Physics 7320/4230 "Electron Microscopy and Microanalysis"

Laboratory 1: Introduction to SEM

August 29th - September 1st, 2016

(Lab reports are due one week from lab session)

Introduction:

This laboratory is designed to be the first part of your hands-on SEM operator's manual. Take notes as you go. The purpose of this first experiment is to familiarize the student with the controls and operation of the SEM. The student will become proficient in the operation of the imaging system and will determine the extent to which the magnification readout of the microscope is correct.

Samples:

1. M3 (0.5 mm) Machine Screw (20 threads per cm).
2. Copper Grid (3 mm diameter, 300 mesh) grid bars meeting at 90° angles and holes where support films (carbon, silicon, graphene) can be laid (Figure 1).
3. Aluminized compact disk – with pits and lands whose sizes are given below (Figure 2).

Objectives:

1. Proper startup and vacuum level checks
2. Specimen exchange
3. Alignment and Focus/Working distance determination
4. Image capture
5. Stage rotation, image rotation, tilting and image distortions
6. Image measurement

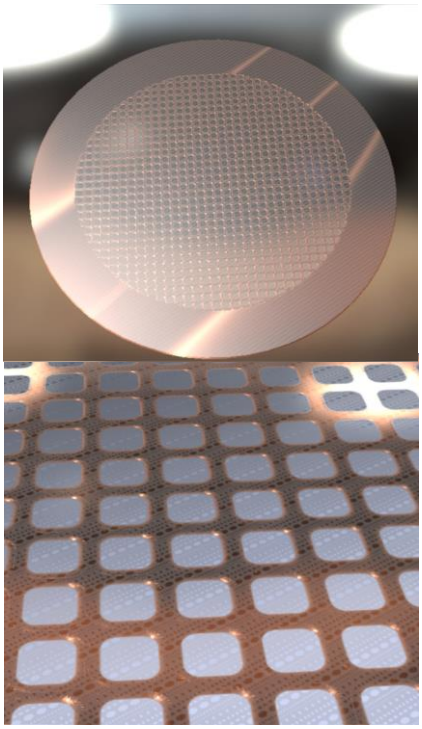

Figure 1. Copper Mesh TEM Grid. Overview (upper) and zoomed in (lower).

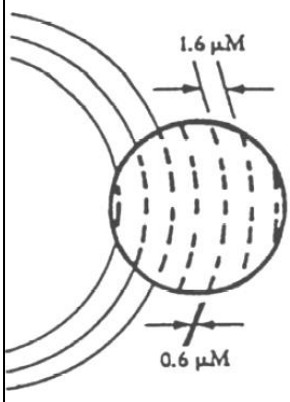

Figure 2. Pits and lands

The pits are 0.12 µm deep and about 0.6 µm wide. The neighboring turns of the spiral pattern of pits are 1.6 µm apart. This spacing corresponds to a track density of 16,000 tracks per inch (tpi), much higher than the figures associated with floppy (up to 96 tpi) or Winchester (several hundred tpi) magnetic disks. Along these turns, pits and the spaces between them (called *lands*) run from 0.9 to 3.3 µm long. (Figure 2 shows these relationships.) Just to put these very small numbers into perspective, here are a couple of gee-whizzers: The total length of the track on a CD ROM disc is almost 3 miles. The total number of pits on a CD ROM disc is almost 2 billion!

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Questions to be answered:

1. Draw a schematic of the SEM, including gun, lenses, apertures, scan coils, specimen stage, detectors. Where are the ion pumps (what parts of the microscope do they provide vacuum to)? Why are the vacuums maintained at different levels? (2 points)

2. How does the scanning electron microscope control image magnification? (2 points)

3. Describe the difference between mechanical and "raster" rotation? Does the secondary electron signal coming from the sample change upon mechanical rotation or upon raster rotation? Why or Why not? (2 points)

4. Set the microscope to a magnification that shows several M3 machine screw threads, determine the number of threads per centimeter. (1 points)

    a. Threads/cm - _____

5. Take an image of your copper grid and measure the following (5 points):

    a. Horizontal, vertical and diagonal distance of a grid opening.

