SCHOOL OF MATERIALS ENGINEERING

Electron Microscopy Facility

a unit of the Purdue Electron Microscopy Consortium

MSE 595T
Basic Transmission Electron Microscopy

Laboratory V
TEM Imaging - III

Purpose

The purpose of this lab is to:

1. Practice the overall alignment procedure for the microscope
2. Obtain lattice images from a crystal of large lattice parameter
3. Check the magnification calibration of the microscope
4. Record images and use the software to check focus and stigmation

Report Requirements

Working individually, in your own session at the microscope after this lab, obtain and submit a computer file in Digital Micrograph format, demonstrating your ability to obtain a high-quality image of today's specimen. This will be checked by computation of its Fourier transform. Also submit your final re-write of the report started in Lab I.

Procedure

Preliminaries

1. Go over the safety checks.
2. Insert a specimen in the specimen holder. (Use a beef catalase specimen.)
3. Check that the stage is *centered*, objective aperture is *out*, EDS detector is *out*, tilts are *zeroed*, then insert the specimen holder in the goniometer.
4. Turn on the beam and perform the basic microscope alignments.
5. Choose an interesting field of view, and focus the image. Check that the objective lens voltage is 7.04.

I. Obtaining a Multi-Beam Image

1. Find a suitable piece of catalase to image.
2. In MAG1 or MAG2 mode, insert and center a selector aperture. Center and spread out the illumination. Ensure that the BRIGHT TILT setting is selected.
3. Select DIFF mode. Focus the diffraction pattern with the DIFF FOCUS knob.

4. Insert the largest objective aperture and center it carefully around the transmitted beam. It will include several diffracted beams. This aperture corresponds to the diameter of the first phase band-pass region in the back focal plane of the objective lens.
5. Select MAG1.
6. Remove the selector aperture.
7. Select a magnification of at least 150,000X, and focus the image.
8. Re-check the bright field beam tilt, using the HT wobbler.
9. Check the objective lens stigmation by looking at the "salt and pepper" contrast from the amorphous carbon specimen support. (You are trying to make the very fine, faint structure appear as homogeneous as possible, with no change in directionality as the objective focus goes from just below to just above exact focus.)
10. Adjust the focus to the Scherzer condition, slightly under the exact focus.
11. Record an image using the computer.

II. Images Measurement

II.i Lattice Spacings

Your image should clearly exhibit crossed grids of lattice fringes from the catalase protein crystal, which has lattice parameters of 8.75nm and 6.85nm.

1. Use the Gatan Digital Micrograph software to measure the lattice fringe spacing. Do this by drawing a line perpendicular to the fringes, using the "ROI Tools," and obtaining its length from the "Results" window. Count the number of fringes crossed by the line.
2. Determine the accuracy of the established magnification calibration.
3. Repeat this for several different magnifications, saving your images as you do so.

II.ii Image Quality

A good check of the quality of your image is obtained from the Fourier Transform. This is obtained using the Digital Micrograph software.

1. Select a suitable area of your image using the "ROI Tools". You need to select an area of 2^n x 2^n pixels, which can be done by holding down the ctrl key as you select the area.
2. From the "Process" menu, select "FFT."

The Fourier Transform that you obtain is similar to the diffraction pattern of the specimen, but it starts with your image instead of the real specimen. Any image imperfections are naturally reflected in the FT.

The FT should exhibit spots corresponding to the lattice fringes in the image, and a diffuse, circular region of intensity centered in the screen. Your image should contain fringes in two directions, so the FT should have crossed grids of spots. If you have any uncorrected astigmatism, the diffuse intensity will be elliptical, or have a "bow-tie" appearance in an extreme case.

Note that you could observe a "live" FFT as you try to focus and stigmate your image.