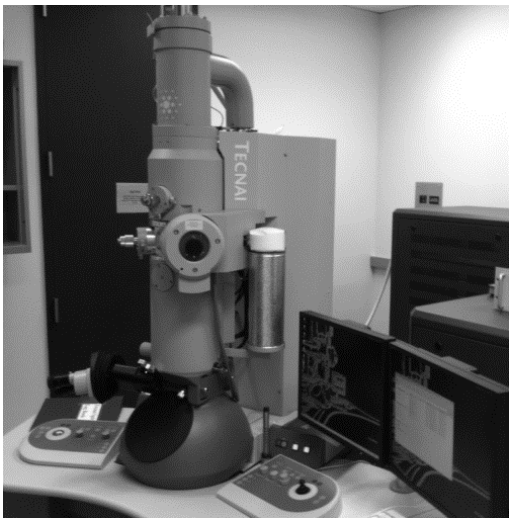
# Introductory Transmission Electron Microscopy Primer

**Bob Hafner  October 2011**

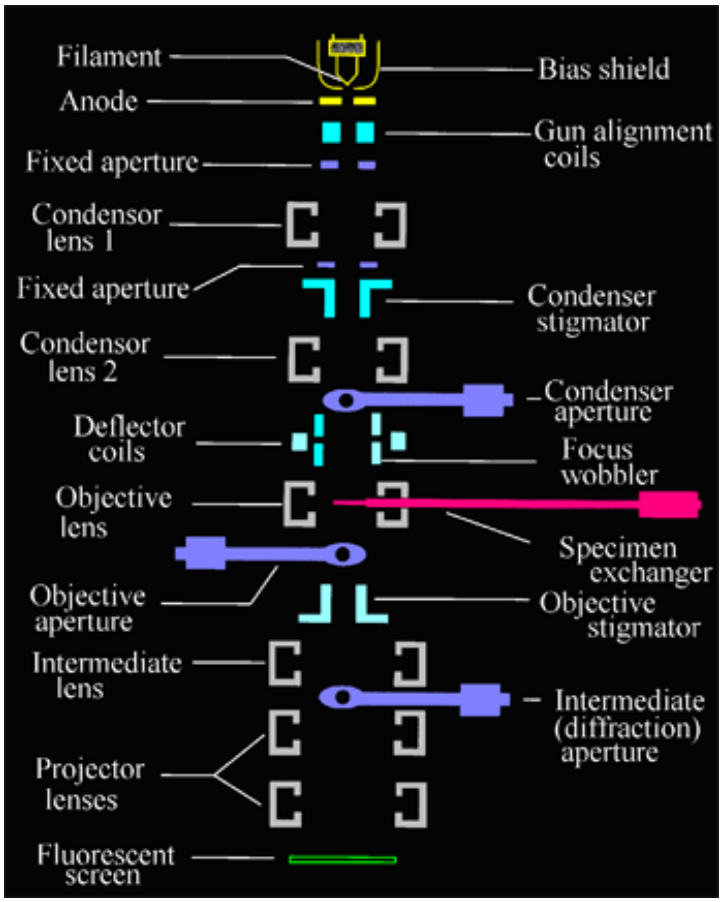
This primer is intended as background for the Introductory Transmission Electron Microscopy training offered by the University of Minnesota's Characterization Facility (CharFac). The primer addresses concepts fundamental to any transmission electron microscope (TEM). Learning this material **prior to** the hands-on training will improve the effectiveness and efficiency of your TEM imaging.




## The Big Picture

To the right is an image of Charfac's FEI Spirit transmission electron microscope.  The microscope column, specimen chamber, and viewing screen are flanked by the monitor and panel controls on the table top.  As an operator you will need to understand what is happening inside this "black box" (microscope column) when a panel or column control is manipulated to produce a change in the monitor image.

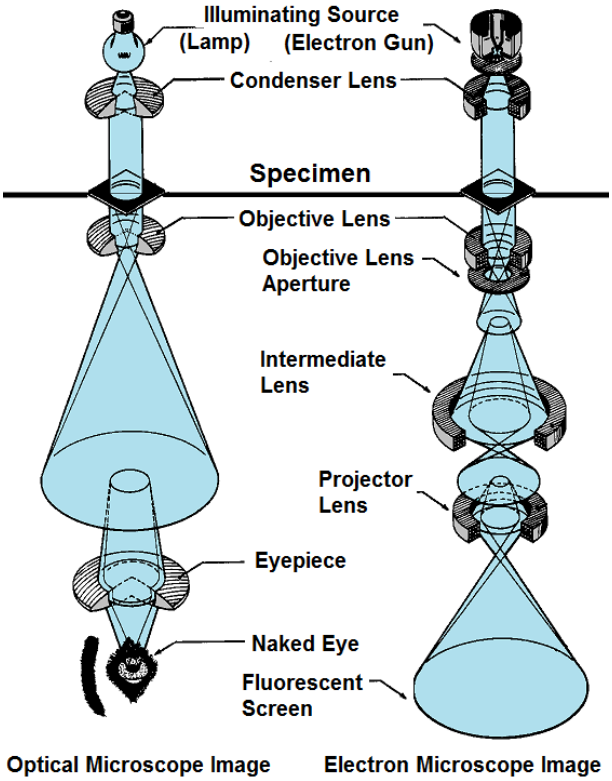
A look inside the black box [*Figure adapted from 1*] reveals quite a bit of complexity. However, we can begin to simplify this generic representation by categorizing the components.  We have a:




- source (**electron gun**) of the electron beam which is accelerated down the column;
- series of **electromagnetic coils** (shifts, tilts, stigmators) which ensure that the electron beam is symmetrical and centered as it passes down the optical column of the microscope;
- series of **electromagnetic lenses** (condenser, objective, intermediate, projector) which act to illuminate the specimen and focus/magnify the specimen on the fluorescent screen/ camera;
- series of **apertures** (micron-scale holes in metal film) which the beam passes through and which effect properties of that beam;
- **specimen holder** which positions the sample in the path of the electron beam. The area of beam/specimen interaction generates several types of signals that can be detected and processed to produce an image, diffraction pattern or spectra.  There are controls for specimen position and orientation (x, y, z or height, tilt, rotation);

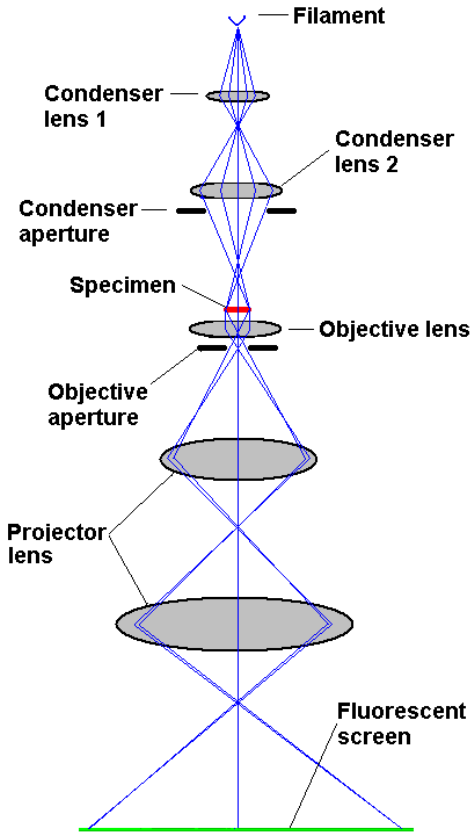
- **fluorescent screen** or CCD camera which converts the electron signal to a form we humans can see;
- all of the above maintained at high **vacuum levels.**

We can provide further structure to this complexity by comparing the arrangement and function of these components to something with which we have all had some experience -- a transmitted light microscope [*Figure adapted from 2*]. Both the electron and light microscope have:




- an **"Illumination system"** (area above the specimen) which consists of: a source of radiation (photons/ electrons) and a condenser lens which focuses the illuminating beam on the specimen -- allowing variations of intensity to be made;
- a **"Specimen stage"**(black line) situated between the illumination and imaging systems;
- an **"Imaging system"** (area below the specimen) which consists of: an objective lens which focuses the beam after it passes through the specimen and forms an intermediate image of the specimen; and, subsequent lenses (eye piece/intermediate and projector lenses) which magnify portions of the intermediate image to form the final image.
- an **"Image recording system"** (eye, fluorescent screen or CCD camera) which converts the radiation.

In addition, the traditional ray diagrams of light optics can be used to display how we magnify/ demagnify and focus the electron beam and how electrons deriving from a point in an object plane can come to a "well-defined" point in the image plane [*Figure adapted from 1*].




One additional point needs to be made at this time:

**You need to take the concept of a "column" seriously and start putting together a picture in your mind of where components are in relation to one another – starting at the top (gun) and working your way to the bottom (fluorescent screen). This is essential to understanding alignment of the microscope as well as everyday operation.**

Having mentioned some of the similarities between optical light microscopy and TEM, the Table below highlights the important differences.

| | **Optical Microscope** | **Electron Microscope** |
|---|---|---|
| **Information carrier** | Light rays | Electrons (have mass, charge) |
| **Wavelength** | 400-800 nm (visible) | 0.0037 nm (100 kV) |
| **Medium** | Air | Vacuum |
| **Lenses** | Glass | Electromagnetic |
| **Aperture angles** | < 60 degrees | < 1 degree |
| **Image visibility** | Direct | Fluorescent screen, CCD camera |
| **Contrast producing mechanism** | Light absorption, reflection, spatial variation in refractive index, scattering, diffraction, birefringence, fluorescence… | Mass-thickness; diffraction; phase. |
| **Resolving power** | 200 nm (visible) | < 1 nm (point to point) |
| **Magnification** | 5—2,000 X | 35—1,000,000 X |
| **Focusing/Alignment** | Mechanically | Electrically |
| **Depth of field/focus** | < 10 um / < 0.1 mm | > 100 um / > 1 meter |

Electrons exhibit both particle and wave characteristics ($\lambda = h/mv$). Just as visible light can be treated as a beam of photons or an electromagnetic wave—so too can electrons be characterized as a succession of particles or treated by wave theory. However, electrons are charged and the Coulomb forces are strong.

The negative charge of the electrons allows them to be:

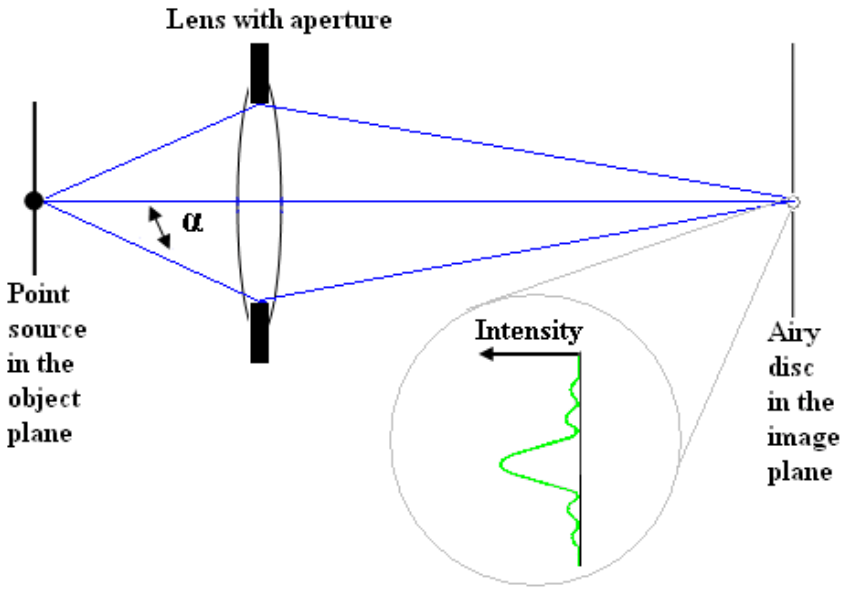
- deflected by means of magnetic or electrostatic fields. This is the basis for how lenses and coils in the microscope work;
- accelerated down the column by experiencing a voltage differential.

The small mass of the electrons necessitate:

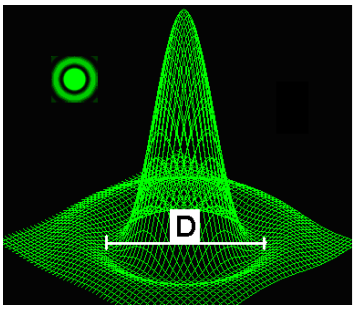
- high vacuum conditions to negate their deflection by air particles;
- ultra thin (<100 nm) specimens to ensure transmission of the beam.

The shorter wavelength of the electron results in a better **theoretical resolution (diffraction limit of resolution)**.

When light rays emanate from a point and pass through a lens of semi-angular aperture α, they form an image which is no longer a point but with the intensity spread out in what is known as an Airy disk.

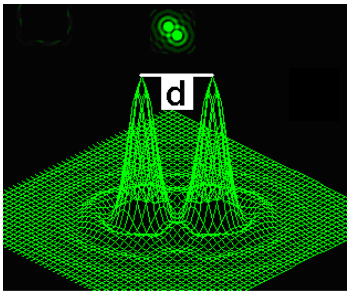


The distance between the two minima on either side of the main intensity peak of the Airy disk is: **$D = 1.22 \lambda / n \sin\alpha$** where:



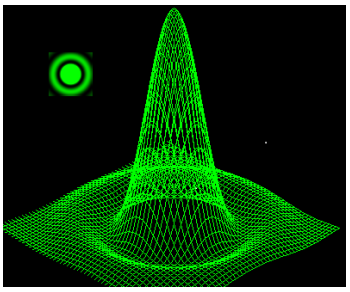

- $\lambda$ is the wavelength;
- n is the refractive index of the material in which the object lies;
- $\alpha$ is the semi-angular aperture.

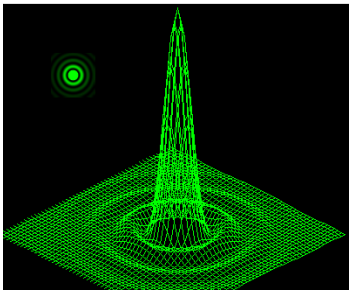
[*Figures adapted from 3*]



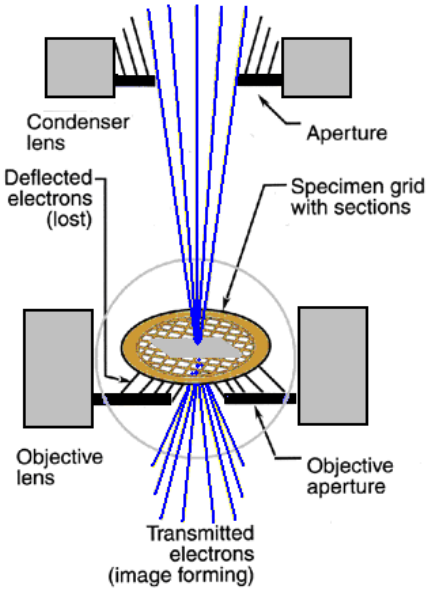

When two emitting points of the of the object lie very close together, the intensity patterns in the image will overlap. **The theoretical resolution of the system is defined as the distance between the maxima when the maximum intensity from one point is coincident with the first minimum from the other point.** This distance is the diffraction limit of resolution: **$d = 0.61 \lambda / n \sin\alpha$**

From this equation one can see that increasing the aperture angle and decreasing the wavelength will enable better resolution. Bottom left Figure: Small aperture /large wavelength; Bottom right Figure: Large aperture / small wavelength.



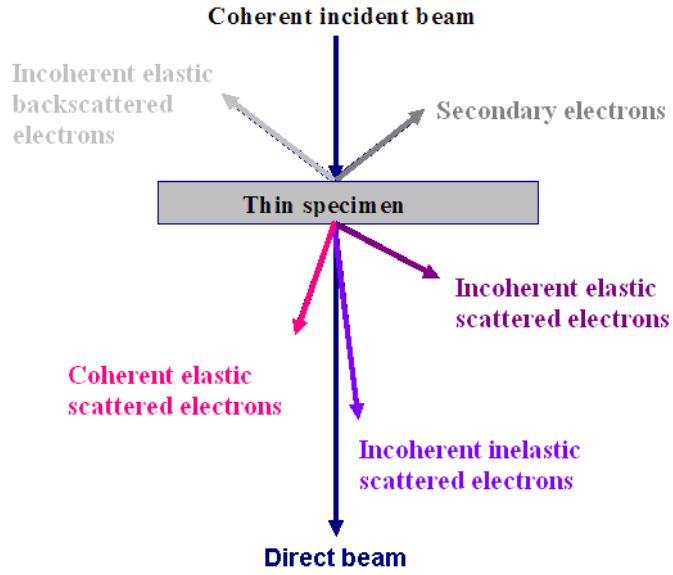


In the light microscope the diffraction limit of resolution is approximately 200 nm. The effective $\lambda$ (nm) of an electron beam can be approximated as $1.22 / E^{1/2}$, where E is the accelerating voltage (eV). The wavelength for an accelerating voltage of 100 kV is 0.0039 nm; 0.0022 for 300 kV. The wavelength of electrons is shorter by orders of magnitude compared to light microscopy, and thus the diffraction limit of resolution is much improved. Our ability to increase the aperture angle in TEM ends up being constrained by aberrations in the electromagnetic lenses (more on this later). Thus the practical, or aberration limited resolution, is less than the theoretical resolution.