        i. _____ _____ _____

    b. The width of a vertical grid bar. _____

    c. Area of a grid opening. _____

6. At 0^o and 40^o tilt, are there differences in your copper grid images? What do you see in your tilted images? Are the squares perfectly square? What is this phenomenon called? (2 points)

7. On your aluminized compact disc, measure and report the pit width and the spacing between rows of pits. (2 points)
    a. Pit width. _____

    b. Spacing between rows. _____

8. Field Emission relies on the ability to induce electron tunneling, as is required for all microscopes utilized this course. Describe this process – include discussion of emitter material, emitter size, and how work function contributes to emission. (4 points)

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Specimen 1 (Machine Screw in Low Mag):

1. Obtain a well-focused and stigmated image of the M3 Machine Screw – save this image named with your lastname_magX_M3screw. Keep in LM mode.
2. Set a magnification that shows several threads, determine the number of threads per centimeter. See questions to give your answers.

Specimen 2 (TEM Grid in Medium Mag):

1. Obtain a well-focused and stigmated image of the copper grid – save this image named with your lastname_magX_grid
2. Measure the length and width of the grid bars and grid squares
    a. Grid bars need to be horizontal and vertical (not at an angle).
    b. Keep this in LM mode. To straighten the grid, rotate image orientation using "raster rotation" (under OPERATION tab) until the grid is square in the display.
3. Using the tilt stage knob, acquire an image at 0^o tilt and again at 40^o. Are there differences in your images? See questions to give your answers.

Specimen 3 (CD in high mag):

1. Navigate to a scratch, use "raster rotation" to obtain horizontal or vertical alignment of features. Obtain a well-focused and stigmated image of the aluminized layer from the compact disc showing the pits and lands – name this image with your last name_magX_AlCD.
2. Measure and report the pit width and the spacing between rows of pits. See questions to give your answers.

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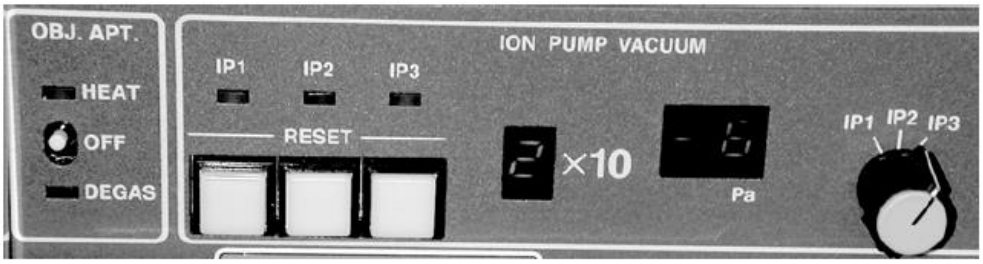
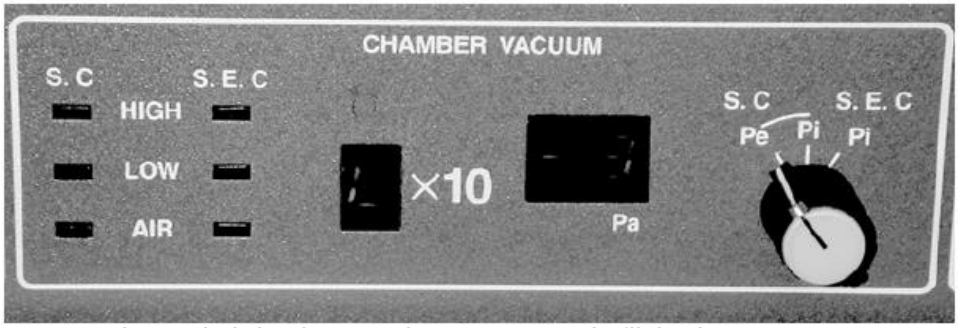
General Operations: **Getting started…**

