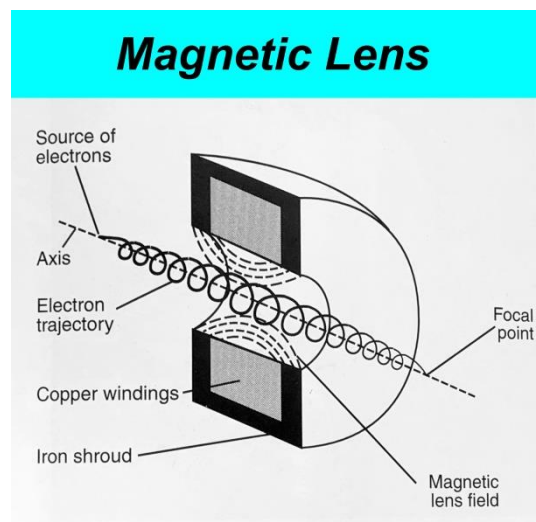
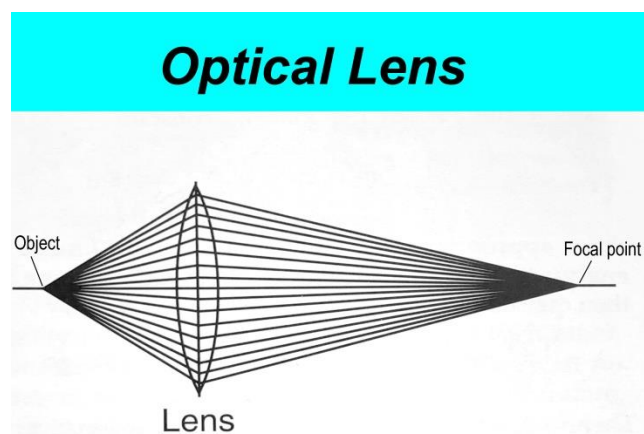


Finding that Focus

Introduction:

Light and Electron Microscopy share many parallel analogies in how images are formed. However, they differ significantly in the principles of image formation. In Light Microscopy, an image is formed after photons of illuminating light, pass through the sample, are focused by an optical lens. The final image is the result of absorption, reflection and refraction when light interacts with the sample. In Electron Microscopy, an electron beam illuminates and passes through the sample. The electrons are then focused by a lens made of coils and magnets. The final image is formed mainly as the result of electron scattering, and to some extent electron diffraction.



In Light Microscopy, and ordinary photography, a perfectly focus image has the highest contrast when compared to an out of focus one. That is how most auto-focus device work in such systems, by detecting the area having the highest contrast, which constitutes the sharpest image. An out of focus image becomes blurry and loses contrast. On the contrary, a perfectly focus image in Electron Microscopy bears the least amount of contrast, while out of focus images actually have higher contrast, leading people to think that the off focus picture they have just taken is in focus. Only a grossly out of focus image looks blurry.

In high magnifications, the image appears grainy when out of focus but the grains seems to suddenly “disappear” when approaching focus. The grainy appearance is also known as “defocus granularity”.

The change from more contrast to less contrast and back to more contrast is subtle and not easy to tell. Moreover, there are often many images lying within this window of “having the least contrast” and they all look identical. Determining which one to be the true focus point can be a real challenge. The followings are some of the tips to help make this daunting task more manageable.

