Microscopy101

Recipes for Consistent Selected Area Electron Diffraction Results: Part 1: Microscope Setup

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Abstract: Electron diffraction is an essential tool for materials scientists to have in their characterization toolboxes. When using the transmission electron microscope (TEM) to perform diffraction experiments, setting up the microscope for both calibration standards and unknown materials in a consistent method will ensure that dependable results are obtained. Care must also be exercised to protect digital cameras from intense transmitted and diffracted beams to avoid damage. A procedure is presented that will allow a microscopist to reproducibly configure the microscope and sample to acquire selected area electron diffraction patterns so that these issues are addressed.

Keywords: TEM, selected area electron diffraction, SAED, calibration

Introduction

As the subject matter expert for electron microscopy at the CCDC-ARL Materials and Manufacturing Science Division at Aberdeen Proving Ground, MD, I work with researchers to help them use the transmission electron microscope (TEM) proficiently in their work. When using the electron microscopes, individual experience levels can vary from limited or none to fully skilled. One duty that I have assumed is to ensure that the TEM has been carefully calibrated for all imaging and diffraction modes. Regardless of their experience level, every user receives individual training on our instruments, tailored to their needs to make them successful. This training obviously includes the procedure for aligning the TEM and ensures that the investigators know the conditions used to calibrate the microscope. This ensures they can duplicate the procedures and rely on their data. Consistency is the key for that reliability. In this three-part series of articles, I will go through my procedures for collecting, recording, and analyzing selected area electron diffraction (SAED) images in the TEM to assure that the data are reliable and presentable. The microscope used for data acquired in this article was a JEOL 2100F TEM/STEM equipped with a Gatan 832 Orius SC1000 camera and Gatan's Digital Micrograph (DM) [1]. The procedures outlined here are somewhat biased toward that platform and may require some modifications if other equipment is used. An advantage of DM is that it has the capability to run scripts, and I describe some simple scripts and readily available scripts that make life a bit easier (pun intended) when processing and saving the SAED patterns.

In Part 1 I will present my procedure for setting up the sample and the microscope for acquiring SAED patterns from both calibration standard and unknown samples. In Part 2, I will present methods for acquiring SAED patterns. In the process, I will try to add a few hints and tricks, while emphasizing techniques that will protect the digital camera. Additionally, I will discuss how to optimize the data, using simple DM scripts, for easy use with electron diffraction analysis software. In Part 3, I will discuss some of the analysis techniques available with existing software, what features the packages should have, and then finally discuss the reliability of SAED measurement results.

SAED Acquisition Introduction

Every user of the TEM has been taught the comparison between the TEM and a light microscope. When training a user I ask a simple question, "How do you focus a light microscope?" The answer, of course, is that you change the distance between the objective lens and the sample, usually by changing the height of the sample stage. Then I ask, "Can you vary the focus of the objective lens of the optical microscope?" and the answer is that you can't for a given objective lens. Then, "How do you fix the focal length of the TEM when you have a knob that can change the strength of the objective lens?" To fix the focal distance of the TEM objective lens, you must set the strength of the lens coils to the same value every time. When that happens, the back focal plane of the lens is fixed in space relative to the lens, just as it is for the objective lens of a light microscope. On modern microscopes, that is done by pushing a button labeled something like "standard focus," and the user can read the hexadecimal value on the computer display and see that it is always the same. On older microscopes, it was common practice to have a five- or six-digit voltmeter read the voltage on the objective lens, and the users would adjust the focus until it read a particular value. The lens strength is usually set so that a sample positioned at the stage's eucentric position is in focus, but be careful; that position can change slightly with different holders. The sample is focused first by setting the strength of the objective lens and then raising or lowering the sample until it is in focus, completely analogous to an optical microscope. If this is the condition in which all of the magnification and diffraction modes are calibrated with a standard sample, users are assured that when they use these modes on an unknown sample, their images and diffraction patterns will also be calibrated.