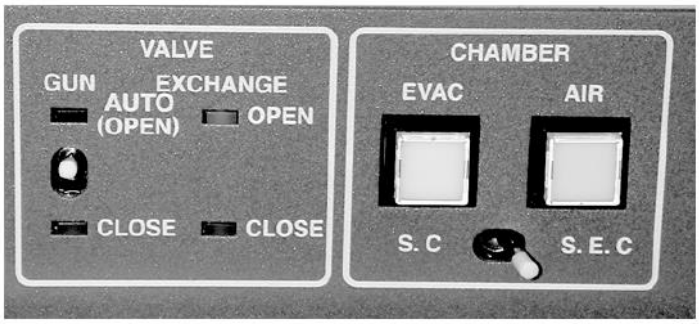
1. Check panel (before every use!)
   a. Write the date, your name, column vacuum levels (IP1, IP2, IP3), note V_{ext} level.
   b. Ensure objective aperture (OBJ APT) switch is set to HEAT
   c. Ensure the ion pump (IP) lamps are lit and vacuum levels are
      IP1 < $2x10^{-7}$ Pa
      IP2 < $2x10^{-6}$ Pa
      IP3 < $7x10^{-5}$ Pa



   d. Ensure the following lamps are lit: DP/TMP (diffusion/turbomolecular pump), water, and Air Pressure
   e. Ensure the top lamps are lit for Specimen Chamber (SC Vac) and specimen exchange chamber (SEC Vac)



   f. GUN valve switch is close and AUTO Lamp is flickering



   g. HV lamp is off

2. Ensure no specimen in chamber in CRT display

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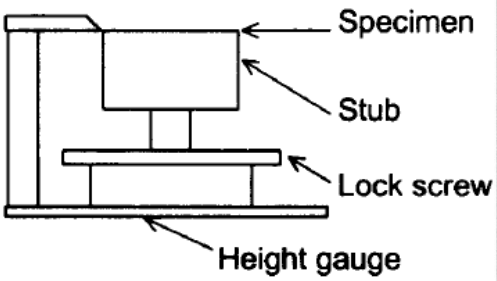
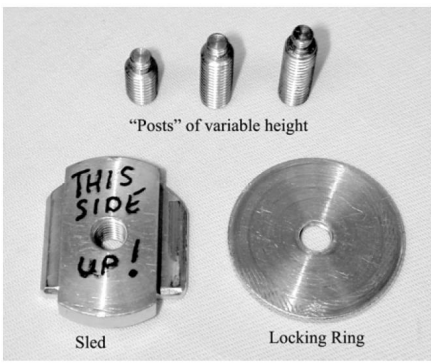
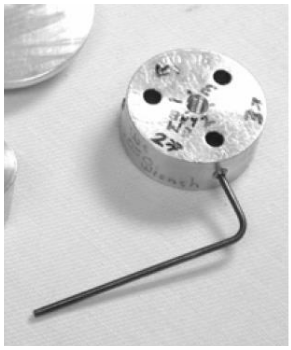
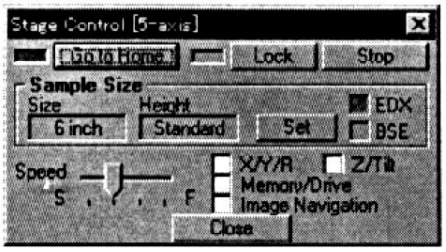
General Operations: **Sample Insertion**







- Check that sample on stub mounted on the sled is below the height gauge. Also ensure that the screw post is not sticking out the bottom of the sled.
- Place stage in the **HOME** position  , and select **GO TO HOME** (X and Y positions should be 12.5)