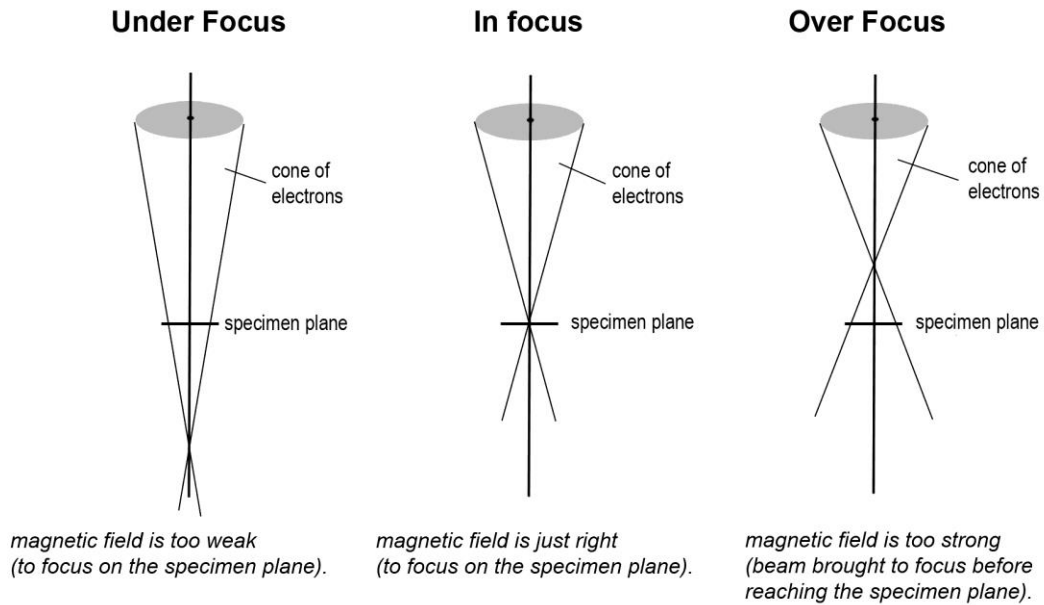
Demonstrating Under Focus, In Focus and Over Focus:

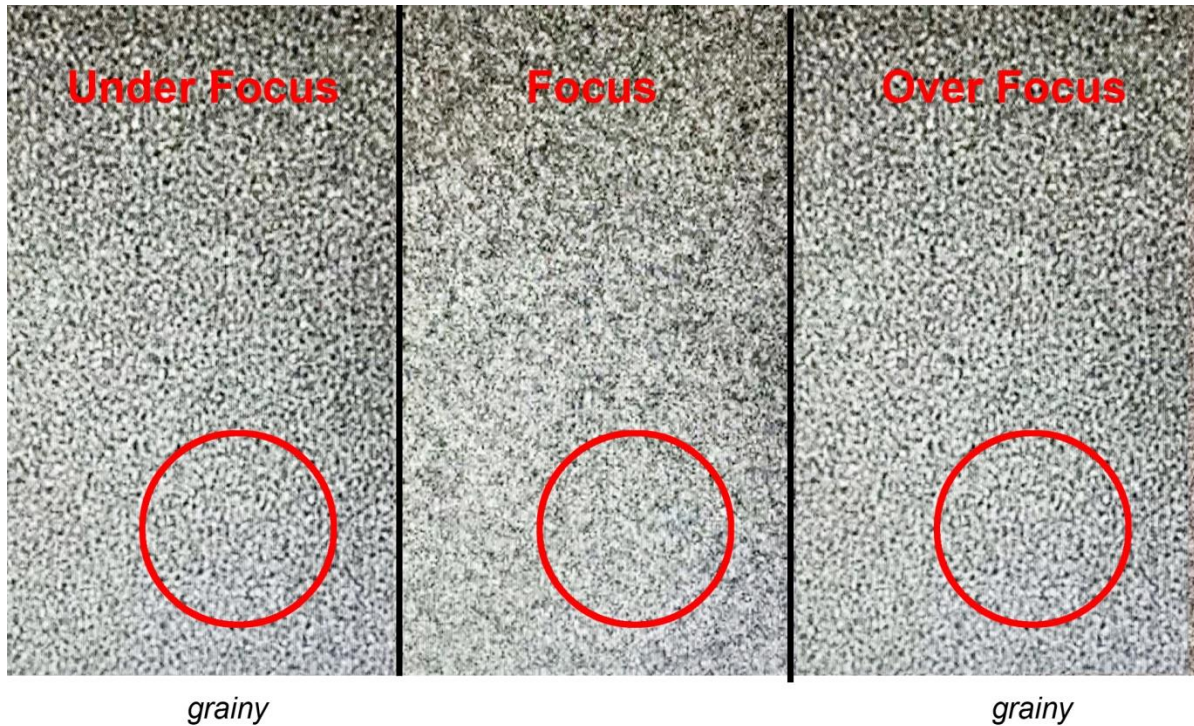
What is meant by Under Focus, Over Focus and In Focus?