For a given microscope, the attainable resolution depends upon the accelerating voltage (higher accelerating voltages produce better resolution) and the design of the objective (focusing) lens. The objective lens has a spherical aberration coefficient. The smaller the lens gap the smaller the spherical aberration coefficient and the better the resolution. However, a smaller lens gap will decrease contrast (more on all of this later).

Finally, the low mass and negative charge of the electrons means that they can easily be scattered (deflected) by passing close to electrons or the nucleus of the specimen atoms.

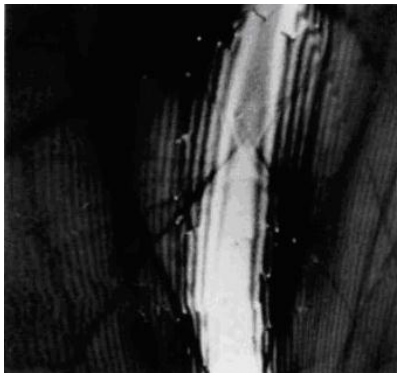



This scattering can be [*4*]:

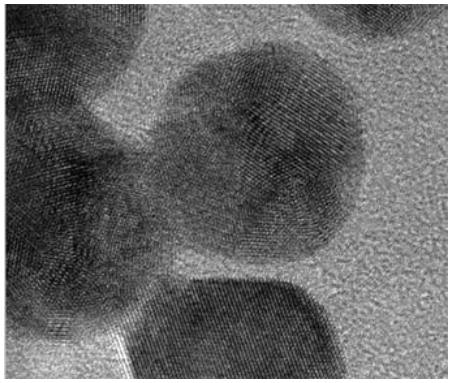
- forward or backward;
- coherent or incoherent; and
- elastic or inelastic (more on this later).

The scattering results in a non-uniform distribution of electrons emerging from the specimen. This non-uniform distribution is the basis of the major contrast producing mechanisms in the TEM:

**Mass/thickness**



**Diffraction**

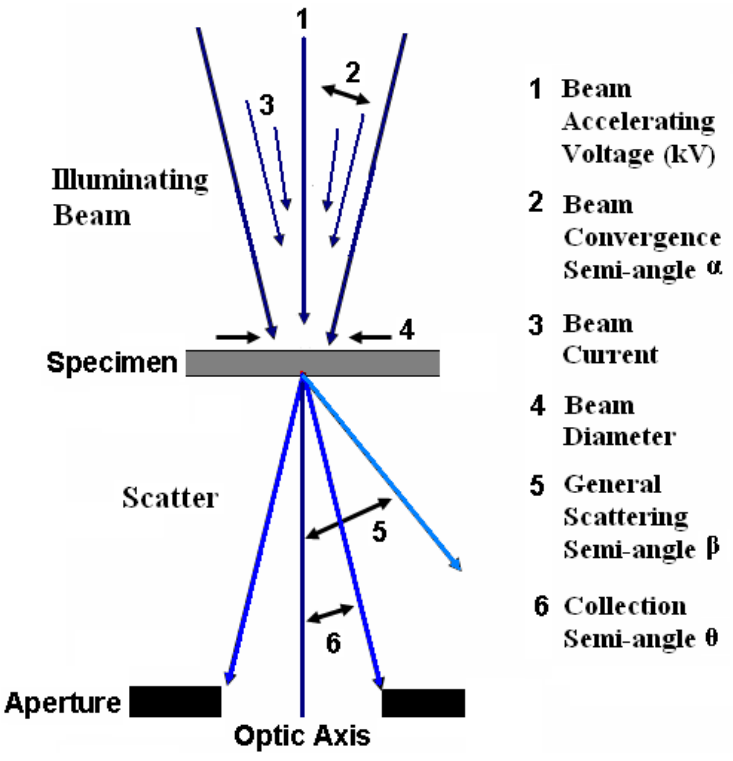


**Phase**

As TEM operators we can selectively choose which aspects of the scatter will transmit and thus contribute to an image [*Figure adapted from 4*]. One common way to do this is to determine the **"collection semi-angle (θ)"** through the use of the objective aperture below the specimen.

The collection semi-angle is a subset of the **"general scattering semi-angle (β)"** —the latter being determined by the nature of the illuminating electron beam and the properties of the specimen.

The illuminating beam has several interrelated properties that we can control: accelerating voltage (kV); convergence semi-angle (α); diameter and current of the beam on the specimen.



In what follows we will work our way down the column -- building a more detailed understanding as we go. We will first address "bright field" and "dark field" imaging that utilizes "amplitude (scattering) contrast". Amplitude contrast includes both "mass/thickness" and "diffraction" contrast. "Phase (interference) contrast" will then be explained and this will allow us to introduce the concept of the "contrast transfer function". Those individuals who wish to have a better conceptual understanding of the alignment of the microscope should consult Appendix A. Appendix B utilizes the contrast transfer function to characterize how the Moos Tower FEI Tecnai Spirit and the Nils Hasselmo Hall FEI Tecnai F30 Field Emission Gun microscopes differ from one another in terms of imaging capabilities.

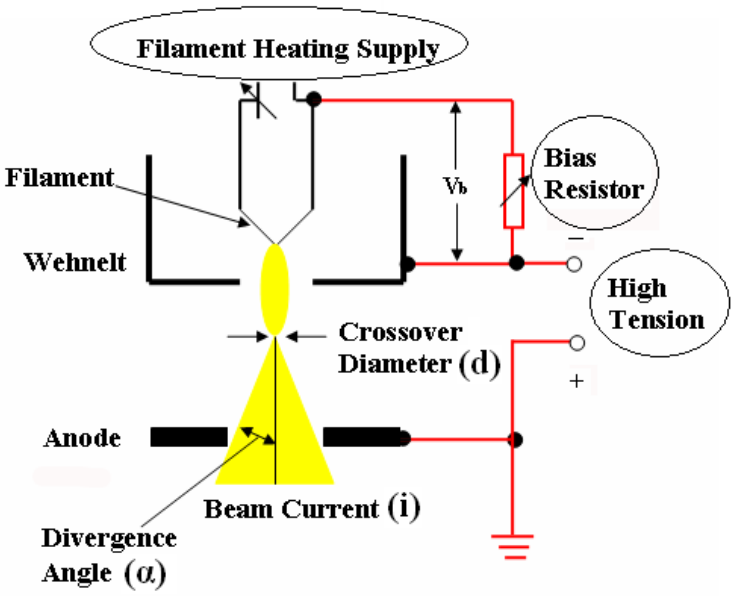
# Illumination System

## Electron Guns

The purpose of the electron gun is to provide a stable beam of electrons of adjustable energy. There are three main types of electron guns:

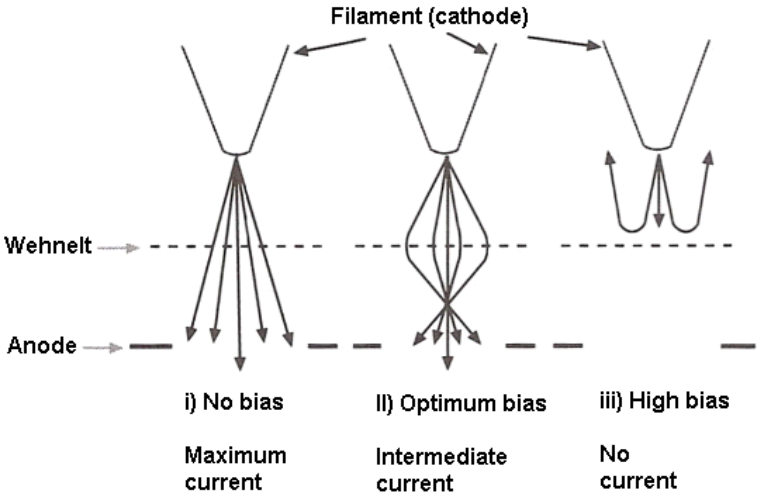
- Tungsten hairpin
- Lanthanum hexaboride ($LaB_6$)
- Field Emission

There are three properties of the Tungsten hairpin and $LaB_6$ filament guns that you have some control over.



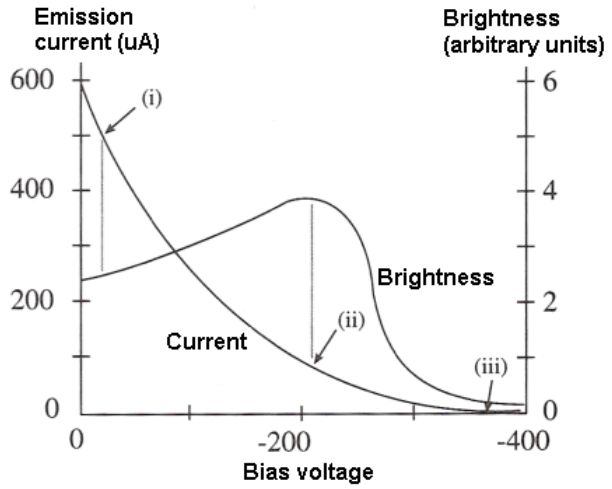

You can:

- heat the filament to facilitate the emission of electrons from it (emission current). The goal is to maximize emission current and minimize filament temperature. The point at which this occurs is termed the **"saturation point".**
- apply a potential difference between the filament (cathode) and the anode of the gun to accelerate the emitted electrons down the column. The **accelerating voltage** we choose will have an effect on the penetrating power of the electron beam as well as its resolving capabilities.
- apply a small negative **bias** between the filament and the Wehnelt cap which houses the filament [*Figure adapted from 4*]. With no bias (i) there is high emission current, but that current has a high divergence angle or spread. A very high bias (iii) will actually retard the emission of electrons. The optimum bias level (ii) will take the emitted electrons and produce a crossover point (gun source) of small diameter -- a saturated electron cloud.



Producing a small gun source or crossover has several benefits:

- An electron beam emanating from a small source size is said to have high **spatial coherency**. That is, the waves emanating from the source point are in phase with one another. High spatial coherence of the illuminating beam will enhance the contrast producing mechanisms in the TEM (we will discuss other ways of enhancing beam coherence later on).
- Also, by using the bias to form a small source size we are putting the emission current into a smaller area and thus maximizing the brightness of the electron beam.




**Brightness** is the beam current per unit area per solid angle [*Figure adapted from 4*]

$B = 4i \ / \ (\pi \, d \, \alpha)^2$ where:

- i = beam current;
- d = diameter of the source (crossover); and,
- α = semi-angle of beam divergence.

With greater brightness we can have a smaller diameter beam on the specimen and still have adequate current for signal production. The result is higher magnification imaging capabilities. Brightness, unlike current, is conserved down the column. Brightness increases linearly with accelerating voltage.

Electron beams can also be characterized in terms of **temporal coherency**. A beam with high temporal coherency will have electrons of the same wavelength. In reality there is a certain **"Energy Spread**" associated with the beam. The energy spread will decrease with: high tension stability; a lower surface temperature of the emitter; and, a somewhat lower emission current (Boersch effect).

The table below highlights the important differences between the various gun types. There is a progression of capabilities from Tungsten to LaB6 to FEG in terms of brightness, source size, and temporal coherency.

| | **Tungsten:** JEOL 1200 & 1210 | **$LaB_6$:** Tecnai Spirit & T12 | **Shottky FEG:** Tecnai G2 F30 |
|---|---|---|---|
| **Brightness ($A/cm^2str$)** | $10^5$ | $10^6$ | $10^8$ |
| **Lifetime (hrs)** | 40-100 | 200-1000 | >1000 |
| **Source Size** | 30-100 um | 5-50 um | <5 nm |
| **Energy Spread (eV)** | 1-3 | 1-2 | 1 |
| **Current Stability (%hr)** | 1 | 1 | 5 |
| **Vacuum (Torr)** | $10^{-5}$ | $10^{-7}$ | $10^{-11}$ |

Along with these capabilities comes the need for better vacuum systems. The advantages of a more coherent beam source will be negated if the beam is interacting with molecules on its path down the column. The table below is provided simply to give you a feeling for what these vacuum levels translate to inside the microscope. For reference: 1 Torr = 133 Pa = 1.33 mbar. "Mean Free Path" is the average distance covered by a moving particle between successive collisions. "Time to Monolayer" is the length of time required, on average, for a surface to be covered by an adsorbate.

| Vacuum | Atoms/cm3 | Distance between atoms | Mean Free Path | Time to monolayer |
|---|---|---|---|---|
| 1 Atm (760 Torr) | $10^{19}$ | 5 x $10^{-9}$ meters | $10^{-7}$ meters | $10^{-9}$ seconds |
| $10^{-2}$ Torr | $10^{14}$ | 2 x $10^{-7}$ meters | $10^{-2}$ meters | $10^{-4}$ seconds |
| $10^{-7}$ Torr | $10^{9}$ | 1 x $10^{-5}$ meters | $10^{3}$ meters | 10 seconds |
| $10^{-10}$ Torr | $10^{6}$ | 1 x $10^{-4}$ meters | $10^{6}$ meters | $10^{4}$ seconds |

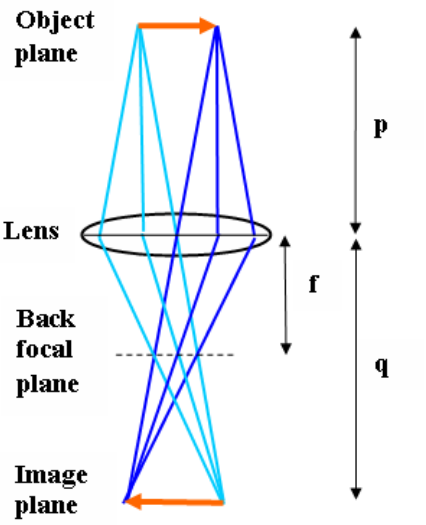
We won't spend time here talking about the various kinds of pumps and gauges associated with the vacuum system since those are maintained by the staff. However, as an operator, **you should be very aware of the vacuum state of the microscope** and ensure that:

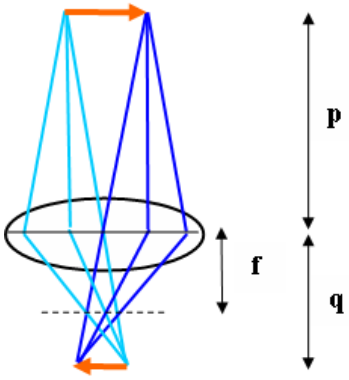
- the microscope is at good vacuum level when you begin your session;
- if there is a "Gun Valve" separating the upper column from the rest of the microscope – that it is closed during sample exchange;
- appropriate vacuum levels are achieved prior to engaging and stepping up the high tension;
- your hands do not touch any part of the specimen holder that is inserted into the column (under high vacuum); and
- your samples are dry and free of contamination and outgassing.

## Electromagnetic Lenses

(Coils [shifts, tilts, stigmators] will be addressed in the alignment section of the primer)

It is useful to reason by analogy with glass lenses used for focusing light to help one understand the operation of electromagnetic lenses. Analogies also have regions of non-correspondence which are equally important to understand. The diagram to the right shows a perfect optical lens. The rays emanating from a point in the object plane (a distance "p" from the lens) come to one common well defined point in the image plane ( a distance "q" from the lens). Parallel rays entering the lens are focused in the "back focal plane" (a distance "f" from the lens). The optical lens has a fixed focal point and the object is in focus at the image plane. The magnification of the lens is q/p (demagnification = 1/magnification)

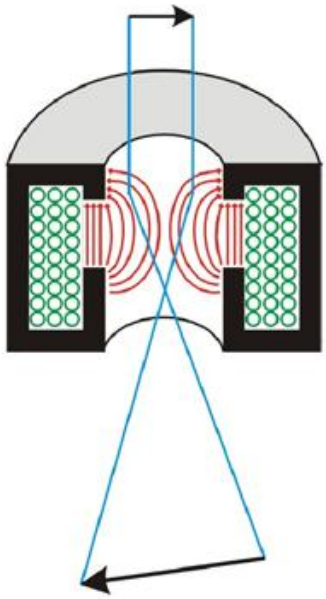



In light optics we exchange lenses to change magnification and adjust the height of a given lens along the optic axis to focus. Electromagnetic lenses in contrast are stationary -- but we can vary their strength by altering the amount of current running through them. Thus electromagnetic lenses have variable focal points. The diagram to the left shows a stationary lens which is stronger (larger). In this instance we have actually demagnified the image.