SAED Acquisition Procedure

1) Follow the manufacturer's or your lab manager's procedures for aligning the microscope. Remember to do this at the standard focus condition. The gun, condenser, tilt compensation, shift compensation, condenser astigmatism, objective astigmatism, and high voltage center (or current

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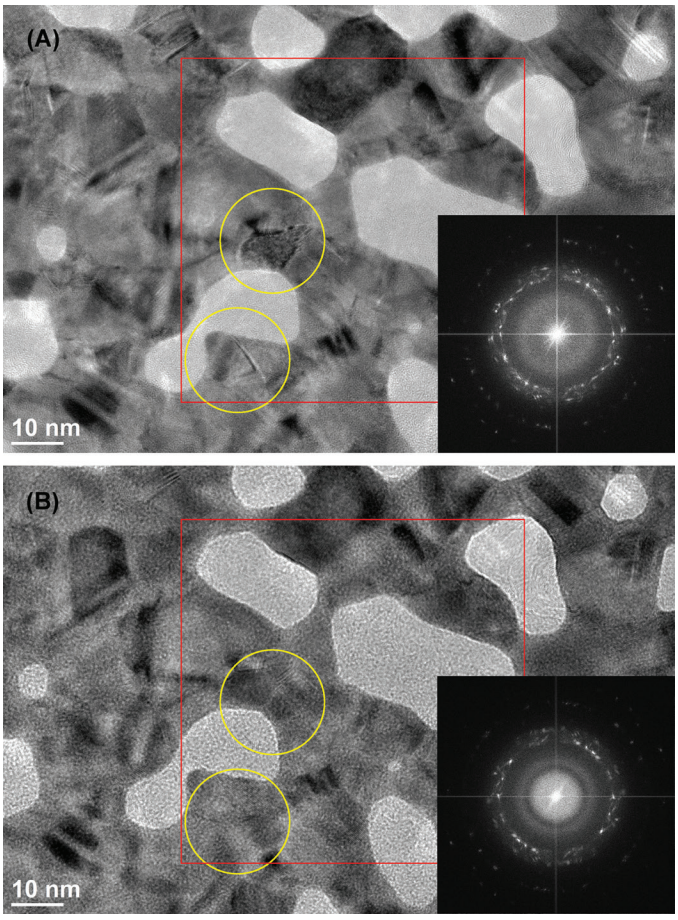

Figure 1: Two focus conditions for a Au film on a carbon support film. A) Minimum contrast condition for the amorphous carbon support film. Note the ghost diffraction images of the Au grains noted by the yellow circles. B) Minimum contrast for a Au crystal film with the difference in height of 37 nm. Note the minimum contrast of the same grains within the yellow circles in (A) and the increased contrast of the amorphous support film. The inserted FFT shows the Thon rings from the carbon support film.

center) should all be set, and the condenser aperture should be aligned.

> **Note:** It is good practice to eliminate hysteresis in the lenses prior to doing the alignment, if your microscope has the capability of doing so. Before I begin my session with the alignment procedure, I switch the microscope to low-mag mode and then back to mag mode and press the standard focus. This turns the objective lens off and then back on and sets the lens to the pre-determined standard focus strength. Next, I turn the brightness knob fully counterclockwise and then fully clockwise before bringing the beam to crossover and centering it. Then I center the condenser aperture.

a. Center the region of interest where the diffraction pattern will be acquired. With the standard focus set and at a magnification above 40 kX, (200 kX is better), raise/lower the sample to get minimum contrast. If using a live fast Fourier transform (FFT) from a digital camera to help with astigmatism, the innermost dark ring of the Thon rings from an amorphous sample will go to a maximum. For a crystalline sample, look for the point where all of the diffracted images coalesce with the bright-field image as shown in Figure 1.