Under Focus: the lens' magnetic field is too weak to bring the beam to focus on the specimen plane.

Over Focus: the lens' magnetic field is too strong and the beam is brought to focus before reaching the specimen plane.

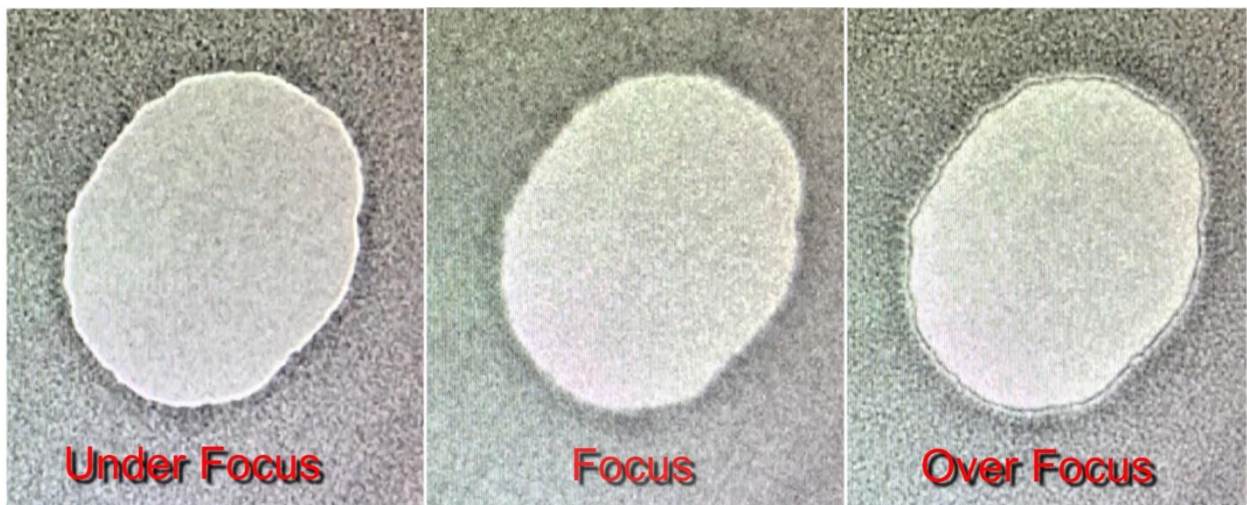
In Focus: just the right amount of magnetic field to focus the beam onto the specimen plane.





Actually, it is very easy to tell between out of focus and in focus images when one is imaging the edge of a hole. Out of focus “rings”, known as fresnel fringes, are distinctly visible. It is the effect of diffraction when the electron beam encounters an edge. The fringes are absent when the image is in focus.