An electromagnetic lens [*Figure from 5*] consists of a coil of copper wires inside an iron pole piece. A current through the coils creates a magnetic field (symbolized by red lines) in the bore of the pole pieces which is used to converge the electron beam.

When an electron passes through an electromagnetic lens it is subjected to two vector forces at any particular moment: a force ($H_Z$) parallel to the core (Z axis) of the lens; and a force ($H_R$) parallel to the radius of the lens. These two forces are responsible for two different actions on the electrons, spiraling and focusing, as they pass through the lens. An electron passing through the lens parallel to the Z axis will experience the force ($H_Z$) causing it to spiral through the lens. This spiraling causes the electron to experience ($H_R$) which causes the beam to be compressed toward the Z axis. The magnetic field is inhomogeneous in such a way that it is weak in the center of the gap and becomes stronger close to the bore. Electrons close to the center are less strongly deflected than those passing the lens far from the axis.



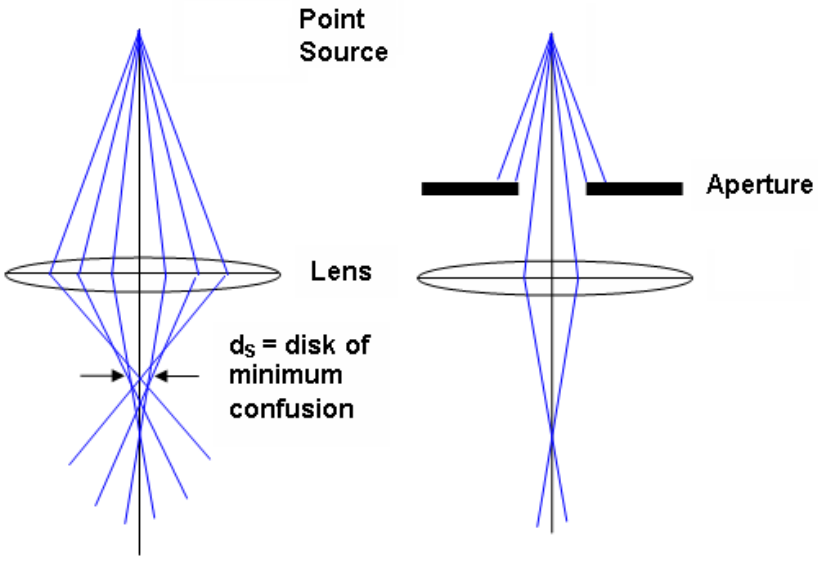
So far we've mentioned that electromagnetic lenses are unlike optical lenses in that they are: stationary; have variable focal points; and cause the image to be rotated. The latter is corrected for in modern TEMs. Electromagnetic lenses also differ in that: the deflection of the electron within the lens is a continuous process (no abrupt changes in the refractive index); only beam convergence (not divergence) is possible; and the convergence angle with respect to the optic axis is very small compared with optical light microscopy (less than one degree!).

Finally, it is important to keep in mind that electron lenses, compared to glass lenses, perform much more poorly. Some have compared the quality of electron optics to that of imaging and focusing with a coke bottle. This is mainly due to the fact that aberrations are relatively easily corrected in glass lenses.

## Lens Aberrations

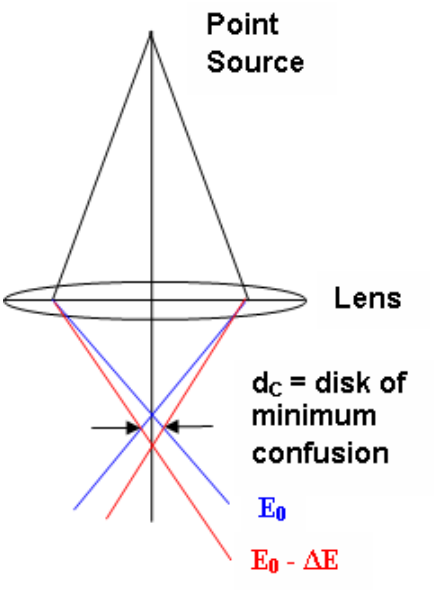
Up to this point, all of our representations depict a perfect lens. That is, all rays emanating from a point in the object plane come to the same focal point in the image plane. In reality, all lenses have defects. The defects of most importance to us here are spherical aberration; chromatic aberration and astigmatism. Rather than a clearly defined focal point, we end up with a **"disk of minimum confusion"** in each instance.

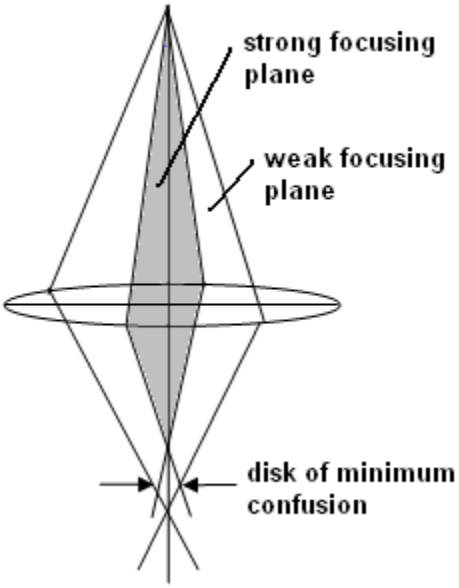
**Spherical aberration**: The further off the optical axis (the closer to the electromagnetic pole piece) the electron is, the stronger the magnetic force and thus the more strongly it is bent back toward the axis. The result is a series of focal points and the point source is imaged as a disk of finite size ($d_S$).



To reduce the effects of spherical aberration, apertures are introduced into the beam path. Apertures are circular holes in metal disks on the micron scale**.** The net effect of the aperture is to reduce the diameter of the disk of minimum confusion. However, that positive effect comes at the price of reduced beam current. Also, a very small aperture will display diffraction effects. The diameter of the aperture used will also affect the convergence angle of the beam and this in turn will affect its coherence as well as image properties such as depth of focus.

**Chromatic aberration**: The electron beam generated by the gun will have a certain energy spread. Electrons of different energies at the same location in the column will experience different forces. An electromagnetic lens will "bend" electrons of lower energy more strongly than those of higher energy. As with spherical aberration, a disk of minimum confusion ($d_C$) is produced.




Astigmatic beam



Astigmatism --corrected beam



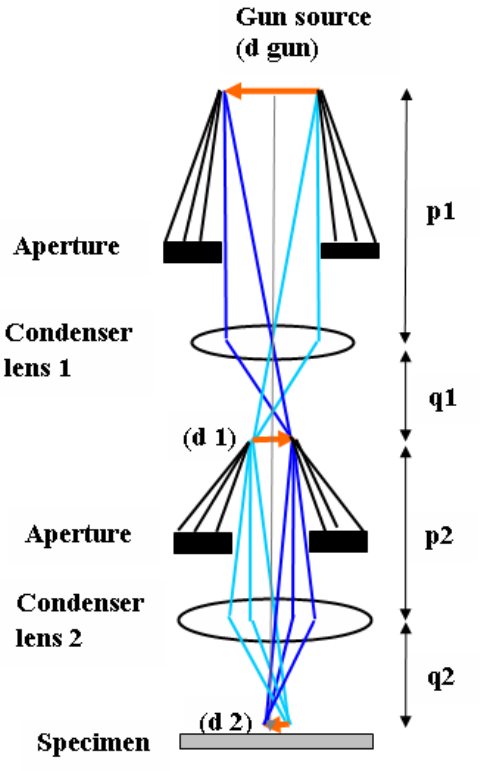
**Astigmatism**: Finally, the electromagnetic lenses used in the TEM can not be machined to perfect symmetry. If the fields produced by the lenses were perfectly symmetrical, a converged beam would appear circular (looking down the column). A lack of symmetry would result in an oblong beam: the narrower diameter due to the stronger focusing plane; the wider diameter due to the weaker focusing plane. The net effect is the same as that of the aberrations above—a disk of minimum confusion rather than a well defined point of focus.

## Illumination system lenses and apertures

Although an understanding of beam -- specimen interactions is necessary to inform user control of the illumination system, we can begin to put that picture together. The illumination system can be set up in two basic modes: Convergent beam and parallel beam. The latter will be our focus in this primer.

### Convergent beam

In convergent beam mode we focus a reduced image of the gun crossover on the specimen to make a small bright probe which would be useful in analytical STEM work or convergent beam diffraction.



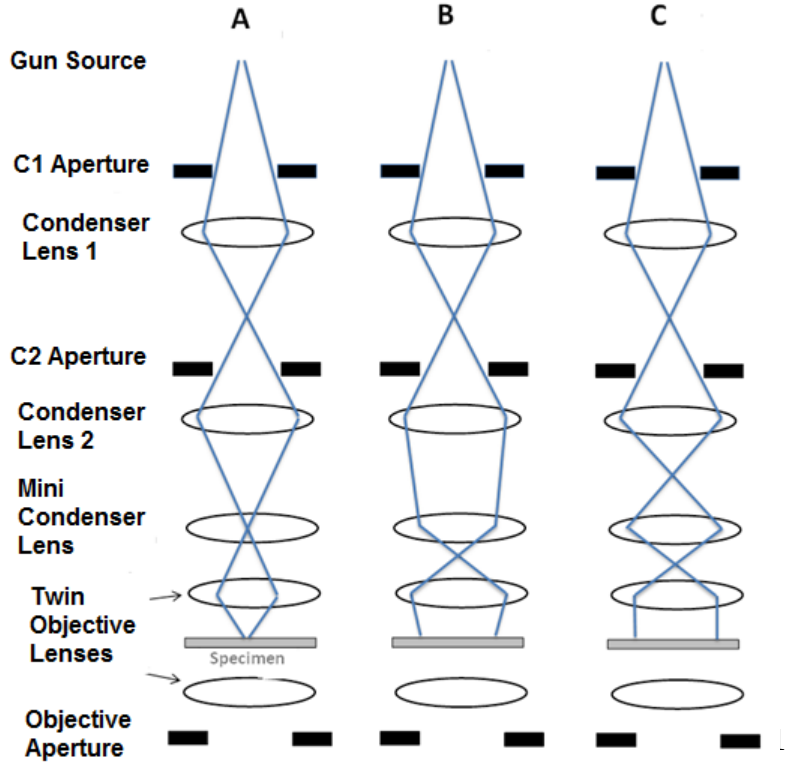

The diagram to the right shows the demagnification of the gun crossover to produce a small probe on the specimen. The actual demagnification is as follows:

- the size of the gun source at d1 = d gun (p1/q1);
- the size of the probe on the specimen is d2 = d1 (p2/q2).

If we wanted a small diameter beam probe we would excite condenser lens 1 (C1) – causing q1 to decrease. Then we would focus condenser lens 2 (C2) to form the smallest spot on the specimen. Smaller C2 apertures would eliminate aberrations – also enhancing a smaller spot size. The size of the C1 aperture is usually fixed. If you have used an SEM, you should be familiar with this arrangement. The cost of a smaller diameter beam; however, is the loss of beam current. (black lines in the diagram). So the operator needs to find a compromise of these factors. Although the principles just elaborated certainly apply, the lens configurations in the FEI TEMs that you will be using are more complex. Option A in the Figure below is a more realistic depiction of those microscopes in convergent beam mode.

### Parallel beam

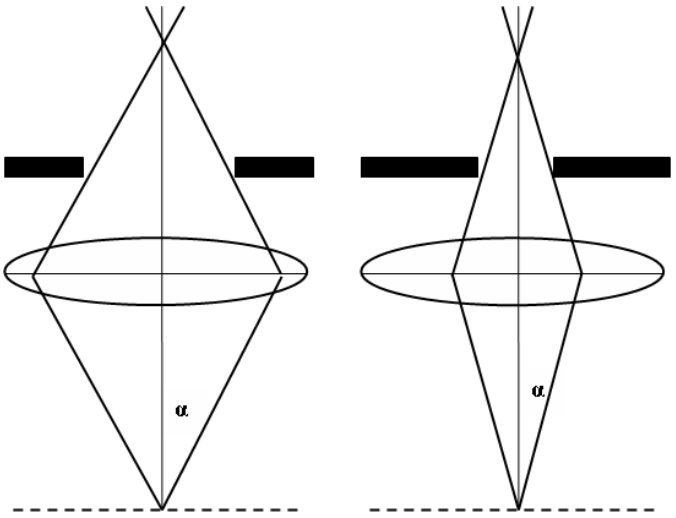
In parallel beam mode we provide a nearly parallel illumination at low intensity with high coherency that would be useful in bright field and dark field imaging, phase contrast imaging, and selected area diffraction. To achieve this we spread the beam on one side of focus (crossover) using the C2 lens. Option B in the Figure to the right shows C2 spread in the **underfocus** direction; Option C the **overfocus** direction. [*Figure adapted from 4*]




The beam is more parallel, and thus more coherent, as it approaches the sample when C2 is spread in the overfocus direction. On the FEI TEMs

this corresponds to turning the "Intensity" knob clockwise from crossover. If we spread the beam too far overfocus we will loose intensity on the screen. In practice we spread the beam to: just cover the fluorescent screen on the microscope; obtain a proper image histogram from a CCD camera; or obtain the sharpest diffraction pattern.

A smaller C2 aperture and/or a more strongly excited C1 lens (small "spot size") will also increase beam coherence. The down side is less beam current illuminating the specimen. Again, the operator needs to find a compromise between these factors. Use the smallest C2 aperture/spot size for which you have adequate illumination at the magnifications being used. One needs both adequate signal and good contrast producing mechanisms. It should be noted that the angles are greatly exaggerated here for pedagogical purposes.




Spherical aberration of the illumination lenses, while important in the convergent beam mode, plays little role in parallel illumination mode.

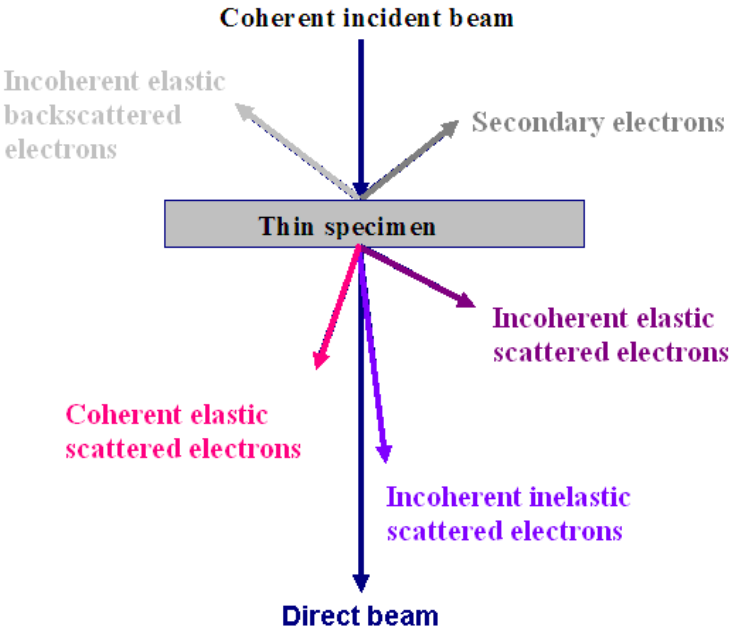
So, we've seen how beam current, probe size, and convergence angle interrelate. What accelerating voltage (kV) should you use? Your choice will, again, depend on knowledge of beam-specimen interactions; however, here are some points to consider concerning higher accelerating voltages:

- brightness increases;
- wavelength decreases and thus resolution capabilities increase;
- some contrast mechanisms will decrease (more on this later);
- beam penetration will increase;
- the heating effect of the beam is smaller (especially with low Z materials).

## Beam-Specimen Interactions

The scatter which results from the electron beam interacting with the specimen can be [*Figure adapted from 4*]:

- forward or backward. We will focus on forward scatter;
- coherent or incoherent. Beam electrons undergoing coherent interactions maintain phase relations and those undergoing incoherent interactions do not. We will utilize both coherent and incoherent scattering;
- elastic or inelastic. Beam electrons undergoing inelastic interactions with the specimen atoms show measurable energy loss and those undergoing elastic



interactions do not. We will focus on elastic scatter. Elastic scatter is the dominant component of the total scatter occurring in the TEM. It is also the principle source of contrast in TEM images and intensity distributions in diffraction patterns.
Inelastic interactions are the basis for a wide range of signals (X-rays, EELS, secondary electrons, cathadoluminescence) as well as beam damage (bond breaking, knockout damage). They are incoherent and have a low scattering angle (θ) – usually less than one degree. The low scattering angle means that they represent only low resolution/structural information. Inelastic scatter which passes through the objective aperture creates a background fog and does not contribute optimally to the in-focus image. This is due to the energy losses and thus increased chromatic aberration in the objective lens.