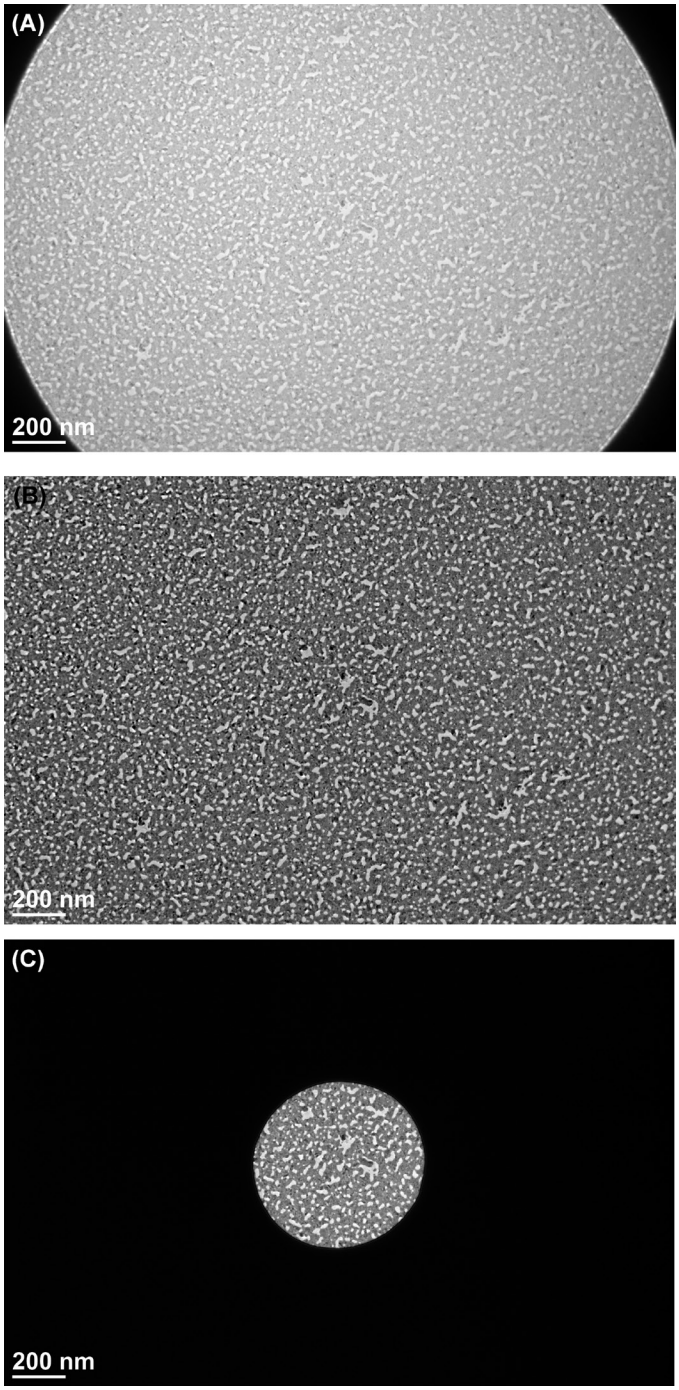

Figure 2: A) Lower camera exposure, condense the beam, and center the illumination. B) Spread the illumination to view the area of interest. C) Insert desired SAD aperture and center it.

b. Notes
   i. With a perfect lens, all of the diffracted images will coalesce, but because of spherical aberration, higher-index reflection images may not coalesce well with lower-index ones.
   ii. If a crystalline material is on a support film, focus on the crystalline material, not on the amorphous

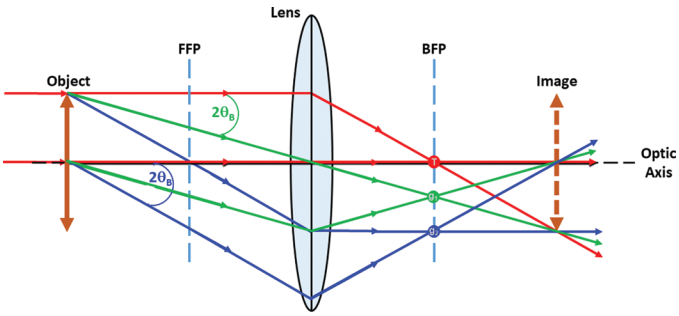

Figure 3: Ray diagram for an ideal lens. Red represents the parallel incident and transmitted beam, while green and blue represent diffracted beams. The diffraction pattern forms in the back focal plane (BFP) of the objective lens.

background that produces the Thon rings in the FFT. See Figure 1.

iii. If there is varying thickness in the selected area of the sample, such as occurs often with nanoparticles on a support film, try to find a portion of the area that is in the center of the thickness to use for determining the sample height. This can be gauged somewhat with the focus knob, but remember to set it back to the standard focus when adjusting the height.

iv. It is possible to immediately tell if a sample is way off the standard focus position by changing the condenser lens control (brightness). If the magnification of the image appears to increase or decrease, the sample is not at the correct height. At the correct position and with the standard focus set, there will be no change seen in apparent magnification as the condenser lens is changed.

