of the specimen.

EELS analysis: electron energy-loss spectrometry (EELS) is the analysis of the energy distribution of electrons that have interacted inelastically with the specimen.

EFTEM: in energy-filtered TEM (EFTEM), some of the beam electrons lose energy upon interaction with the specimen (inelastic scattering). Strong energy losses result in image blurring. In EFTEM, the microscope is equipped with an imaging filter that separates the electrons according to their energies, making it possible to filter out the blurring effects or retrieve the chemical or physical information from the specimen.

electron: a negatively charged particle that can be deflected by means of magnetic and electric fields.

electron energy-loss spectrometry (EELS): the analysis of the energy distribution of electrons that have interacted inelastically with the specimen.

emission current: the total current emitted by the electron gun.

exposure state: in Low Dose mode, sets up the conditions for the exposure and defines the exposure time.

F

FEG (field emission gun): a rotationally symmetric, thin rod that tapers to a point and is located along the optic axis. It pulls heated electrons out of a very fine tungsten tip using an extremely high electric field. With a FEG, users gain electron densities up to 1,000 times those of tungsten emitters without a FEG.

field emission gun: *see FEG*

FFT (fast Fourier transform): an efficient algorithm to compute the discrete Fourier transform (DFT) and its inverse.

flyout button: arrow button on the far right of a control panel title bar that gives access to flyout panels; also known as *flap-out* button.

flyout panels: additional panels that appear to the right of the main control panel for access to less frequently used microscope settings; also known as *flap-outs*.

focus state: in Low Dose mode, the setup to allow image optimization (focusing, correction of astigmatism) at a high magnification and high intensity at an off-axis part of the specimen.

G

gain image: an image taken with uniform illumination and then recalculated to the inverse of the maximum intensity in the image.

Gatan DigitalMicrograph: an image acquisition, analysis, and processing program produced by Gatan.

Gatan imaging filter (GIF): a device attached under the camera chamber that allows Electron Spectroscopic Imaging (ESI) and Electron Energy Loss Spectroscopy (EELS). The GIF incorporates sophisticated electron optics and an energy selecting "slit" that allows the beam to be filtered to reveal additional information in the form of energy filtered images, diffraction patterns, or spectra.

H

HREM image: high resolution electron microscope image

L

Lorentz force: the force on a charged particle moving in electric and magnetic fields, equal to the particle's charge times the sum of the electric field and the cross product of the particle's velocity with the magnetic flux density.

low angle diffraction (LAD): imaging of the diffraction pattern formed by the objective lens in the SA plane. The objective lens current is the same as in LM mode

Low Dose mode: mode for minimizing the amount of beam damage (minimum dose of illumination) to the specimen prior to recording. Has three states: Search, Focus, and Exposure.

M

mass-thickness contrast: an image contrast mechanism created when only the electrons within the aperture contribute to the image, thus the thin, light part in the specimen looks more bright in the image than the strongly diffracting or absorbing part.

micro-micro-diffraction (μμD) pattern (also called nano-diffraction pattern): pattern obtained in Diffraction mode (in Nanoprobe mode), used for characterizing crystalline materials at the lower end of the submicron scale.

Microprobe mode: the normal TEM mode where a nearly parallel beam illuminates the specimen.

N

nano-diffraction pattern: see *micro-micro-diffraction (μμD) pattern*

Nanoprobe mode: provides a very small probe with high beam intensity, which can be utilized for X-ray analysis because it gives a better spatial resolution and a stronger signal. However, it is not an ideal mode for imaging since the area illuminated is small and, normally, the beam is convergent.

normalization: in image processing, normalization is a process that changes the range of pixel intensity values. Applications include photographs with poor contrast due to glare, for example. Normalization is sometimes called contrast stretching. In more general fields of data processing, such as digital signal processing, it is referred to as dynamic range expansion, the purpose of which is to bring the image, or other type of signal, into a range that is more familiar or normal to the senses, hence the term normalization. Often, the motivation is to achieve consistency in dynamic range for a set of data, signals, or images to avoid mental distraction or fatigue.