Elastic scattering can be understood from a particle view (scattering from isolated atoms) or a wave view (scattering from the specimen as a whole) [*4*].

**From the particle view**: beam electrons can interact with the electrons or the nucleus of a specimen atom through Coulomb forces.

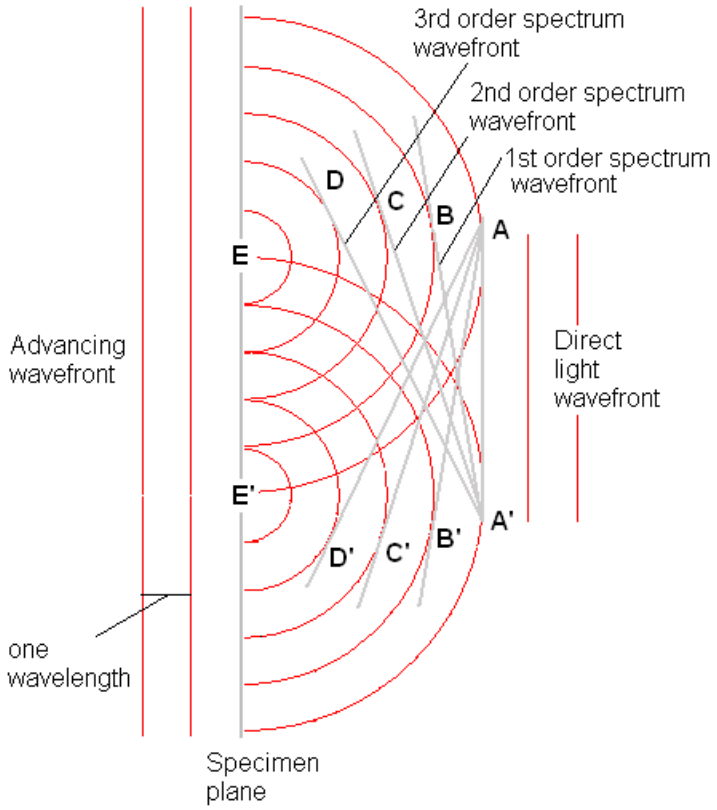
Electron-electron interactions result in relatively low θ and are primarily a function of accelerating voltage – more interactions at lower accelerating voltages.

Electron-nucleus interactions result in higher θ which is incoherent (termed **Rutherford scattering**). Above approximately 5 degree θ, all of the elastic scattering from a particle view can be considered Rutherford incoherent. The probability of this type of interaction increases with:

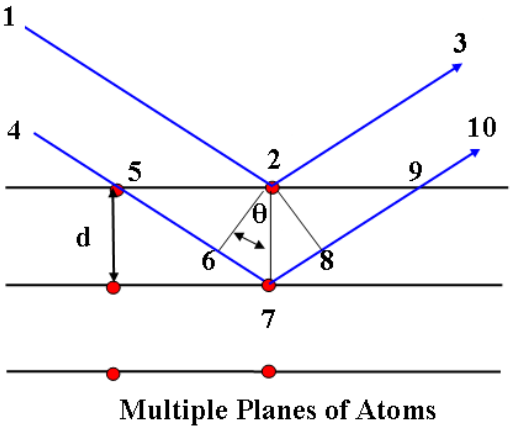
- higher atomic number elements;
- lower accelerating voltages;
- greater specimen thickness; and
- lower θ.

**From the wave view**: the electron wave can interact with many atoms together within a crystalline specimen resulting in collective coherent scattering (**diffraction**).

Each atom in the specimen (E and E') acts as a source of secondary spherical wavefronts. These new wavefronts are coherent and will therefore interfere -- reinforcing one another in certain angular directions and canceling in others. The points on the wavefront marked A, A' are in phase and will reinforce. This will give rise to the powerful central part of a diffraction pattern. AB' and A'B reinforce (due to interference between wavefronts separated by one wavelength) to give the two separate patterns on either side of the central band—called first order spectra. Second order spectra are due to interference AC' and A'C….. and so on.



Diffraction is controlled by the angle of incidence of the electron beam to the atomic planes in the specimen and the spacing of those planes. Diffraction occurs when Bragg's law is met.



Multiple Planes of Atoms

Bragg's Law: $n\lambda = 2d \sin\theta$ where:

- λ is the wavelength of the incident beam;
- d is the spacing between atomic planes; and
- θ is the angle of the incident beam to the atomic plane.

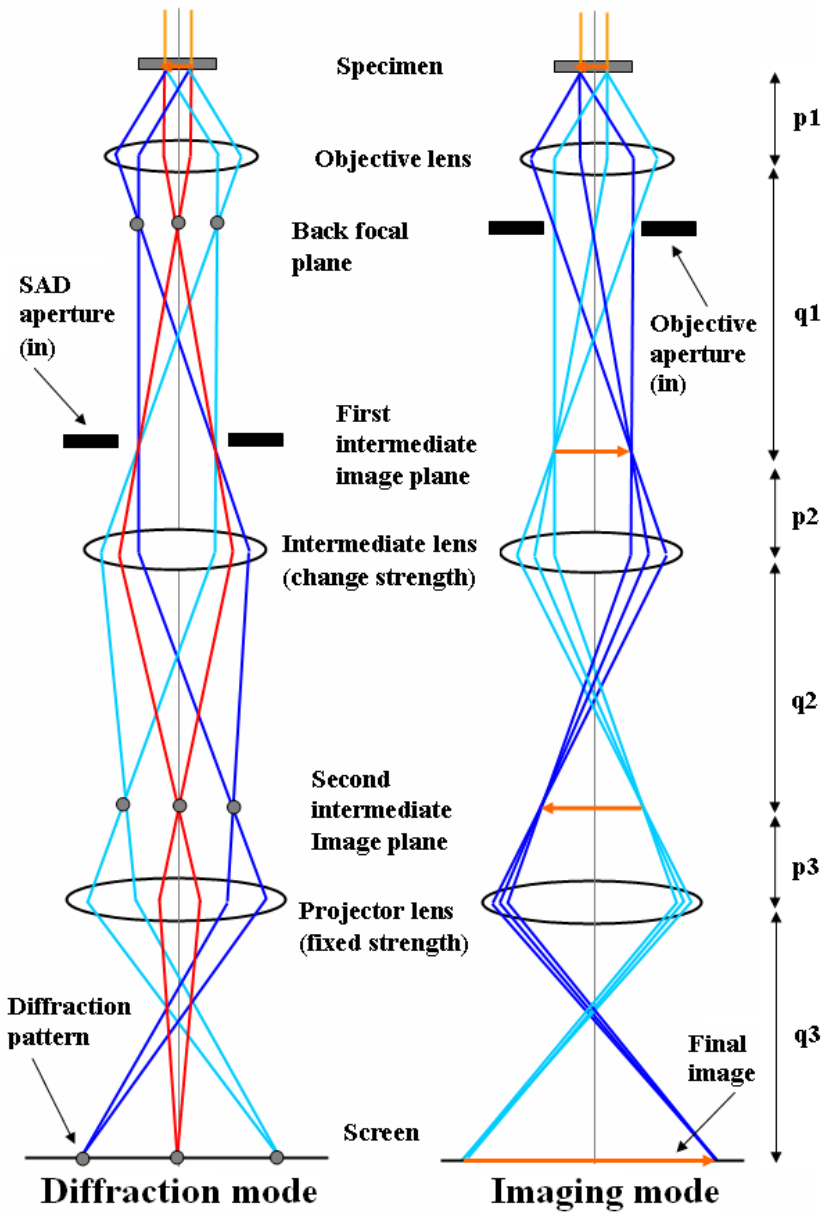
In the diagram to the right, the extra distance the bottom beam travels is 6—7—8. If this extra path length is some multiple of the wavelength (nλ), the two beams at 3 and 10 will constructively interfere to produce a diffraction pattern. The distance 6—7 = d sinθ; the distance 6—7—8 = 2d sinθ. Thus, diffraction will occur when: $n\lambda = 2d \sin\theta$. [*Fig courtesy of 6*]

## Imaging System

The forward elastic scattering taking place within the specimen results in an emerging non-uniform distribution of electrons which is the basis of the major contrast producing mechanisms.
[*Figure adapted from 4*]

In **imaging mode**, the scatter is focused by the objective lens to the first intermediate image plane which subsequently acts as the object plane for the magnifying lenses. Magnification can be calculated in the same manner as we did for the illumination system in convergent beam mode.

"Rays" emanating from a given point in the specimen plane come to a focused point in the image plane. The adjacent Figure shows rays coming from two different points of the specimen as dark and light blue.



An objective aperture is situated within the beam path just below the objective lens. The objective aperture is important for several reasons. The aperture will:

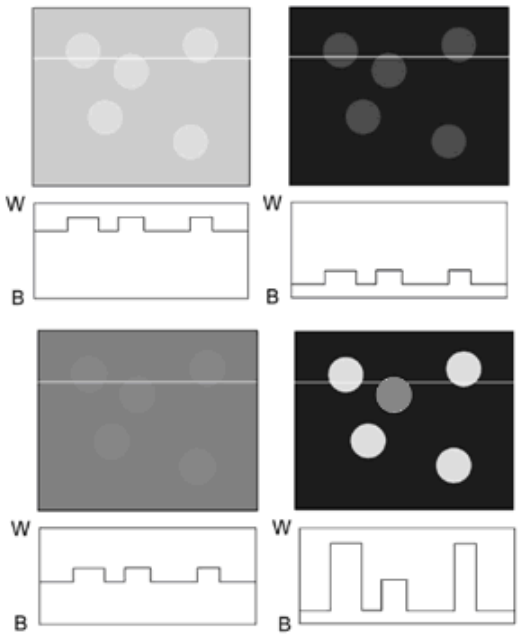
- allow for signal selection (this will depend upon what we see in diffraction mode—see below);
- provide for contrast within the image ( a lower accelerating voltage will also increase contrast);
- decrease objective lens aberrations, spherical and chromatic, which will degrade image resolution. The objective aperture is optimally chosen as a compromise to limit both spherical aberration and diffraction error defects. However, the energy range of electrons leaving the sample can be significant (15-25 eV). Thus, a smaller objective aperture may be necessary to alleviate chromatic aberration effects. Ensuring a thin specimen will also help;
- affect depth of field in the image – a smaller aperture giving better depth of field.

In **diffraction mode,** rays emanating from the specimen that are parallel to one another come to focus in the back focal plane of the objective lens. The previous Figure shows parallel rays with different colors: dark blue, light blue, and red. The diffraction pattern which occurs in the back focal plane of the objective lens arises simply as a consequence of image formation in the image plane of that lens. When we enter diffraction mode on the TEM, we are adjusting the strength of the intermediate lens so that this objective back focal plane becomes its object plane. We remove the objective aperture and insert another aperture further down the column—a selected area diffraction (SAD) aperture, to select a portion of the sample from which the diffraction pattern arises. That SAD aperture acts as a virtual aperture.

## Contrast

A high resolution signal is worthless if adequate contrast is absent. Contrast can be defined as $(S_2 – S_1) / S_2$ where $S_2$ is the signal from the feature of interest; $S_1$ is the background signal; and $S_2 > S_1$. For most users the minimum useful image contrast level is about 5 % (contrast lower than this can be enhanced via image processing).

The figure to the right shows a simplified screen image under different brightness and contrast conditions. Below each screen image is an intensity profile across that image. The X axis corresponds directly to distance; the Y axis represents the dynamic range of the signal. The base level of the signal is the **brightness;** the spread of the signal up from this baseline is the **contrast**. The contrast should span as much as possible of the dynamic range because this produces the most useful and pleasing images.

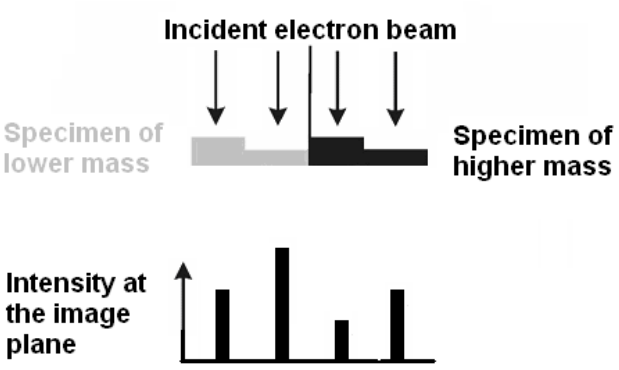


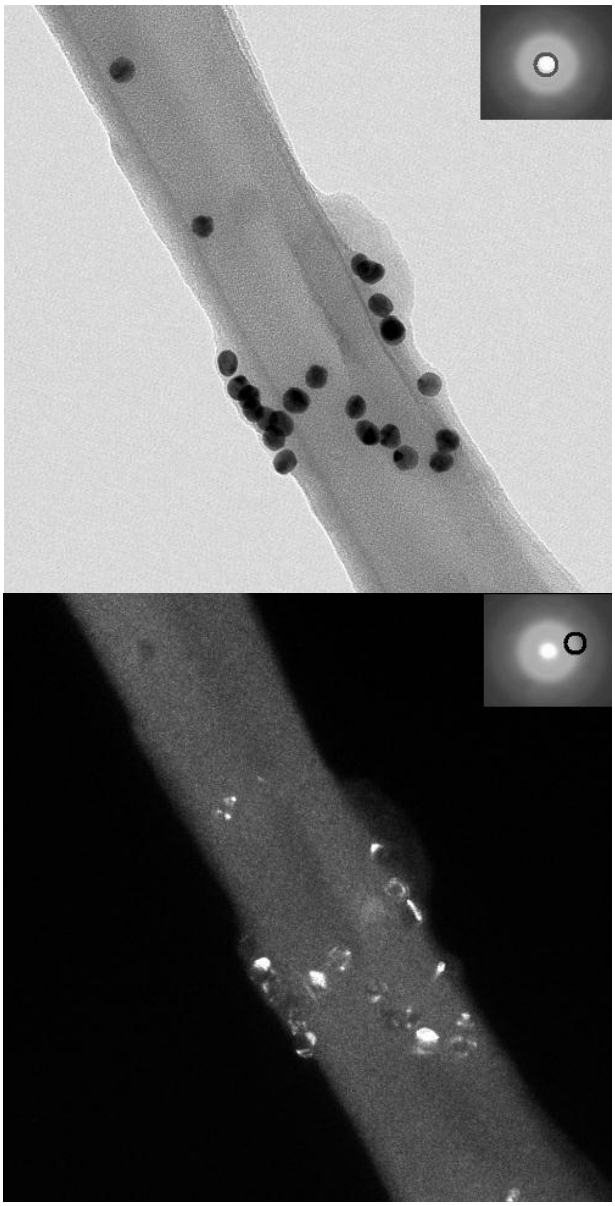

In what follows we will take a closer look at the amplitude contrast mechanisms (mass/thickness and diffraction) and how they contribute to image formation. Differences in intensity due to **amplitude (scattering) contrast** result from the scattered electrons being intercepted by the objective lens/aperture and thus not contributing to image formation. Next we will discuss phase contrast. Differences in intensity due to **phase (interference) contrast** arise from interference effects between scattered and unscattered electrons which pass through the objective lens/aperture. A slight amount of underfocus will enhance the phase contrast in our images.

## Bright and dark field images showing mass/thickness contrast

Consider an amorphous biological or polymer sample. We can neglect collective coherent elastic scattering due to the lack of crystallinity. The principle scattering mechanism in our sample will be Rutherford incoherent elastic scattering. That scattering is enhanced by atomic number, density and specimen thickness and the contrast produced is thus termed **"mass – thickness contrast"**. Given their composition, biological and polymer samples are often stained with heavy metals to enhance this type of contrast.

Those areas of the sample that are thicker or of higher atomic number will proportionally scatter more. If we introduce an objective aperture directly below the specimen then that scatter will not be allowed to travel down the column and those areas of scatter will appear darker in the image. A smaller objective aperture will intercept more of this scatter and thus will enhance mass—thickness contrast.



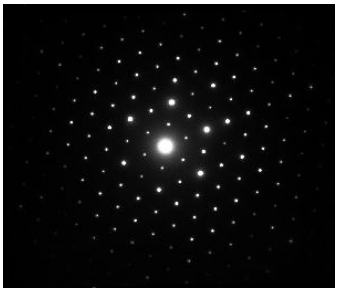



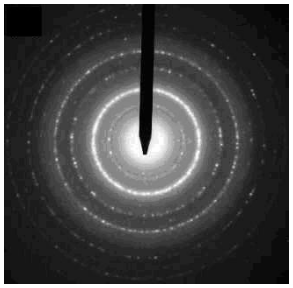
The images to the left are of a filamentous carbon membrane with 15 nm gold particles attached. The top image is referred to as **"bright field"**; the bottom **"dark field"**. When we go to diffraction mode with the beam on the specimen we get a diffuse diffraction pattern with an intense central spot (upper right image insets). If we position our objective aperture (dark circle) around this high intensity, low θ scatter—then a bright field image is produced when we subsequently go back to imaging mode. Only the scatter within this circle will contribute to image formation. Thus the gold particles appear darker as compared to the carbon membrane in the bright-field image due to the fact that the particles have higher density (atomic number) and the beam travels through 15 additional nanometers of this material. Both of these properties result in proportionally more higher-angle scatter which does not make its way through the objective aperture. A third property of the gold, its polycrystalline nature, also contributes to the higher angle scatter (next section).