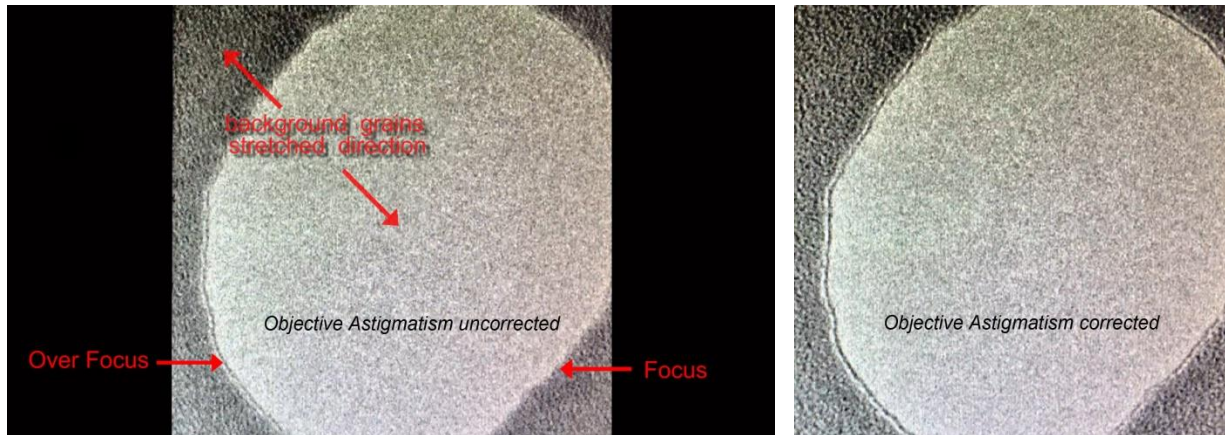
(video). At under focus, there is a white ring outside the edge of the hole. (that is, on the side facing the empty hole). At focus, the ring disappears. At over focus, the white ring appears on the inside of the edge. (that is, on the solid side of the hole). At the same time, the edge appears to turn into a black ring. Since this black ring is very prominent, people often refer to “seeing a black ring equates to over focus”; verses “seeing a white ring being under focus”.



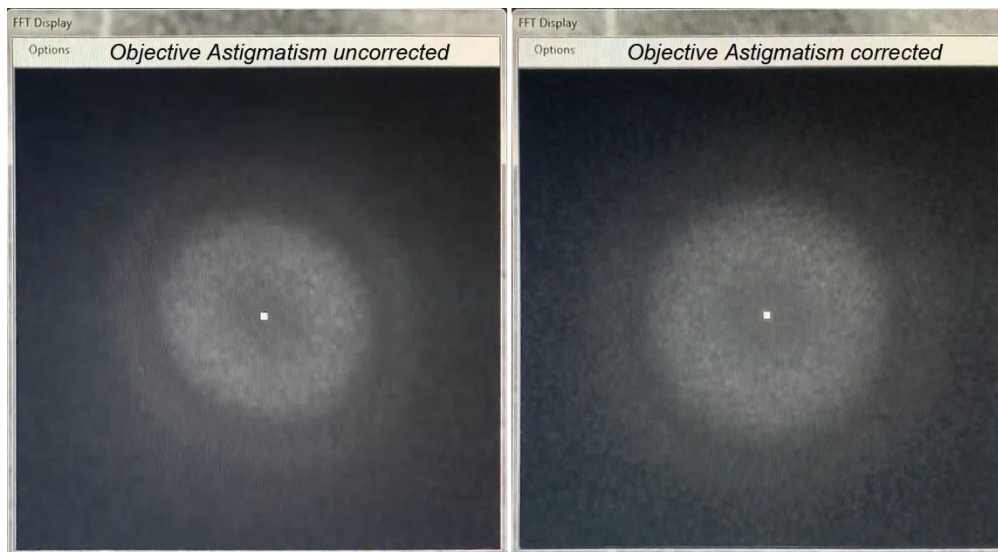
Objective Astigmatism:

With the presence of objective astigmatism, the hole appears to be in focus in one direction but out of focus in the other direction, (in this example it is over focus) with the background grains appearing to be directionally “stretched”.

Meaning: part of the image is in focus while the other part is out.



If this happens, it is relatively easy to make corrective adjustments with the Objective Stigmata so that the ring uniformly covers the edge of the hole. It will disappear uniformly when approaching focus. This task can also be done with the help of the F.F.T. image. (elliptical halo = presence of astigmatism; round halo = no astigmatism)