2) Lower the magnification to observe the desired area of interest, and center the area of interest on the screen. If an objective aperture is inserted, remove it.
   a. Your sample is now focused, the region of interest is selected, and the diffraction pattern is formed in the back focal plane of the objective lens. If there is parallel illumination, then a spot or ring pattern from the sample is first formed there, as discussed below.
   b. Note: Whether doing imaging or diffraction, the sample is now in the calibrated position. This should be the position that both the imaging and diffraction modes were calibrated with using a calibration sample.
3) Center your illumination.
   a. If using a digital camera, please be careful. Lower your exposure to an appropriate level. (If using DM, three or four clicks of the down arrow will drop the exposure by a factor of 8 or 16 and is usually sufficient.)
   b. Condense the beam until it is seen in the camera window as shown in Figure 2A, and center using the beam shift controls. If you are not using a digital camera, then center the condensed beam on the phosphor screen.
   c. Spread the beam and restore the camera exposure to what it was (in DM, three or four clicks with the up arrow (see Figure 2B).
4) Introduce the appropriate size of diffraction aperture that selects the area of interest, and center it as shown in Figure 2C.
5) Turn the brightness knob fully clockwise. If you have access to viewing the microscope's hexadecimal lens values, the condenser lens should read "FFFF." Regardless, most microscopes beep when you have changed a control to its maximum or minimum. Just listen for the beep.
   a. At this point the illumination is as parallel as possible, and the diffraction pattern is formed at the back focal plane (BFP) of the objective lens. Ideally, the transmitted beam (*T*) and diffraction spots (*g*) are points, ignoring spherical aberration. There is also a plane where the