If we somehow (next section) allow only the high θ scatter to contribute to the image-- then a dark field image is produced. Just remember that those areas in an image that are darker correspond to scatter which was intercepted by the objective aperture and not allowed down the column.

## Bright and dark field images showing diffraction contrast

Consider a specimen which has a crystalline nature. The incident electron beam interacting with such a specimen will result in coherent elastic as well as incoherent elastic scattering. The former type of scatter will occur at special (Bragg) angles controlled by the crystal structure and the orientation of the specimen with respect to the incident beam. At these angles the specimen, whether mono (left) or polycrystalline (right), will produce a diffraction pattern.

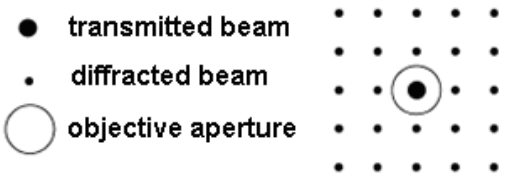


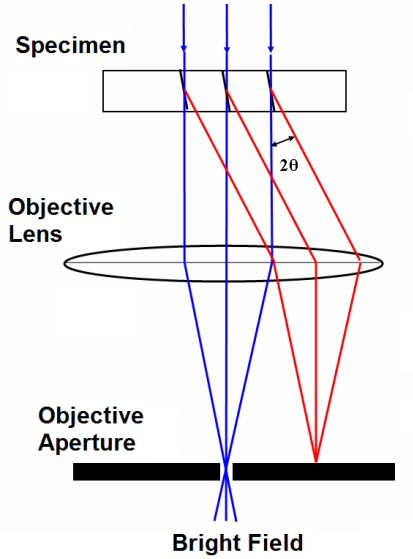


To obtain a bright field image of a crystalline specimen we will:

- ensure the beam is on the specimen;
- enter diffraction mode;
- position the objective aperture around the transmitted beam (shown blue in the ray diagram) in the diffraction pattern;
- enter imaging mode.

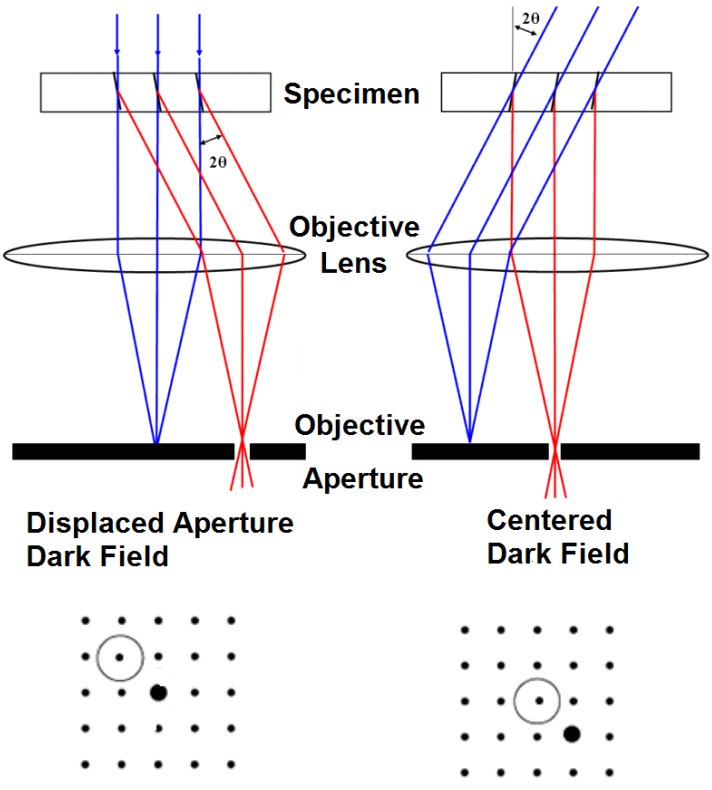
[*Figures adapted from 4*].




A dark field image results from allowing one of the higher order diffraction spots (shown red in the ray diagrams) to constitute the signal for the image. There are two ways of doing this. We can:

- move the objective aperture off-axis so that it selects one of the higher order diffracted beams as the signal. This situation, which we term **"displaced aperture dark field imaging",** enhances off-axis lens aberrations and thus is not desirable;
- keep the objective aperture stationary on axis and tilt the beam to achieve the same result. This is termed **"centered dark field imaging"** and is the choice of microscopists.

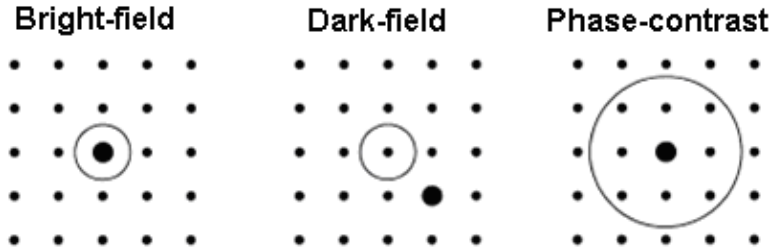


The coherent elastic scattering from this type of sample gives rise to diffraction contrast. Diffraction contrast will co-occur with mass—thickness contrast, but we can select/accentuate the contrast arising from particular specimen planes by selecting the corresponding diffraction spot as the source of our image.

## Phase Contrast and the Contrast Transfer Function

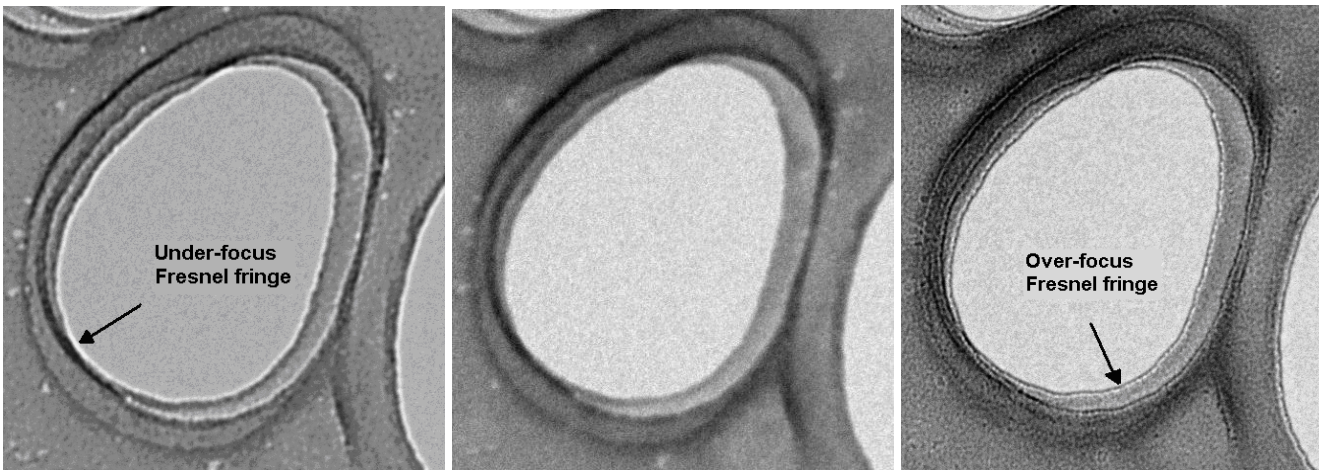
The phases of elastically scattered electrons are shifted as they pass through the specimen. The amount of shift is proportional to the electron density of the specimen atoms. This is analogous to how the refractive index affects a light wave. **Phase contrast** in images arises from differences in phase between scattered and unscattered rays in different parts of the image and interference between these rays.

Using a monocrystalline sample as an example, a bright or dark field image requires that we select a single beam using the objective aperture. A phase-contrast image requires the selection of more than one beam. Generally the more beams collected, the higher the resolution of the image. [*Figure from 7*]




In general, both amplitude and phase contrast will coexist when imaging most samples. However, amplitude contrast is dominant for structures with large mass thickness, while phase contrast increases in importance for small and thin structures and becomes the dominant source of contrast for very small objects of low atomic number.

Phase contrast results in the formation of strong **Fresnel fringes** about any part of the specimen where there is a rapid change in mass thickness. The fringes serve to enhance these specimen areas by delineating them with a bright line when the objective lens is in the **under-focused** position and a dark line in the **over-focused** position. The Fresnel fringes virtually disappear at the point of "exact" or **"minimum contrast" focus**. The relative amount of amplitude contrast can be determined by focusing the specimen to "minimum contrast focus" where phase contrast is at a minimum. The image below is of a hole in a thin amorphous carbon film at under-focus, minimum contrast true-focus, and over-focus respectively.



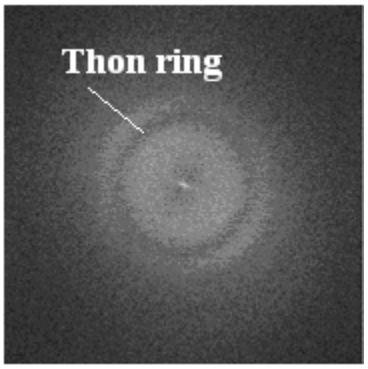
In order to image a phase object, we need to shift the phases of the scattered electrons additionally so that they will be 180 degrees out of phase and can thus interfere destructively with unscattered beam electrons**.** This additional phase shift of the scattered electrons is caused by the objective lens spherical aberration coefficient (fixed microscope value) and the amount of defocus of the objective lens (variable value under control of the operator)

This **phase shift as a function of spatial frequency** is: $X(k) = \frac{1}{2} \pi C_s \lambda^3 k^4 + \pi \Delta f \lambda k^2$ where:

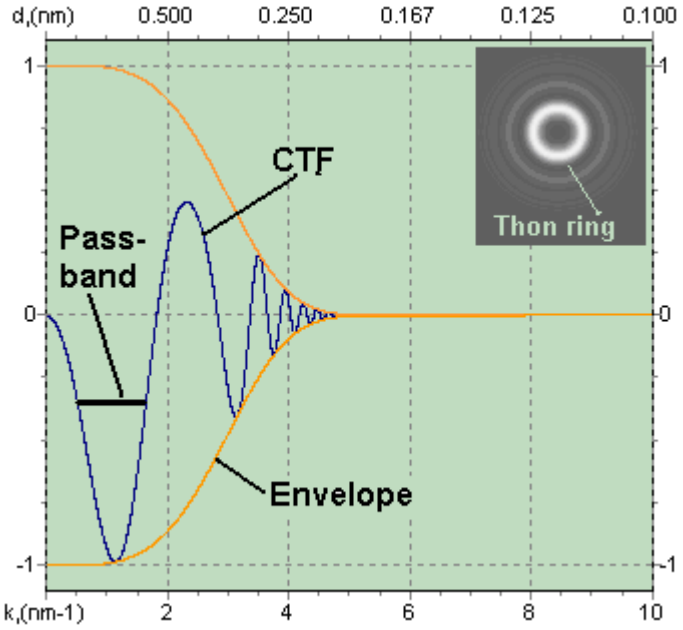
k = spatial frequency;
$C_s$ = spherical aberration coefficient of the objective lens;
$\lambda$ = electron wavelength defined by accelerating voltage;
$\Delta f$ = defocus value of the objective lens.

**–sin X(k) is termed the Contrast Transfer Function (CTF)**. The CTF is displayed simply and directly in the **Fourier Transform (FT)** of the image in the back focal (diffraction) plane of the objective lens. The FT corresponds to a frequency analysis of the image. The image is considered to be the sum of a set of sinusoidal waves of varying frequencies: the higher the frequency of the wave, the finer the detail it refers to. In FT reciprocal space, high spatial frequency (resolution) corresponds to long distances from the optic axis.

The image to the right is the FT of an amorphous thin carbon film taken at under-focus on our Tecnai T12. **The FT is the CTF with envelopes** (more on envelopes later). Actually, since only intensity is displayed, the CTF is $-\sin^2 X(k)$.




The figure below shows a simulated CTF from the program CTF Explorer [*8*]. This simulated CTF is of the Tecnai T12 under the conditions which generated the FT to the right. Also shown below (upper right inset) is a simulated FT corresponding to this CTF.

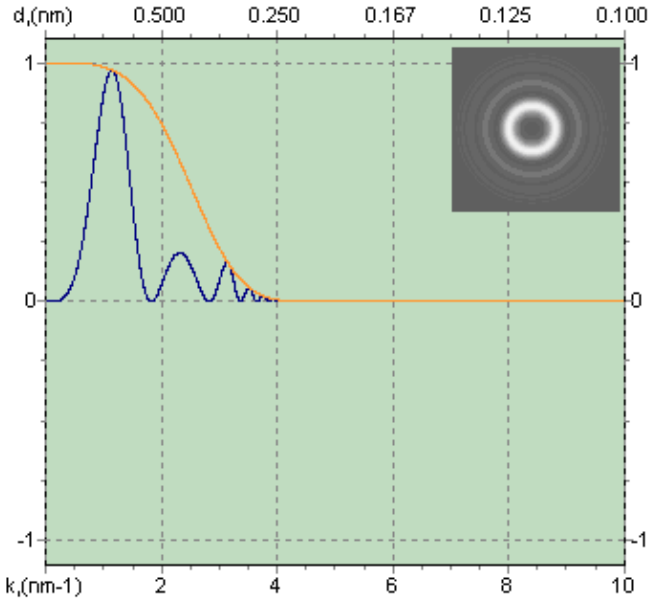



The Y-axis displays contrast and the X-axis spatial frequency. The CTF is oscillatory. When it is equal to zero, there is no contrast (information transfer) for that spatial frequency. Those spatial frequencies without contrast are characterized as **Thon rings** on the FFT. Likewise, there are **passbands** where there is good "transmittance" of spatial frequencies. The oscillation (and thus contrast) eventually dies off due to envelope functions associated with temporal and spatial coherency.

Negative contrast values correspond to the type of contrast we are used to seeing with scattering or amplitude contrast, ie a subtraction from the background electron intensity over regions of high mass density; positive values reverse this intuitive relation.

If we map intensity, the CTF looks as follows. The relation between the FT and this characterization of the CTF is more direct and thus understandable:

- Bright concentric areas of the FT map to CTF spatial frequency ranges displaying high intensity contrast;
- FT Thon rings correspond to CTF spatial frequencies of zero transmittance;
- Higher spatial frequencies occur farther to the right on the CTF X-axis and farther from the FT center.



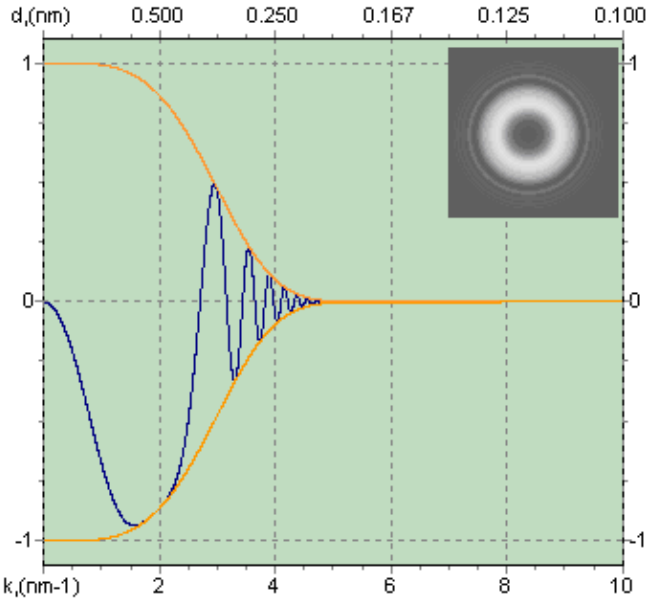

The best CTF is the one with the fewest zeros and with the broadest passband of good transmittance (where CTF is close to -1).