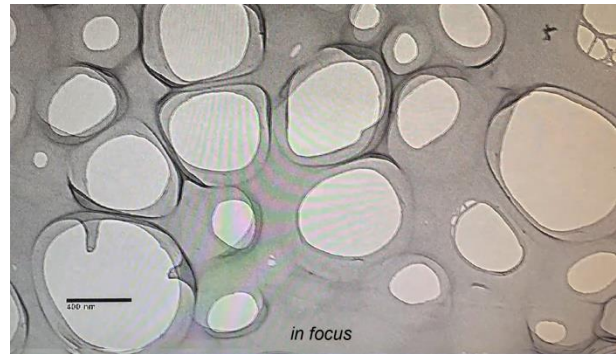
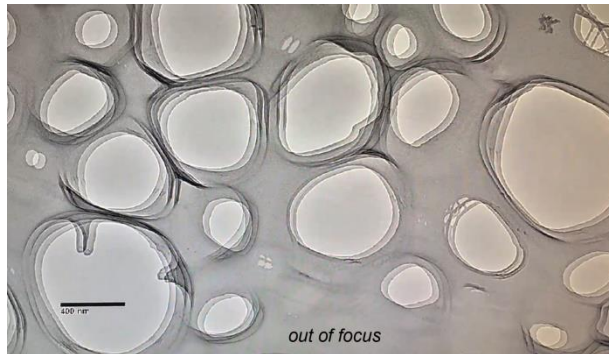
Important: Objective Astigmatism must be properly corrected for, or it is impossible to get a focus image.

Practical Tips in finding focus:

(Note: the following suggestions are general guidelines only. The results are highly dependent on the sample and the magnification. You should familiar yourself with each technique and come up with a strategy that works for you).

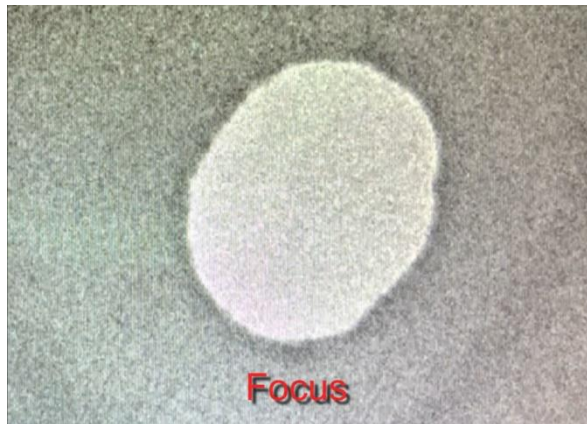
At Low magnifications:

For magnifications that can **use the Wobbler**, use the Wobbler, it is the easiest. An object splits into two when focus is out. Focus is achieved when the split image merges back into one. However, wobbler may not work well at magnifications higher than 15 to 20000X.



At High magnifications:

Method 1. Focus on a hole or an edge: if you can find a hole on your sample near where your area of interest. That would be the best. Focus on the edge of the hole as demonstrated above.



After that, move the hole away from the viewing field to take the picture. The objective lens has enough depth of field to allow the slight shifting of image sideways and still remains in focus. But don't shift too far away though.

Warning: avoid imaging the edge of a torn Formvar film as such area is unstable resulting in movement and image drift.

Method 2. Focus on the background grains: this is likely the situation we face most of the time when there is no hole near the area of interest. Background grains maintain higher contrast when out of focus but lose contrast when in focus, (looks as if the well-defined structure has “faded” away). But that is focus.

The following trick aims at detecting out of focus grains, as they are easier to see. And use that to deduct where the true focus point is.

The goal is to identify the first under focus change to the first over focus change, and vice versa. Then, true focus is somewhere in between. Take note of which direction the focus knob is being turned to determine over focus or under focus. (that is clockwise towards over focus. And anti-clockwise towards under focus).

Do a through focus run: Let’s start at an under focus point that is relatively close to focus. Turn the fine focus knob clockwise through focus to over focus and then backwards to under focus again. Remember the contrast change when the grains just turn over focus and just under focus.