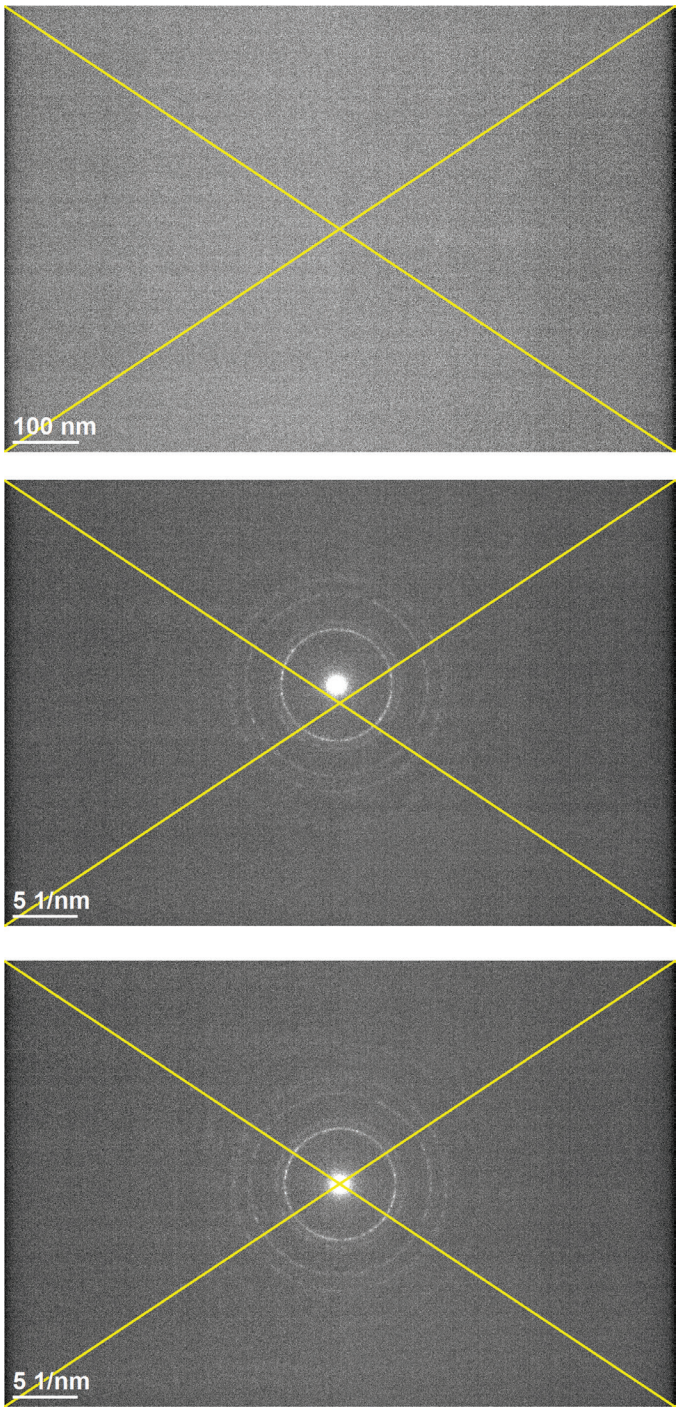

Figure 4: Top: Lines are drawn on the view screen to find the center of the digital image. Center: With a low exposure, the screen is lifted, and the transmitted beam should be close to center. Bottom: Center the transmitted beam to the center as closely as possible. Lower the screen. (In the example here, the exposure was a little higher than normally used so that some rings could be seen.)

image is reconstructed (Image); just like all of the elementary ray diagrams show (see Figure 3).

b. For imaging mode, the projector lens system grabs and magnifies the image plane; while in diffraction mode, the projector lens system grabs the diffraction plane.

6) Lower the screen to protect the camera from the bright transmitted beam, and change the microscope mode to selected area diffraction (SA Diff).
7) Change the camera length to an appropriate value.
8) The pattern must be focused and the spot checked for astigmatism. This is best done with the screen down and using the binoculars. As shown in Figure 3, the diffraction pattern is formed in the BFP of the objective lens. The projector system must grab that as its object. The first intermediate lens (IL1) of the projector lens system is adjusted with the diffraction focus control to make the BFP of the objective lens coincide with its object plane. This is done by focusing the transmitted spot (or any diffraction spot) to the smallest point possible. The diffraction pattern is now in the calibrated position.
   a. While focusing the IL1, note whether there is astigmatism in the spot by going through the diffraction focus. If there is, it should be eliminated with the IL1 stigmator controls.
9) At this point, the diffraction pattern may not be centered on the screen or on the camera. If it is not, center the pattern using the projector alignment controls.
   a. If you are not using a digital camera, simply center the transmitted spot on the center of the phosphor screen.
   b. Important!: If using a digital camera, care must be taken to not expose the camera to an intense transmitted beam with a long exposure. The following procedure is recommended for reproducibly in centering the pattern, which can help when using diffraction analysis software.
      i. With the phosphor screen down, draw two lines from opposite corners of the viewing window as shown in Figure 4A. A simple script to do this is given below:

```
DM Script: X-MarksTheCenter.s
// This short script draws two yellow lines from corner to corner to indicate the center of the camera.
Image img
Number Sx, Sy
Img := GetFrontImage ()
Getsize (Img, Sx, Sy)
Component display=Img.ImageGetImageDisplay (0)

//Draw yellow line from top left to bottom right
Component XLine1=NewLineAnnotation(0, 0, Sy-1, Sx-1)
XLine1.componentSetForegroundColor (255, 255, 0)
display.componentAddChildAtEnd(XLine1)

//Draw yellow line from bottom left to top right
Component XLine2=NewLineAnnotation(Sy-1, 0, 0, Sx-1)
XLine2.componentSetForegroundColor (255, 255, 0)
display.componentAddChildAtEnd(XLine2)
```

      ii. Decrease the exposure of the camera drastically, lift the phosphor screen, and very quickly center the transmitted beam where the two lines intersect with the projector lens alignment controls as shown in Figure 4B and 4C.
      iii. Lower the screen, and restore the exposure.
10) Insert and center the beam block.
11) Raise the screen.
12) Determine the exposure.
13) Record the SAED pattern.

In Part II of this series, to be published in the next issue of *Microscopy Today*, acquisition of SAED patterns and optimization of SAED data will be discussed.

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Reference

[1] DigitalMicrograph™, Gatan, Inc., Pleasanton, CA 94588, USA.

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