Scherzer suggested an optimum defocus condition occurring at:

$\Delta f = -(C_s \lambda)^{1/2}$

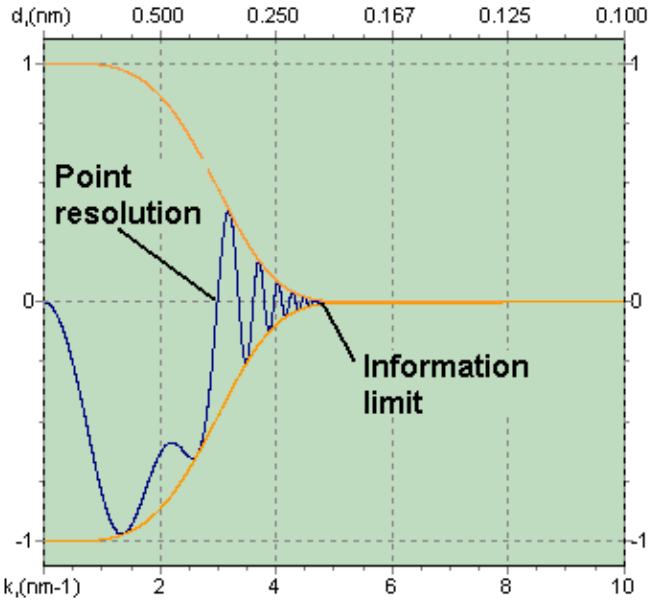
The resolution at this defocus, now called **"1 Scherzer"**, is:

$r = 0.66\, C_s^{1/4}\, \lambda^{3/4}$




**"Extended Scherzer" (1.2 Scherzer)** provides even broader band of transmittance with CTF still close enough to -1. The crossing of the k-axis at "extended" Scherzer defocus corresponds to **point-to-point resolution of the microscope.** The point where envelope functions damp the CTF to zero corresponds to the **information limit of the microscope.**

The envelope function acts as a virtual aperture. The envelope shown in our figures combines a temporal coherency envelope and a spatial coherency envelope. The former is caused by chromatic aberrations, focal and energy spread, instabilities in the high tension and objective lens current; the latter by the finite incident beam



convergence

**Phase contrast images are directly interpretable only up to the point resolution**. If the information limit is beyond the point resolution limit, one needs to use image simulation software to interpret any detail beyond point resolution limit. On LaB6 filament instruments such as the Tecnai Spirit there is relatively little information limit beyond the point resolution.

## Operator control

**Specimen thickness:** Thinner specimens are better! Increased specimen thickness results in the elastically scattered electrons being inelastically scattered as well. As a result, image resolution is reduced due to chromatic aberration of the objective lens and there is a loss of contrast. The scatter angle of inelastically scattered electrons will also increase with specimen thickness draining out higher resolution detail

**Beam coherence:** Contrast mechanisms are enhanced when we use a parallel illuminating beam which is itself coherent. We've seen that we can increase the spatial coherency of that illuminating beam by: lowering the magnification when possible; using a smaller spot size; using a smaller C2 aperture; and by spreading the beam in the under-focus direction. The down side of increasing beam coherency by these means is the reduction in beam current on the specimen and thus signal/illumination. This can be compensated to some extent with longer camera exposure times. So, you are balancing beam current and the degree of coherency of the beam. FEG sources provide a very strong improvement in spatial coherency extending the information limit to very high frequencies

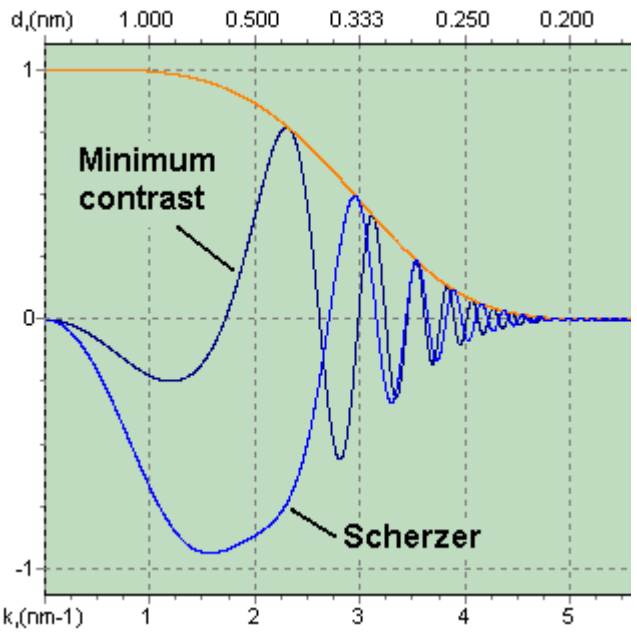
**Accelerating voltage:** A higher accelerating voltage has many advantages including: greater brightness, increased resolution; and better beam penetration. The down side is that proportionally the amount of scatter decreases with increasing accelerating voltage. Samples with inherently little mass – thickness contrast (many biological and polymer samples) may require lower accelerating voltages to increase the angle and amount of scatter and thus the necessary contrast. So, you are balancing resolution and contrast when deciding upon the accelerating voltage to use. Use the highest accelerating voltage for which you have adequate contrast.

**Objective aperture:** The size of the aperture you use will depend on a number of factors. If your sample is adequately thin and has high inherent mass-thickness contrast, then an intermediate size aperture that balances aberration and diffraction effects would be appropriate. If your sample is thicker (prone to producing scatter of a high energy spread – thus displaying chromatic aberration) or has less mass-thickness contrast, then you may wish to use a smaller aperture. Larger aperture sizes (or no aperture) may be required when doing high resolution phase contrast imaging. Higher spatial frequency information is farther from the central diffraction spot axis and thus may be excluded from producing phase (interference) contrast in the image. In other words, the objective aperture acts as an envelope function in the CTF. If you are interested in high resolution imaging, you must also understand the nature of the spatial and temporal envelope functions on your microscope as they may attenuate the contribution of higher spatial frequency information. The relationship between objective aperture size and spatial frequency (maximum obtainable resolution [r]) can be approximated by:

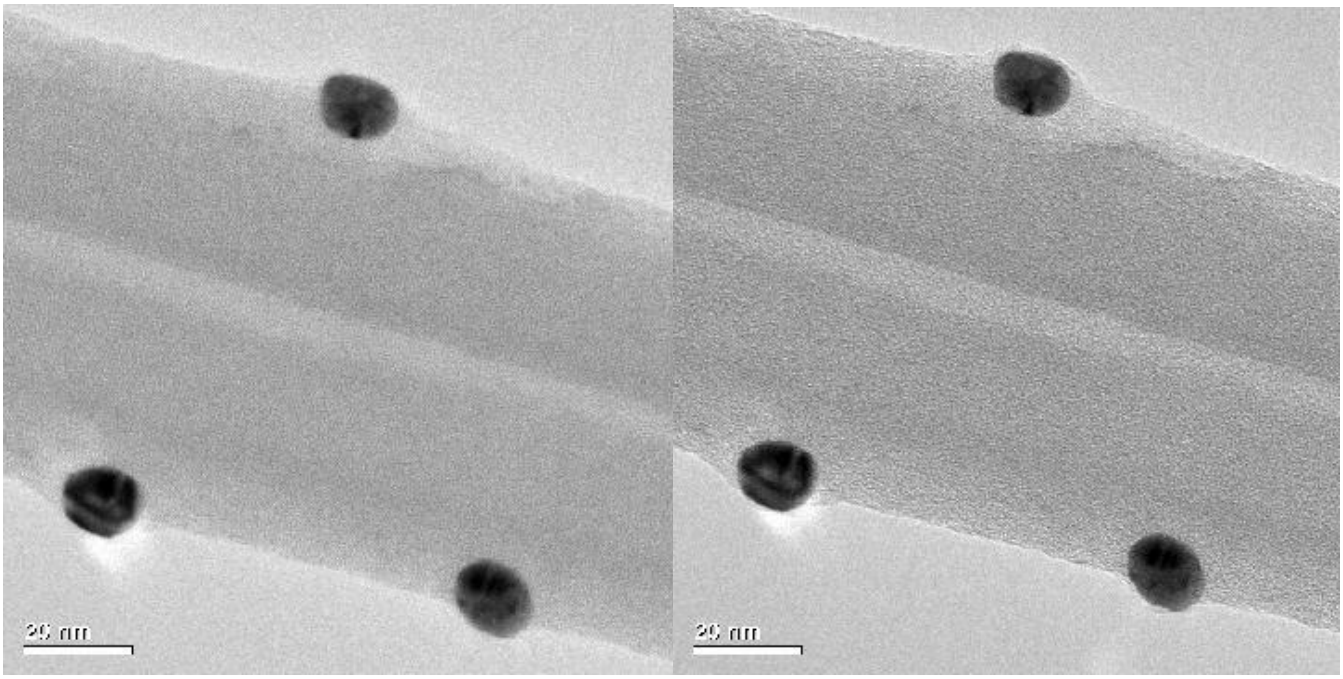
$r = 2\lambda$ (focal length / aperture diameter). The Table below shows corresponding values for the Tecnai Spirit

| Aperture Diameter (microns) | 20 | 40 | 70 | 150 |
|---|---|---|---|---|
| r (nanometers) | 2.04 | 1.02 | 0.58 | 0.27 |

**Focusing and spatial frequency**: As mentioned before, most images are a combination of phase and amplitude contrast. The degree of amplitude contrast can be assessed by focusing to minimum contrast with the assistance of a Fast Fourier Transform (FFT). Fresnel fringes may also be used for this purpose but their accuracy is not as good.




Minimum contrast is a reference point from which to determine your optimal under-focus. As shown in the image to the right, a slight amount of under-focus (here Scherzer) cancels the effect of objective lens spherical aberration and will increase the point resolution and enhance phase contrast over a broad range of spatial frequencies. You need to keep in mind that amplitude contrast is not being represented in this CTF image. The apparent lack of transmittance at lower spatial frequencies will be compensated by amplitude contrast at those frequencies. So, for many samples, amplitude contrast is enhanced with phase contrast at slight under-focus. The images below of gold nanoparticles on amorphous carbon where taken close to minimum contrast (left) and slight under-focus (right).

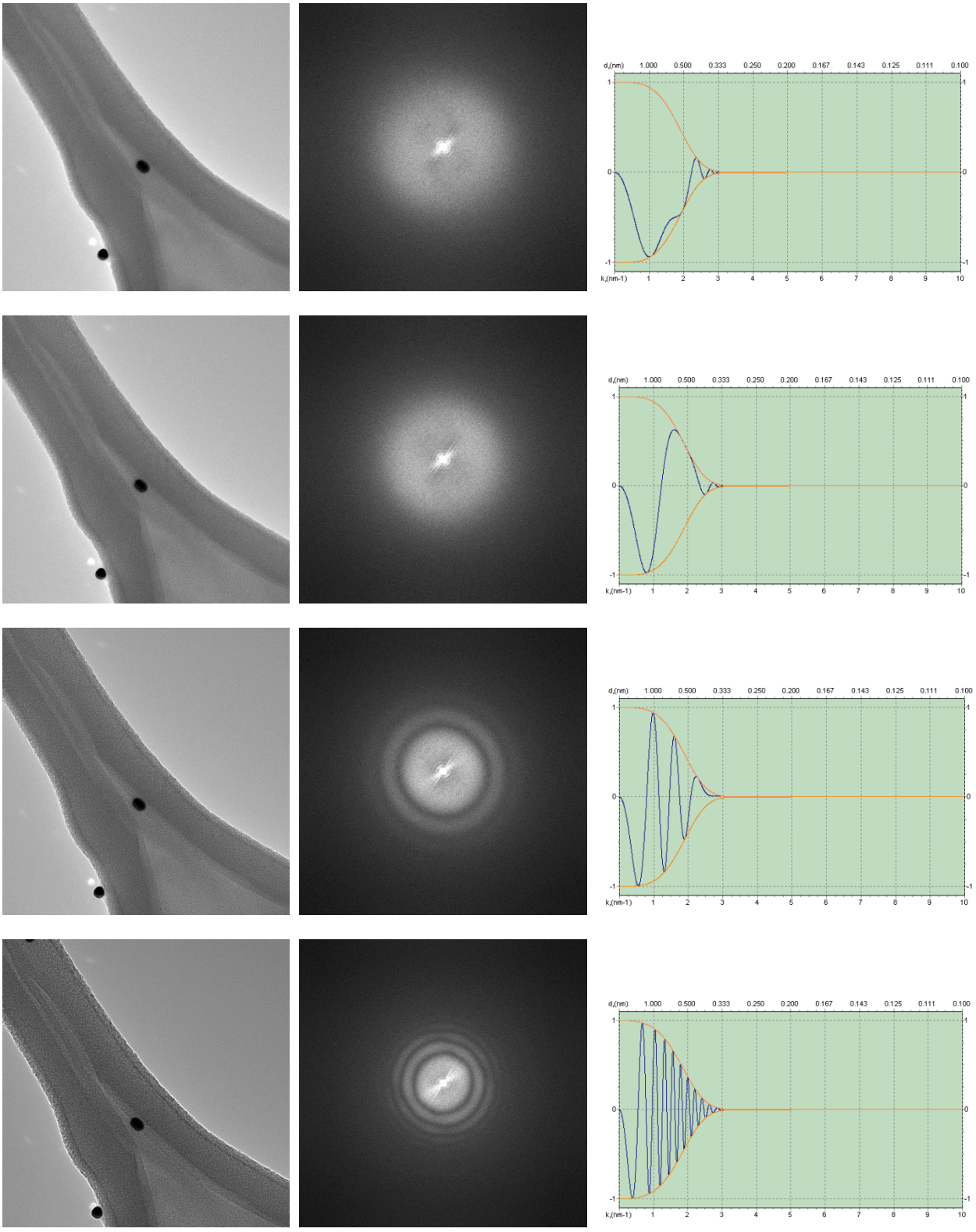



The choice of under-focus depends on the resolution expected from a specimen and becomes more critical as the resolution improves. The optimum defocusing can be specified as that which puts the first zero of the transfer function at a value somewhat greater than the highest spatial frequency or resolution of interest. This will produce maximum overall contrast with enhancement of higher resolution details.

Maximum phase contrast for a specimen spacing of r will occur at defocus: $\Delta f = r^2 / 2\lambda$. The table below shows point resolution and the spatial frequency of maximum phase contrast for various underfocus values on the Moos Tower Tecnai Spirit TEM.

| Underfocus (nanometers) | -174 | -250 | -500 | -1000 | -2000 | -3000 |
|---|---|---|---|---|---|---|
| Spatial Frequency of Maximum Phase Contrast (nanometers) | 1.08 | 1.3 | 1.8 | 2.6 | 3.7 | 4.5 |
| Point Resolution (nanometers) | 0.45 | 0.81 | 1.26 | 1.81 | 2.58 | 3.15 |

The Figures below show TEM images and corresponding CTFs and FFTs at -174, -250, -500 and -1000 underfocus on the Tecnai Spirit.



# Appendix A: Alignment of the Microscope

As an operator you should expect the following from the microscope. The:

- brightness and resolving capabilities of the beam should be maximized;
- field of view should stay centered on the screen when changing magnification;
- illumination should stay centered and even on the screen when you vary it;
- image should not move on the screen when you try to focus it; and
- illumination should not be lost when switching from one mode of operation to another.

These expectations are met only when the microscope is well aligned. Microscope alignment is multilayered: some aspects are so fundamental and detailed that they are done only by service engineers; others advanced users will need access to. We will focus here on basic alignment capabilities that the beginning user should master.

**Think in terms of an optic axis.** That axis can be defined by (travels through) the field center of the objective lens and the center of the viewing screen. We want the gun, all other lenses and apertures centered with respect to that optic axis. We will be using deflection coils to ensure proper alignment since the lenses are obviously stationary. We also want the beam to be symmetric as it passes down the column. Thus there are additional coils associated with the various lenses that correct for lens astigmatism.

If you give it a little thought, hopefully it is apparent why you would want to start alignment at the top of the column and work your way down. That's what we will do -- align the illumination system first and then the imaging system. The former does not require the specimen to be in the beam path.

## Gun alignment

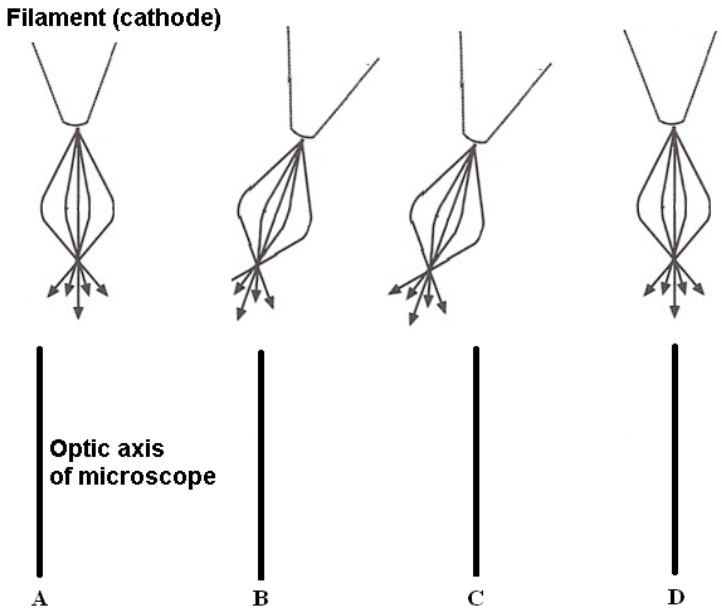
Earlier we talked about maximizing the brightness of the gun by adjusting the bias at saturation. To take advantage of that setup the electron beam should be adjusted so that it travels directly down the optic axis of the column.