O

objective lens: provides an image of the specimen with high magnification before being further enlarged by the magnifying system.

P

photons: visible light electrons are converted to photons when they strike fluorescent material.

plasmons: the collective oscillation of weakly bound outer-shell electrons, commonly the conduction electrons existing in a metal. The energy causing the oscillation is transferred from the incident electrons when their Coulomb fields disturb the stability of this electron gas. In electron microscopy, plasmons are not measured directly but indirectly through the transmitted electrons that excite plasmons and lose the corresponding energy.

plate camera: located immediately underneath the viewing screen.

popup panels: control panels that are too large to be assigned to worksets.

pole piece: a piece of magnetic material forming one end of an electromagnet or permanent magnet, shaped to control the distribution of magnetic flux in the adjacent air gap.

R

Rutherford scattering: the theory, named after Ernest Rutherford in 1909, that states that the elastic electron scattering power depends on the specimen density (Z number) and its thickness. It led to the development of the Rutherford model (planetary model) of the atom. This is the principle of mass-thickness contrast.

S

scalar: a quantity (as mass or time) that has a magnitude described by a real number and no direction.

Scherzer focus: the optimum underfocus, at which all the phase distortions by the optical system are approximately equal.

scintillator: a material that emits light when struck by electrons.

Search state: in Low Dose mode, the setup for searching for suitable areas, usually at a low magnification (or defocused diffraction) and a small spot size to reduce beam intensity.

selected area diffraction (SAD): this technique offers a solution to obtain crystallographic information of a small area by introducing an aperture in the first intermediate image (the image plane of the objective lens) in the selected area (SA) plane.

selected area plane: the object plane of the imaging system. By adjusting the objective lens current, you can position the objective-lens image accurately in the selected area plane, so that the image on the fluorescent screen is focused.

SmartCam: the fluorescent screen camera that looks onto the TEM image.

spectroscopy: the analysis of an object from its spectrum; the study of the interaction between radiation and matter as a function of wavelength.

spherical aberration: the inability of a lens to focus all incident beams from a point source to a point. This is a major resolution-limiting phenomenon.

STEM mode: in the STEM (or Scanning) mode of the microscope, images are acquired by moving a focused beam in a raster across the specimen and collecting a signal at each x,y pixel coordinates. The signals for all pixels together make the STEM image.

stigmators: used to correct astigmatism. They act on the electron beam by pressing it from opposite sides and pulling it from both sides in the perpendicular direction, i.e., modifying an ellipse into a circle.

T

TEM: Transmission electron microscope; also a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

thermionic: pertaining to the emission of electrons as a result of heat.

TIA: Thermo Fisher's trademarked TEM Imaging and Analysis software

tilted beam method: allows high resolution dark-field imaging by all electrons with a specific scattering angle of θ_B, achieved by automatically rotating the tilted beam about the optical axis. This technique is especially useful in imaging polycrystalline or amorphous specimens.

Thon rings: rings visible in the power spectrum of micrographs obtained by bright-field electron microscopy. These rings can be explained as the effect of the contrast transfer function, which modulates the Fourier transform of the object in a defocus-dependent way. Presence of axial astigmatism is evident from deviations of the Thon rings from perfect circular symmetry, defocus is gauged from the placement of the rings, and resolution can be gauged from the range of the rings. For these reasons, electron micrographs are routinely inspected by optical diffraction, prior to scanning and digital analysis.

triode: a three-electrode electron tube containing an anode, a cathode, and a control electrode.

W

Wehnelt: an electrode between the cathode (filament) and the anode (earth) in an electron gun that focuses and controls the electron current in the beam and keeps it constant. Pronounced *vay-nelt*.

workset: the combination of up to three control panels forms a workset, defined by name and accessible through a tab in the workset selection window above the control panels themselves.

Workspace Layout control panel: provides the tools for customizing the worksets: control panel selection, color selection, etc.