Tips on Focusing Transmission Electron Microscopes

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Recently, there was a thread on the microscopy listserver about focusing TEMs, in particular whether to focus at cross-over, or at the illumination level used for recording images. When setting the image focus there are a number of problems that may complicate matters.

1) To try to focus at condenser cross-over is absolutely the wrong thing to do!

i) At cross-over there is the poorest illumination coherence. This will lead to soft images, not out of focus but not optimized either.

ii) At cross-over there is a space charge effect (too many electrons in a small area, and they repel each other) which may cause a false focus. Just look at any image at crossover and then overfocus the condenser - more detail can be seen!

iii) To focus at crossover and then change to a lower intensity is asking for trouble, as the change in heating effect is likely to cause specimen drift and flexing which may change the contrast of material science specimens.

2) Focusing must be done at the photographic magnification. Focus is the matching of the focal lengths of the objective and diffraction lenses. If focusing is done at a higher level and there is a diffraction lens change when dropping the magnification, a matching objective correction will be required or the image will be out of focus.

3) Why is best focus not always true focus? To a materials scientist it will almost certainly be true focus, no Fresnel fringes, as determined by the focus wobbler. They will set their intensity in advance as an intensity change may cause the material to flex and change in contrast. To the biological scientist it will be a degree of underfocus determined by the specimen's organelle density, its thickness, the kV, the objective aperture size and most important, the magnification; this is optimum under focus, the defocus where the (white) Fresnel fringes enhance the high contrast areas!

When taking a photograph, the ideal conditions (as used in all the manufacturers' demo laboratories that I have worked with, Hitachi, JEOL and ISI) require the photograph to be taken at the same intensity level as when the focus is set. Why? Once an intensity is set, the operator may focus, and during the focus procedure he/she should be concentrating sufficient to spot any image drift. It is most important to see the image under the conditions that the micrograph will be taken. So what will the excuses be?

1) "It is too dim to focus."

i) Increase the emission current. Many people run at too low a current and make the task of optimizing the instrument settings much more difficult.

ii) Increase the kV which would also increase the intensity.

iii) Recalibrate the photographic system to allow a focus in tensity that is suitable for most operators

2) "The negatives are to dark."

The denser the negative the higher the contrast, so possibly one could increase the kV, have a more stable specimen and an

even brighter image?

3) Difficult one this - "The pathologists like a very bright image." Yes, I know. I guess it comes from looking down light microscopes all day?

i) Try 1 or 2 above - "Yes, but the pathologists like a high screen contrast." Try to explain that no one publishes the screen. If it is the photographic record that is the data, we can get stacks of contrast from modern printing/publishing systems.

Regarding the reported overlap in currents between condenser and objective lenses, it is true, however the result is usually more of an image shift than a focal change in my observations, but yet another reason to focus and stigmate at the photographic intensity.

So to conclude I would:

1) Always focus and stigmate at the photographic illumination level. For biological specimens, determine and plot out the optimum under-focus for the material over the normal magnification range.

2) Always use the second condenser over-focus from crossover for high coherence and combine this with a 2 micrometer spot size for biology.

3) Set up the instrument's photographic procedure to suit as many operators as possible, so that they too may focus at the photographic level.

4) Increase the emission current to give a higher brightness, making point 2 far easier to use.

5) Always use a higher kV. If 60, wow, please go to 80; if 80 try 100 and work to obtain the best results on a photograph not trying to optimise for the screen.

6) To focus at low magnifications, try removing the objective aperture and focusing without the binocular microscope or a wobbler, looking for the very strong minimum contrast effect. ■