A: the gun is shifted horizontally off-axis.

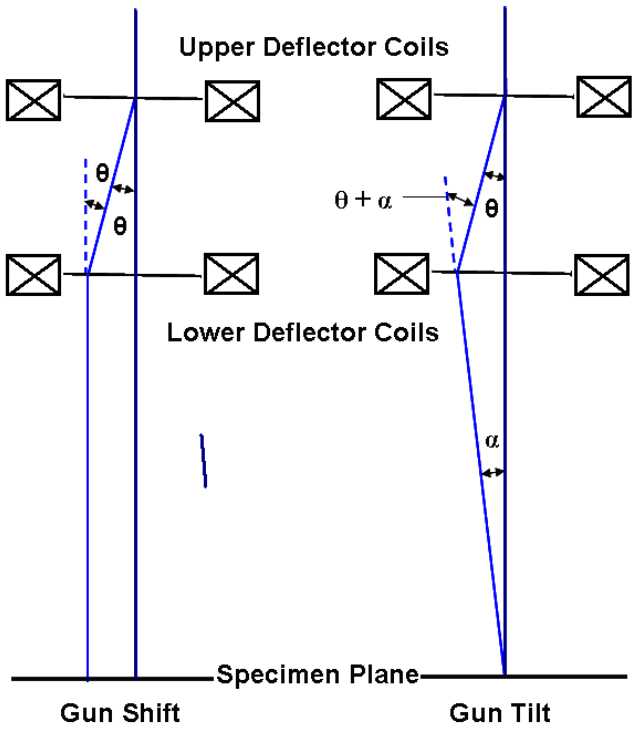
B: the gun is tilted off-axis.

C: the gun is misaligned with respect to both shift and tilt.

D: proper gun alignment.



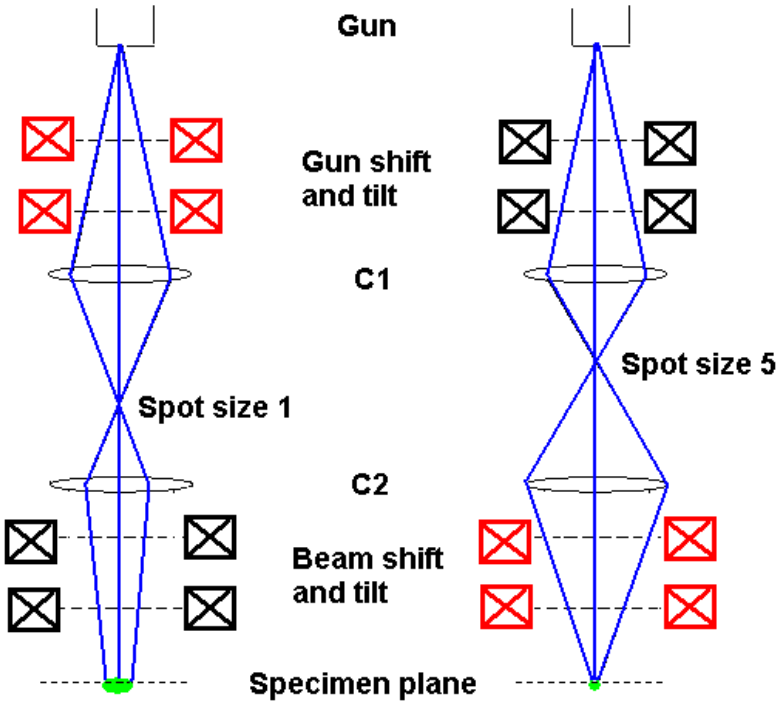
Now we don't move the gun physically. Instead we use a pair of electromagnetic coils (gun shift and tilt) just below the gun to achieve the same result. If we want to horizontally shift the beam, we apply current to the coils in such a manner that the tilt angle created by the first coil is "compensated" by the tilt angle produced by the second coil. Tilting the beam requires an additional angular tilt applied to the second coil.




Gun alignment involves bringing the beam to its smallest spot (crossover), de-saturating the beam to reveal some filament structure, ensuring symmetry of illumination with gun tilt, and centering the beam on the screen with gun shift. Remember that there is no specimen in the beam path so that what we are imaging here is the gun source.

## Spot size alignment

We want the beam to stay centered when we change current to the C1 lens in order to change spot size (smaller number—bigger spot.). Spot size alignment uses an additional set of electromagnetic coils (beam shift and tilt) located beneath the C2 lens [*Figure adapted from 5*]. These coils operate in the same manner as the gun coils above. The gun shift and tilt coils align the gun with C1. The beam shift and tilt coils align the beam exiting C2 with specimen plane and the rest of the electron microscope.



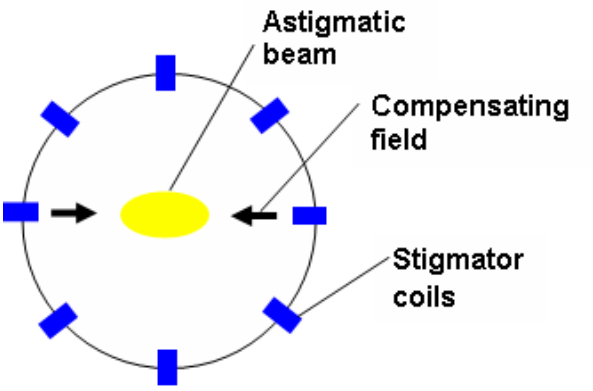

When the C1 lens strength is weak and thus the spot size is large (spot 1), it magnifies any error in the gun position. Because we know that the phosphor screen is reasonable well lined up with the C2 lens, then getting the illumination back to the center of the screen is accomplished with the gun shifts.

When the spot size is small (spot 5), the cross-over below C1 lens is close to the center of that lens. The C1 spot acts as the source for C2 lens and we align it with the screen center through the use of beam shifts. We go back and forth between spot sizes, manipulating gun and beam shifts, until the beam crossover remains centered on the viewing screen.

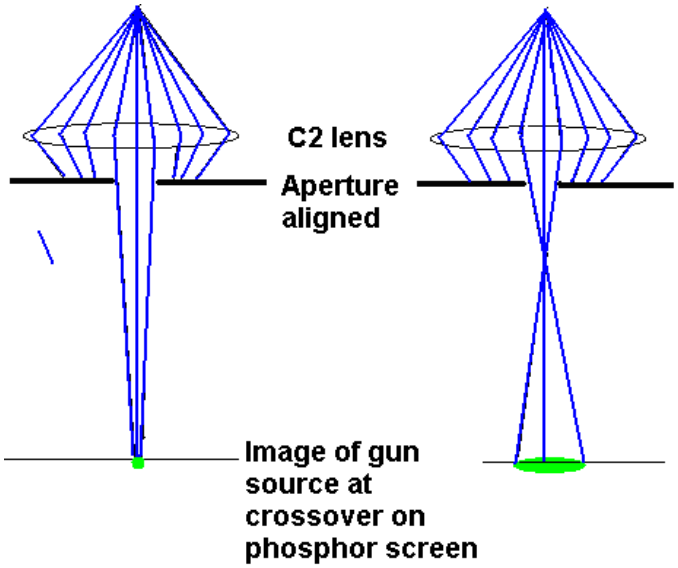
## C2 astigmatism correction

If the C2 lens is astigmatic, the beam will elongate as you move from crossover in either the underfocus or overfocus direction. Again, there is no specimen in the beam path so we are viewing the gun source on the viewing screen. The adjacent image shows an exaggerated (hopefully!) astigmatic beam condition on the viewing screen.


If astigmatism exists then we apply current differentially to a series of stigmator coils around the C2 lens to compensate--thus creating a symmetrical beam



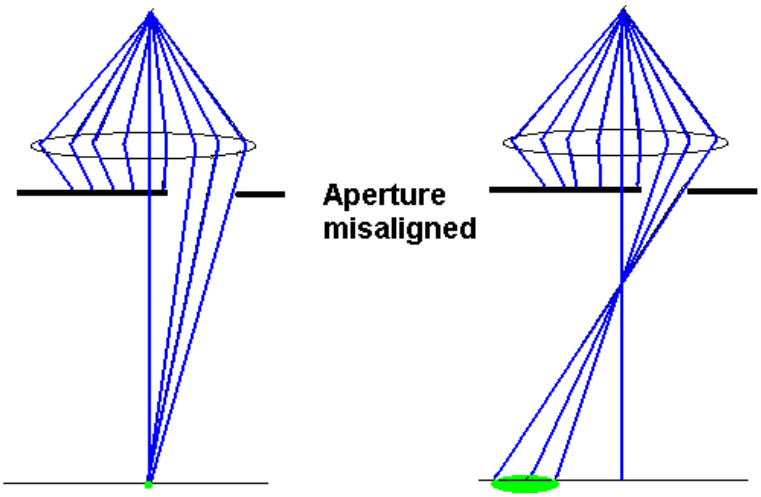

## C2 aperture centering




When we insert the C2 aperture we want it to be positioned so that the beam travels through its center. Changing the focus of C2 will move the focal point of the lens vertically up and down. The beam will remain centered and expand uniformly when the C2 aperture is centered [*Figures adapted from 5*].

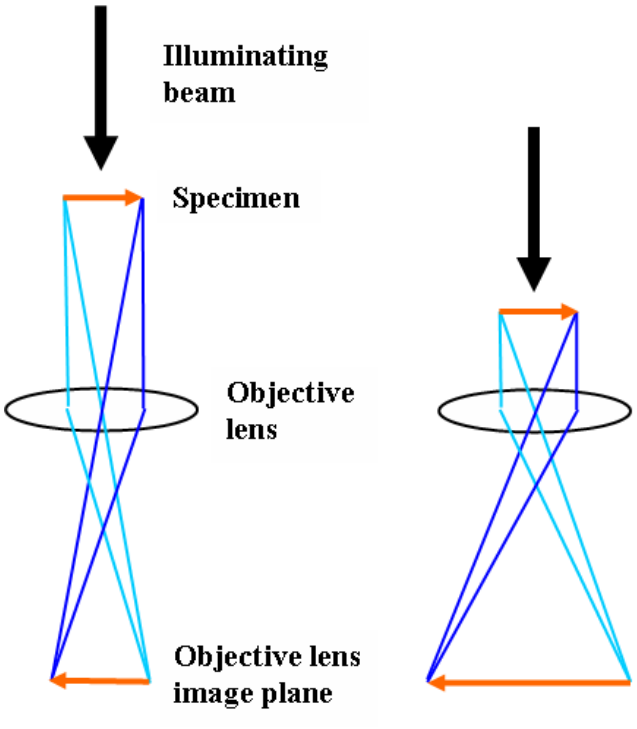
If the C2 aperture is slightly off then the beam will touch one end of the viewing screen before another as it is spread. In the case of extreme misalignment, the whole expanding beam will move off the screen.

The beam is brought to crossover, centered with the beam shifts, and spread. If the beam does not spread uniformly on the screen, the C2 aperture has X and Y adjustment knobs which can be used to center it.



## Eucentric (Z) height adjustment

The adjacent figure shows that the height of the specimen within the column will affect the magnification level of the objective lens.




It is common practice to adjust the specimen height (Z-shift) so that the middle of the specimen lies on the rotation axis of the specimen loading arm. This has the convenience that the point you are observing remains stationary when the specimen is tilted. In this condition the specimen is said to be at the **eucentric height**.

Many aspects of the performance of the microscope depend upon the eucentric height of the specimen:

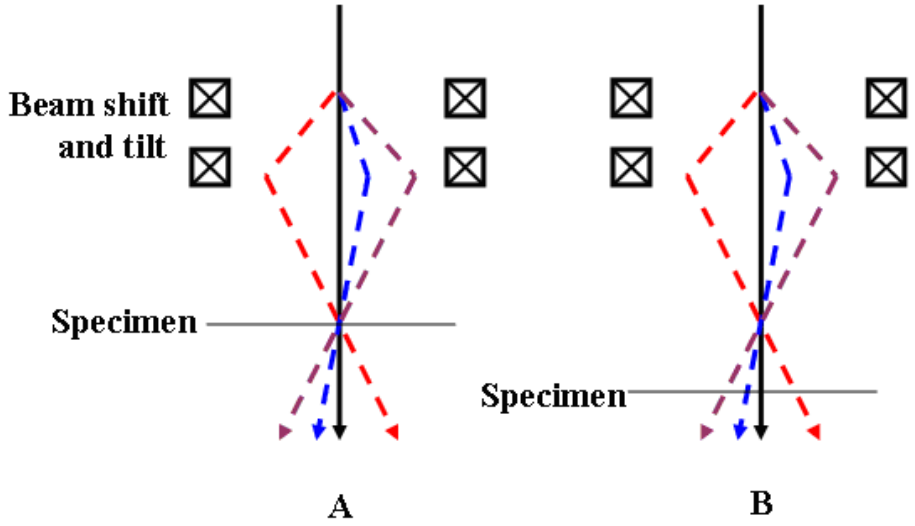
- magnification and spot size are indicated for this height;
- measuring is calibrated for this height;
- objective and selected area apertures are located optimally;
- maximum tilt can be obtained.

There are a variety of ways of ensuring that the specimen is at the eucentric height and your training sessions will provide the specifics. But you should remember to set the eucentric height for each of your specimens/grids.

## Pivot point of beam tilts and shifts

Each pair of coils in the microscope (gun, beam, imaging) needs to be aligned by setting pivot points for shift and tilt. The gun and imaging pivot points are set by advanced users; however, you will need to become adept at setting the beam pivot point—specifically tilt. In "Rotation Centering" (below) we will be aligning the whole illumination system with respect to the field center of the objective lens. We will use the beam coils to do this and thus ensuring the correct pivot point of the beam tilts is critically important.

**A:** Depicts beam tilt with different colored rays. Here the pivot point is set at the specimen level. Rays representing different tilts converge at the same point on the specimen. Rays emanating from that point on the specimen will focus to one point in the image plane of the objective lens.

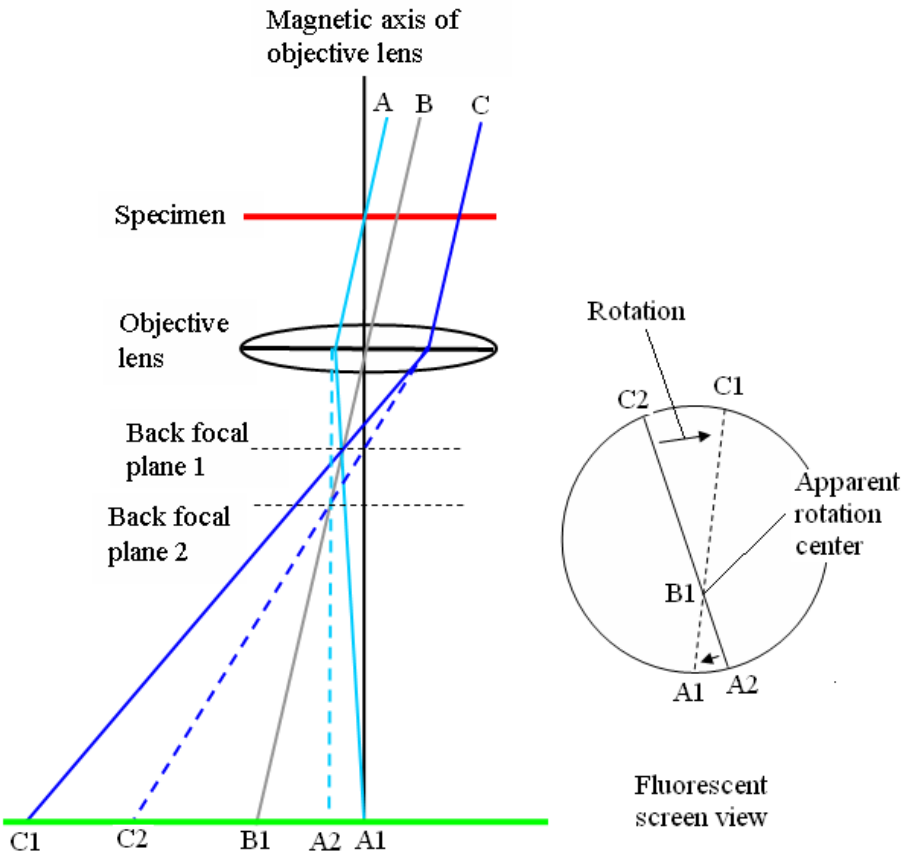


**B:** What happens if we change the height of the specimen? The beam tilt is off due to the fact that the different beam tilt settings put the beam at different positions on the specimen. This will correspond directly to different positions in the image plane—an undesirable scenario.

