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Electron Diffraction

(contributed by Becca Weiner, with modifications by D. Morgan)

The following is a brief description of how to collect electron diffraction data using the JEOL JEM 3200FS. Start by finding the area you want to examine, adjusting the strength of the electron beam to an appropriate level (see below for ways to do this) and making sure that an image recorded under these conditions is focused properly (close to focus but slightly under-focus) when the objective lens is set to standard focus. Then follow the steps that start below in order to put the 3200FS into diffraction mode, deal with the properties of the electron beam and record the diffraction data. If you decide to change the size of the electron beam or the spot size, or if you insert a selected area aperture (see below), you will need to redo some of these first seven steps.

1) Make sure that the optical axis of the microscope is centered (cycle between the spots sizes, return to the one you want to use and center the beam using beam shift) and that the object from which you want to record diffraction information is centered within the beam).
2) Switch into diffraction mode using the "SA DIFF" button on the right-hand knobset.
3) Focus the diffraction pattern using the "DIFF FOCUS" knob, also on the right-hand knobset. A focused diffraction pattern will have very sharp diffraction spots and the unscattered beam (aka the undiffracted beam, F(0), F(0,0), *etc.*) will be as small and point-like as possible.
4) If the unscattered beam shows signs of astigmatism (elongation in one direction that rotates by 90° as the diffraction pattern passes through focus), remove the astigmatism using the intermediate lens stigmators (toggle the "IL STIG" button in the Alignment window in TEMcon and use the "DEF/STIG" knobs on both knobsets to minimize this elongation). The

E

intermediate lens astigmatism will change whenever the camera length is changed.

5) Adjust the camera length (the magnification knob in normal imaging mode) to make the diffraction pattern larger or smaller. Camera length is measured in cm (TEMcon) or mm (DigitalMicrograph), and a shorter camera length corresponds to a smaller diffraction pattern. The smaller the pattern, the easier it is to block the strongest part of the unscattered beam. This protects the CCD camera from damage due to over-exposure.
6) Center the diffraction pattern using the projector lenses (toggle the PL button in the Alignment window in TEMcon and move the pattern using the "DEF/STIG" knobs). Place the unscattered beam as close as possible to the black dot on either the small (focusing) or large phosphor screen.
7) Move the beam-stop (the knob on the left side of the viewing chamber) so that the tip of the beam-stop blocks the unscattered beam. The position of the beam-stop can be adjusted by turning the knob on the side of the viewing chamber (moving the beam-stop more or less up or down as you look into the viewing chamber) and by pressing or pulling on the knob (moving the beam-stop in the direction you push or pull). You may find it easiest to move the tip of the beam-stop so that it is very close to the unscattered beam, and then to use the projector lenses to move the unscattered beam so that it is exactly on the beam-stop.

You are now ready to record images of the diffraction pattern. Parts of the diffraction pattern (*e.g.*, the unscattered beam) are very bright and can damage the sensor of the CCD unless care is taken to minimize the interaction between the beam and the sensor. This starts by controlling the exposure time:

8) Change the camera exposure time to 0.05 or 0.1 s

There is also an issue with short exposures such that the movement in the electron beam as it is un-blanked can be seen. This can be fixed by changing how the camera is shuttered:

E

9) In DigitalMicrograph's Record window, click first on Setup and then on Advanced Settings in the new window that appears. One option there is to toggle between the types of shuttering (pre-specimen vs post-specimen shuttering). Make the change to post-specimen shuttering and close all the new windows. If for some reason you find that the shuttering is already set to post-specimen shuttering, leave it there but let the EM Center staff know that you found things this way. Also keep in mind that when you are done recording diffraction data, you will need to set the shuttering back to pre-specimen shuttering.

You are now ready to record an image. Remember that the first image you take after changing the exposure time causes DigitalMicrograph to collect a new dark reference image, which means that the camera will appear as if it is acquiring two sequential images.

10) Record images as usual.

You will need to adjust the contrast of the image of the diffraction pattern by right-clicking on the image, selecting ImageDisplay and changing the two values in the "Remove Lowest/Highest % of outliers" line from 1.0 to 0.0. This is the same change that is often made for STEM images. After you have made this change, the contrast in the image of the diffraction pattern can be adjusted using the mouse in the histogram area (in the upper left corner) of DigitalMicrograph. You may want (or need) to change the exposure time (longer or shorter, keeping in mind that you do not want an exposure long enough to damage the CCD), the strength of the electron beam and/or the position of the beam-stop.

If you notice that the beam-stop appears to be out of position in the recorded images but seems OK on the view screen, it means that the electron beam is travelling down the column at a slight angle. This should not be the case if the TEM alignment has been done properly, but it may happen. In such cases, move the beam-stop so that it more fully blocks the unscattered beam in the recorded image (and protects the CCD sensor from beam damage), even though it may appear out of position on the viewing screen.

E

11) When you have finished recording the diffraction information, remember to return the shuttering to "pre-specimen shuttering," the exposure time to 1 s and the microscope to normal imaging mode, and to remove the selected area aperture if it was used and set the CLA back to the largest aperture (#1).

Other things to think about when recording diffraction data

1) **Finding a zone-axis view**: If you are trying to record the diffraction pattern from a particular zone-axis view of a nano-particle, you will often need to tilt the specimen so that the beam travels "down" the zone-axis view you need. All the holders for the JEOL JEM 3200FS can tilt some amount around the axis that runs along the rod of the specimen holder, but in order to find the proper zone-axis view, it is often necessary to tilt both in that direction and in the direction normal to that tilt axis. This is the reason the dual tilt beryllium holder is used when recording diffraction data from many nano-particle preparations. When tilting any of the holders, set the tilt increment to 0.5° or 1° in the Stage tab of the Operations window in TEMcon and tilt the holder in single steps around x (the tilt axis parallel to the rod) and/or around y (the second tilt axis that is accessible when using the dual tilt beryllium holder).

