

Outline of Suggested Protocols for Acquisition of Tomographic Data from Highly Beam Sensitive Specimens

Motivation:

Samples that are very sensitive to electron beam induced damage need special care when trying to collection tomographic data sets. Very low dose imaging techniques must be employed while surveying the specimen for appropriate areas from which to acquire tomograms. The very low dose technique requires a very wide spread minimal electron beam to minimize sample damage. This is best accomplished while maximizing image contrast in defocused diffraction mode. This very low dose survey should be done before launching SerialEM, with the JEOL 3100 Minimum Dose System. Although this description is specific to the JEOL 3100 with SerialEM, it is easily adapted to any TEM and low dose tomography package.

1. Initially Set up the JEOL MDS conditions before inserting a sample into the column.

Suggested conditions would be:

Search- With spot size 5 at about 5kx, spread the beam to at least fill the view screen; the more spread the beam the larger the area viewed and the lower the electrons/cm² dose to the specimen. Insert objective aperture so aberrations of the lens are cut in the defocused diffraction pattern, otherwise a caustic figure is observed.

Focus--spot size 5 at 6kx with beam spread to fill viewing screen. This would be similar to the view mode in SerialEM, with zero image shift and very low dose beam condition.

Record--spot size 5, 20kx with beam spread to fill the viewing screen. This would be a low dose but plenty good for an image binned by two to give a real good look with a half second exposure.

2. Insert specimen into column and survey in MDS. In SEARCH mode: set the sample to eucentric height.

3. Use SEARCH mode to scan the sample and find potential areas. Acquire and save images in Digital Micrograph, or whatever digital camera system is on the TEM, to document various areas of the sample. Name the images of areas consistently with the saved coordinates in the JEOL specimen location window. Record some binned images at the focus and record settings. The RECORD image should provide a good look at the area to decide if it has the features of interest. The FOCUS image will be a good reference for comparison to VIEW mode images in SerialEM.

4. Note: It would probably be wise to test and note the tilt angle range at each possible area of interest in the low dose SEARCH mode. Using the digital camera to record a SEARCH mode image at several sample tilts should help here, while keeping the dose to a minimum.

5. Move the specimen to an open grid square or remove from the column to the side exchange chamber.

6. Launch SerialEM and collect gain reference at the proper spot size for data collection. This is needed for the program to determine your dose in the various modes.

7. Set up the Low Dose mode in SerialEM.

8. Verify the Camera settings: binning, and exposure for the various conditions.

9. Re-insert specimen into column and set to eucentric height, saved earlier.

10. Proceed to saved areas of the grid and examine with SerialEM.