We want to ensure that our beam tilt pivot point is on the specimen. There is a button on each of the microscopes for 'pivot points' which makes the beam jump (wobble) between two tilt settings. If the pivot points are wrong, you see two beams (converged to crossover) separated laterally on the screen. It is then a simple matter to adjust a couple of correction knobs until the two beams are coincident. The correction knobs adjust the ratio of excitation of the two sets of deflection coils.

## Rotation centering

To this point we have the entire Illumination system aligned – we can think about it as one collective entity. We also have the specimen at eucentric height, and the beam tilt pivit points set for that Z-height position.

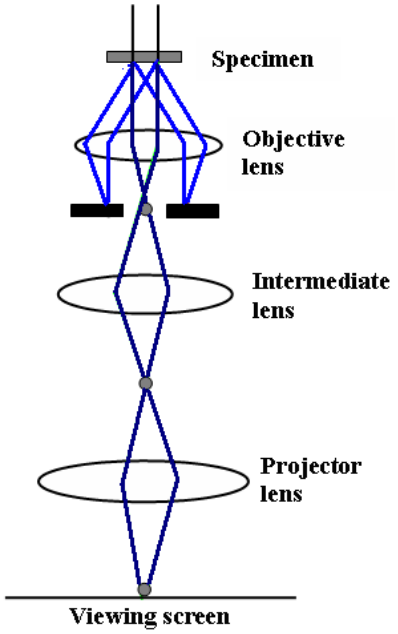


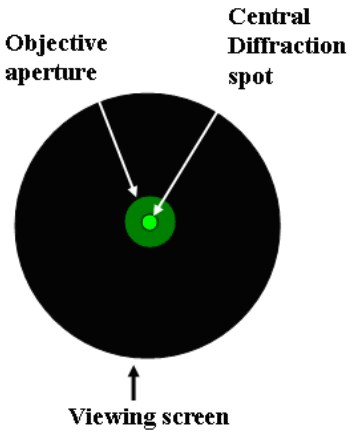

Now we need to align the transmitted signal from the specimen with the field center of the objective lens. If the field is off center then the electrons will move off axis and the image will rotate about a position off axis as you change focus with the objective lens [*Figure adapted from 4*]. A common means of rotation centering involves varying (wobbling) the voltage to the gun. The beam tilts are then adjusted to ensure that electrons remain on axis through the lens as their energy varies.

## Objective aperture centering

In order to center the objective aperture we:

- ensure that the beam is on the specimen;
- put the microscope in diffraction mode, and focus and center the central diffraction spot;
- insert the objective aperture;
- center the aperture around the central diffraction spot with the aperture X and Y knobs; and
- return to imaging mode.

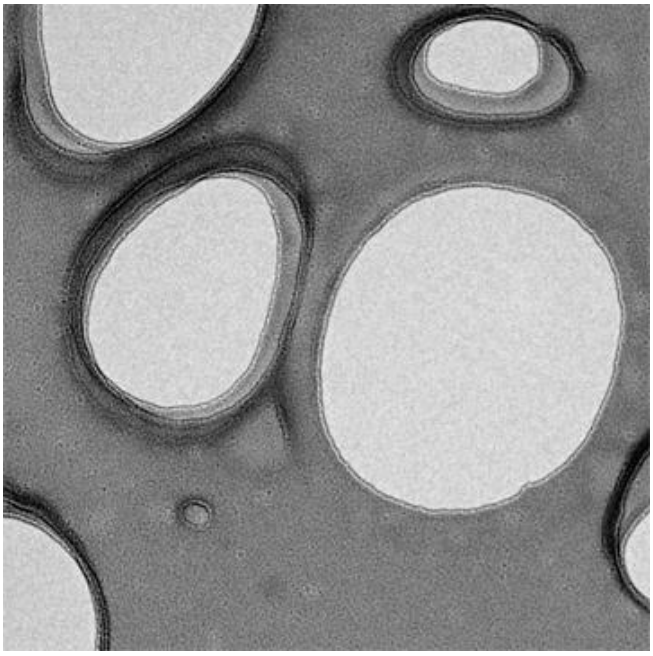


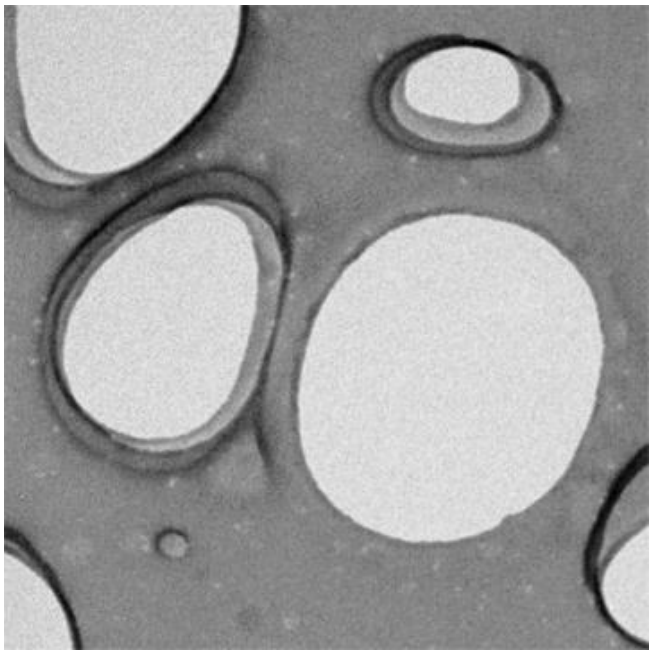

## Objective lens astigmatism correction

Objective lens astigmatism correction is best done utilizing a CCD camera at high magnification (>>100 kX) on all three microscopes. As with the C2 lens, there are a series of stigmator coils around the objective which we can provide current to differentially—thus compensating/correcting for the astigmatism present. There are a number of methods for correction:

We can use a pure carbon film specimen which contains holes of widely varying sizes. The holes have clean edges and show clearly defined Fresnel fringes when the objective lens is slightly under-focused (a white fringe) or over-focused (a black fringe).

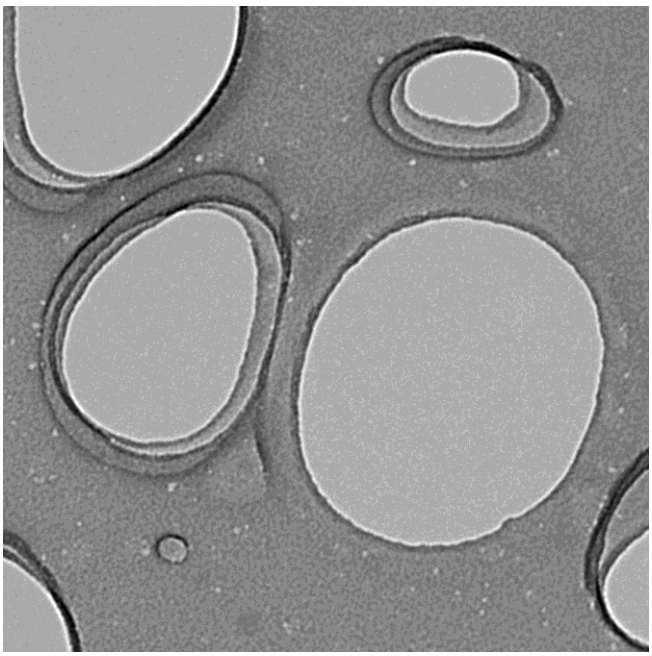


**Over-focus: Dark Fresnel fringe uniform. No astigmatism**

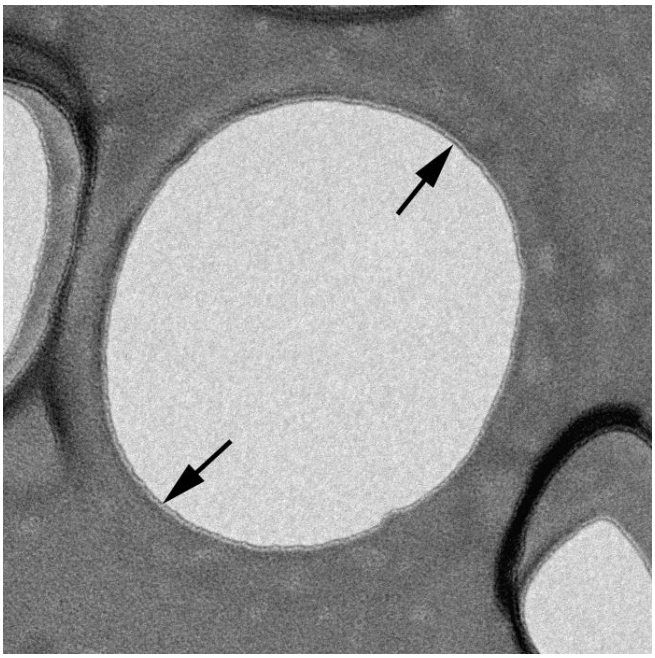


**True focus: Neither dark nor light fringes. Lack of contrast**

By observing the symmetry of the fringes while the objective lens is adjusted from under-focus to over-focus, the degree of astigmatism present can be determined. The astigmatism can then be corrected by adjusting the direction and strength of the objective lens stigmator. With a correctly adjusted lens, the black over-focused fringe will appear in its entirety around the inside edge of a hole as the objective lens is adjusted from under-focus to over-focus. The fringe will be of a constant width. With an uncorrected objective lens, the black fringe appears at two opposite parts of the circumference of a hole first, then as the objective lens is further over-focused, the remaining parts of the hole show a black fringe. However the fringe is not of constant width.

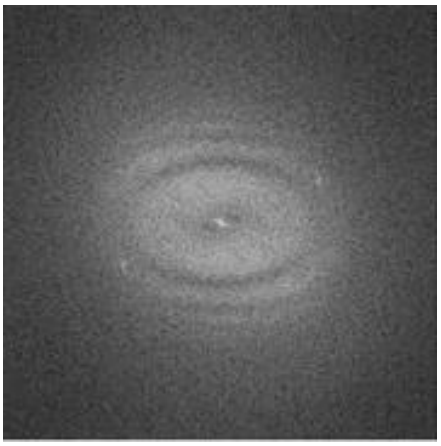


**Under-focus: Light Fresnel fringe uniform. No astigmatism**

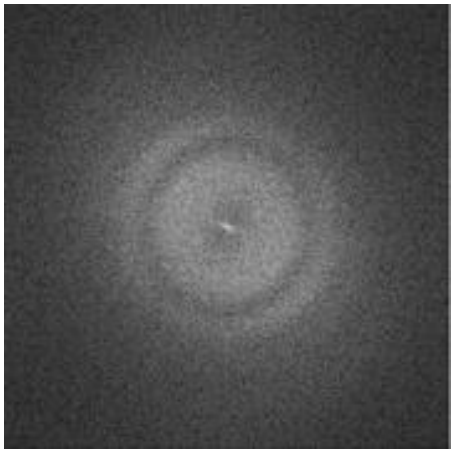


**Astigmatism: Dark Fresnel fringe not uniform.**

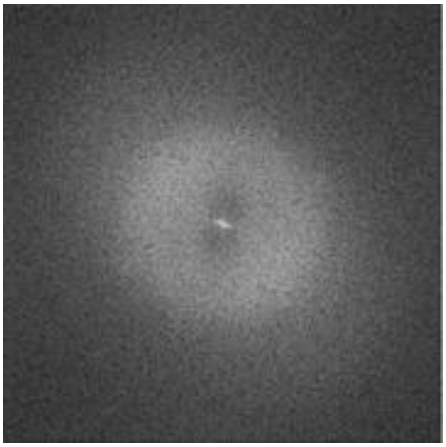
Alternatively, you can compensate for astigmatism by selecting an area of the holey carbon film that shows uniform density (no holes) and ensuring that a live Fast Fourier Transform is spherical at a high magnification



**Under-focus; Astigmatism**



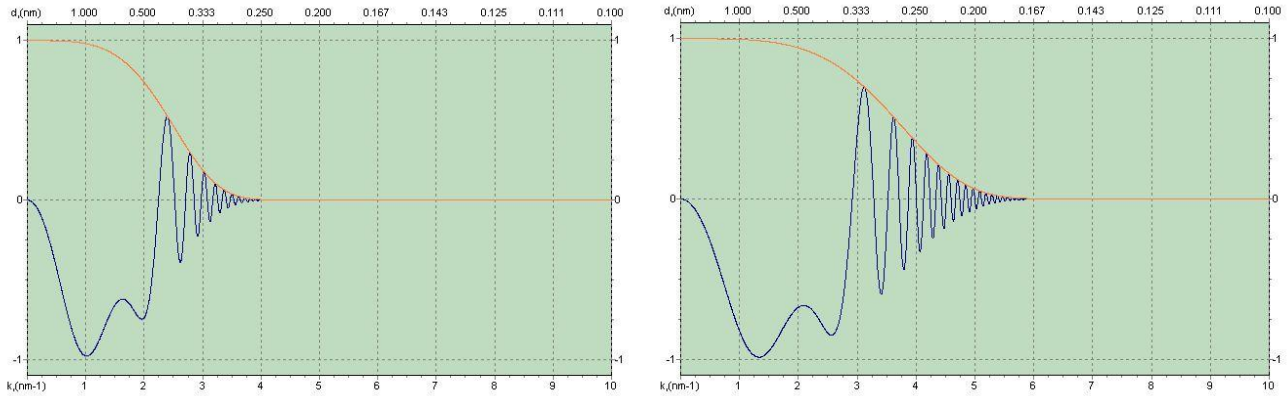
**Under-focus: No astigmatism**



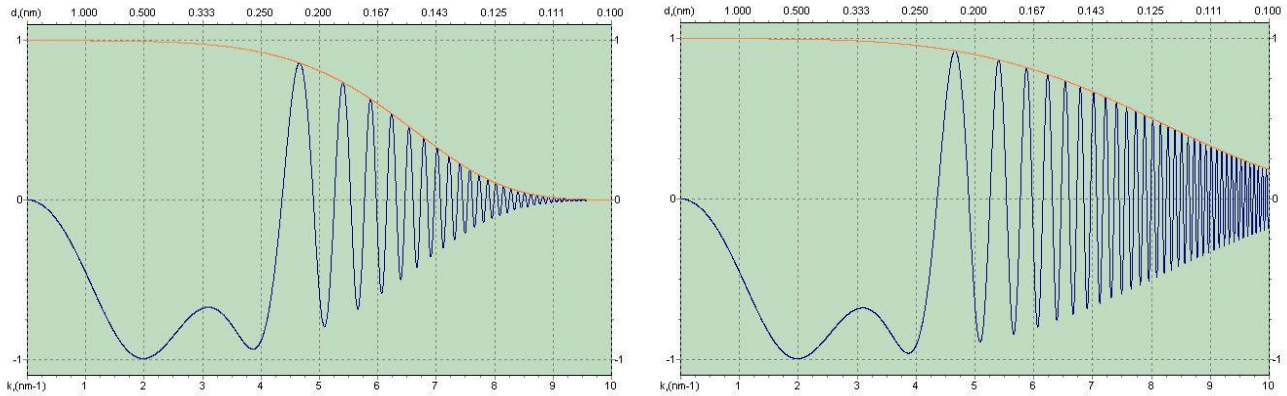
**"Focus": No astigmatism**

You can also focus on specimen fine structure at very high magnification. Astigmatism will show itself as image stretching on both sides of focus—much like it does in the SEM.

# Appendix B: Using the CTF to compare the Moos Tower Tecnai Spirit and the Nils Hasselmo Hall Tecnai F30 Field Emission Gun TEMs.




A B




C D

The above CTFs were simulated at 148K magnification and extended Scherzer underfocus. The CTFs graphically show the differences in point resolution and information limit.
**Figure A**: the Moos Tower Tecnai Spirit.
**Figure B**: the Moos Tower Tecnai Spirit if it had the focusing lens pole piece of the NHH FEGTEM.
**Figure C**: the Moos Tower Tecnai Spirit if it had the focusing lens pole piece of the NHH FEGTEM as well as 300 kV accelerating voltage.
**Figure D**: the NHH FEGTEM: higher resolution pole piece of the focusing lens; 300 kV; and better spatial and temporal coherency associated with the field emission tip.

## References

[1] J. J. Bozolla and L. D. Russell, "Electron Microscopy: Principles and Techniques for Biologists"
[2] JEOL LTD., "Invitation to the SEM World".
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[7] Leonid A. Bendersky and Frank W. Gayle, "Electron Diffraction using Transmission Electron Microscopy", J. Res. Natl. Stand. Technol., 106, 997-1012, 2001.
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