   The diffraction pattern will change as you tilt the specimen and the goal is to tilt the particle so that you see the diffraction pattern from a particular zone-axis orientation. The problem is that it is impossible to set the goniometer so that it is eucentric around both the x- and y-tilt axes, and this means that as the specimen is tilted, the particles will move significant amounts when tilting around one of the tilt axes. When

E

working with small nano-particles and small selected area apertures (see below), this means that the particle will move out of the electron beam, and the diffraction pattern you want to see will vanish. You can watch a particle move by either switching from diffraction to imaging mode (and back again after finding the particle in imaging mode and re-centering it), or you can defocus the diffraction pattern so that the unscattered beam forms a high contrast image. The second approach is more convenient than switching between modes (but there are still times when you will likely need to return to imaging mode). However you tilt and track the nano-particle of interest, the goal is to tilt around the two axes until the desired diffraction pattern is seen.

NOTE: When looking at a field of particles in TEM imaging mode, the darkest particles are in or near zone-axis views. They may or may not be in the zone-axis view you want, but such dark particles should be close to a zone-axis view: some of the electrons scattered to particular Bragg angles are lost from the final image, making it appear that such particles were not illuminated with as many electrons as nearby nano-particles that are not in zone-axis orientations.

2) **Reducing the strength of the electron beam**: The unscattered beam contains most of the electrons in the beam, and thus is very bright and can damage the CCD sensor. For this reason, you will generally want to use a much weaker beam to record electron diffraction information than you would use for normal imaging. The strength of the beam above the specimen (and thus the brightness of the unscattered beam) can be controlled by changing spot sizes (where larger numbers correspond to a less bright beam) and/or changing the condenser aperture (the CLA setting on the left-hand knobset, where, as is always the case for the JEOL JEM 3200FS, larger numbers correspond to smaller apertures and for the CLA, a less bright beam). Each change in spot size reduces the beam intensity by a factor of about 2, while the change from CLA #1 to CLA #2 is much larger than changing from #2 to #3 or #3 to #4. As the beam that

E

is used to produce the diffraction data gets weaker, it is possible to use longer and longer exposures without damaging the CCD. However, always start with an exposure no longer than 0.1 s and increase the time from there if needed. Remember that when you change spot size and/or the condenser aperture, you will need to re-center the CLA.

You may also find it necessary to reduce the strength of the electron beam when trying to record diffraction information from nano-particles that are sensitive to the electron beam. Virtually all materials will damage if exposed to the electron beam for long enough, but some particles (especially smaller ones) damage significantly over the course of a relatively short exposure. The only way to deal with such particles is to reduce the strength of the electron beam as described above.

3) **Using selected area apertures**: A diffraction pattern comes from everything that the electron beam touches. In other words, everything that is exposed to the beam will contribute to the diffraction pattern. This can include things such as multiple small (or large) particles, areas of amorphous carbon and the edge of a grid bar. You can control what the beam touches by making the beam larger or smaller (Brightness knob on the left-hand knobset). However, this is often not the best way to make the area that contributes to the diffraction pattern smaller. In all aspects of TEM, you want to work with an electron beam that is as parallel as possible as it passes through the specimen, and if you make the beam very small (so that, for example, it covers a single, small nano-particle), it become very convergent. This is bad for both imaging and diffraction work. The best way to avoid this problem for diffraction work is to spread the beam so that it is as parallel as possible when it interacts with the specimen and then to introduce a selected area aperture (SAA) below the specimen. This is done using the SAA settings on the left-hand knobset: While still in imaging mode, spread the electron beam so that it is roughly parallel as it passes through the specimen. Then adjust the magnification

E

so that you can still clearly see the particle from which you want to record diffraction information.

Make sure the optical axis of the microscope is centered, that your particle is centered within the beam, and introduce the largest of the selected area apertures (#1). You should observe that much of the electron beam is blocked by this aperture (and if you do not notice this, switch immediately to the #2 aperture). Center the aperture using the motorized aperture controls (left-hand knobset). You should see the particle of interest and the area surrounding it. If there is still a large area around the particle (or if you see multiple particles within the part of the image that the SAA allows to pass through), switch to the next smaller aperture, and center it. Repeat this process of switching to smaller and smaller apertures until you have reached aperture #4 or you find an aperture where your particle of interest just fills the image. At this point, you can switch the microscope into diffraction mode (step 2 above), adjust the beam and record an image of the diffraction pattern.

How does the SAA work? The selected area apertures sit at the image plane of one of the lenses below the objective lens. The image at this point is a copy of everything that the electron beam touches and the SAA's simply block some of the electrons in this image from passing further down the column of the microscope. The electrons that remain have all the properties of the electrons that interacted with the specimen (such as the fact that the beam was parallel and the nano-particle caused some of the electrons to experience Bragg diffraction) and end up in the diffraction pattern as if the SAA were not there. However, the electrons blocked by the SAA do <u>not</u> continue into the diffraction pattern. This lowers the total number of electrons that interact with the CCD, reduces the background diffraction from the amorphous carbon substrate and eliminates Bragg diffraction from other nano-particles that are in areas of the image blocked by the SAA. All of this contributes to a better diffraction pattern that would be obtained without using the SAA.

E

NOTE: The absolute diameter of the electron beam in the JEOL JEM 3200FS when it is parallel will depend on the alpha setting of the microscope, where a setting of alpha 3 generates a wider parallel beam than a setting of alpha 1. This means that for diffraction work that involves use of the SAA, it can also be useful to adjust alpha so that more (or fewer) electrons are in the beam that passes through the SSA and into the diffraction pattern.