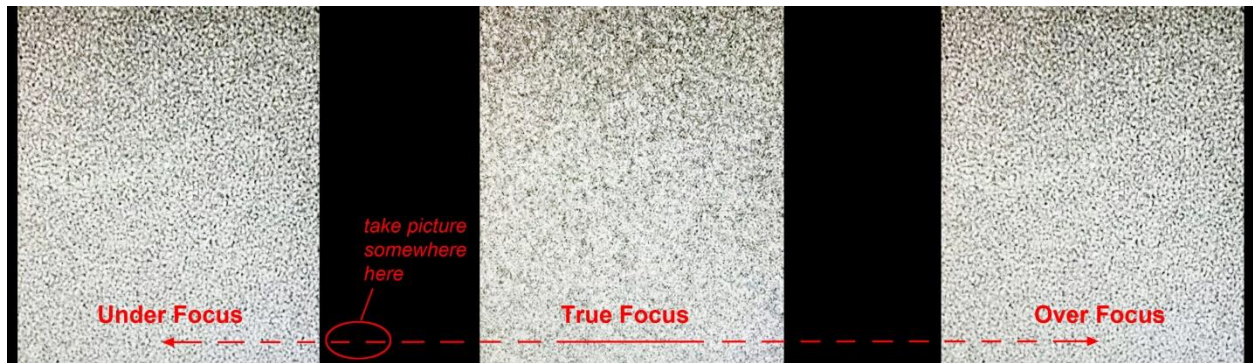
Repeat the procedure several times and carefully determine the first detectable off focus contrast changes from both ends, and the number of clicks needed to move between them. For example, if it takes 20 clicks to go between the two end points. then, at the middle, which is ten clicks from either end, is the true focus point.



The “under focus trick”:

A perfectly focus image usually bears little contrast and looks “plain”. Therefore, It is a common practice to purposely take a picture that is ever slightly out of focus enough, (to add to the contrast), while still maintaining enough details of a focus image. To that end, a slightly under focus image is preferred. Thanks to the edge sharpening effects of the white fringes, under focus grains always appear “sharper” than over focus grains (having black fringes). However, be aware that going too far under focus will result in notable artifacts on your image.

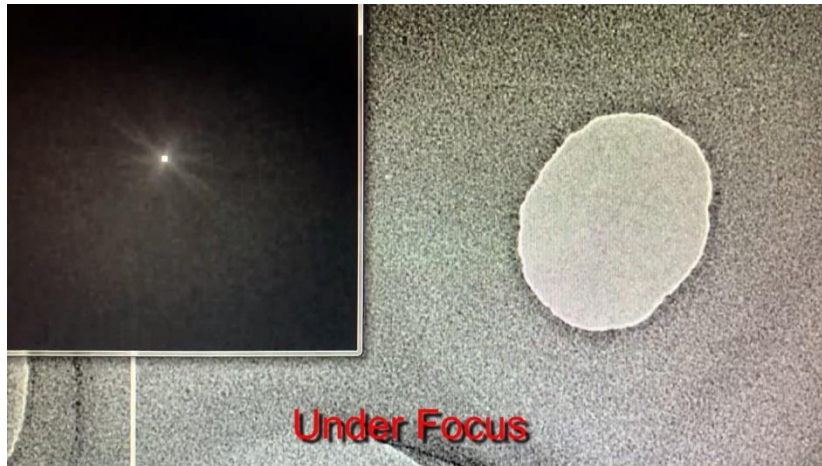
So: if it needs 20 clicks to move from the first detected under focus to the first detected over focus, instead of moving 10 clicks to the perfect focus point, move only 3 or 5 clicks gives a “sharper” final image. There is no fix answer as to how much defocus one should apply to the final image. It all depends on the sample and magnification, and obviously personal preference.



Bottom line: try taking several pictures, from the estimated true focus to slightly under focus. Compare them side by side. Whichever “looks” best for you will be your “best focus” point applicable to your sample at similar magnifications.

Final Remarks: the F.F.T. function may not be sensitive enough as a fine focusing tool.

You can easily detect out of focus fringes, and background contrast changes, well ahead of seeing the distinct out of focus halo.



Therefore, the ability to distinguish subtle contrast changes in background grains is the ultimate goal in fine focusing at high magnifications!