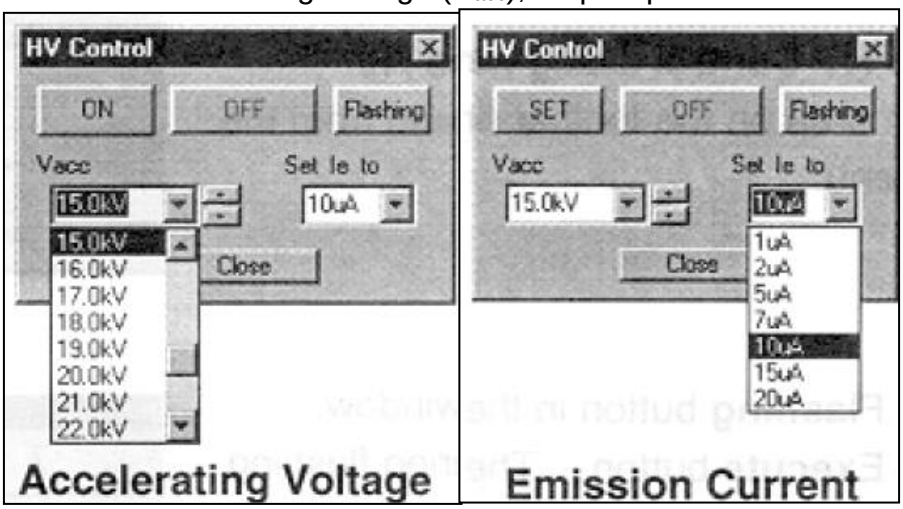
- On panel under **CHAMBER**, verify the **SC/SEC** switch is set to **SEC** and depress the unlit **AIR** button
- The sample exchange chamber (SEC) will vent and open within 5-10 seconds. Open the door gently (don't use the rod to open the door)
- While holding door, push rod in gently to unlock it. Screw specimen sled onto rod and orient upright. Lock rod into place by pulling all the way out.
- Close door and depress unlit **EVAC** button.
- Wait until the top (High) lamp on the SEC Vac is lit and the SEC/Pi reads $4x10^0$ or less – this should not take more than a minute.
- Open exchange valve lever (**MV1**) using one finger.
- Using rod, push specimen sled into chamber while watching on the CRT.
- Engage the sled in the stage, and slide sled until docked into the holder fork. Once in securely, unscrew rod from sled. Retract rod all the way back to lock position.

(**NOTE**: get help from a staff member if having issues getting your specimen docked)

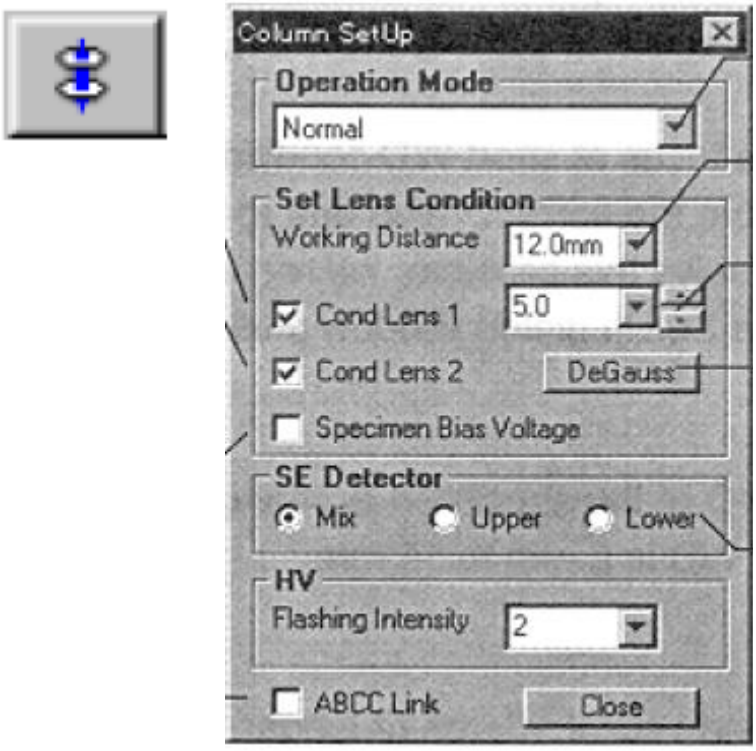
- Close exchange valve lever (**MV1**).

General Operations: **Operating conditions**

1. 5 kV = Accelerating Voltage (V_{acc}), 10 µAmps = Emission Current (Ie)



2. Normal Operation Mode, 12 mm = Working Distance, 8 = Condenser lens 1 setting (range: 1-16 with higher numbers giving a smaller spot), Mixed = both SE Detector



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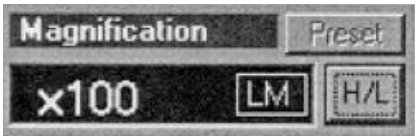
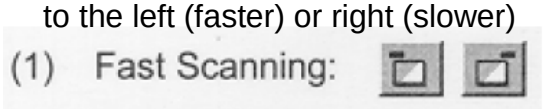
General Operations: **Initial Imaging and Focusing**



1. Ensure low magnification (100x) is selected using the H/L button
2. Acquire an image
    a. There are 8 scanning modes for imaging in the **Scanning Image** Window
        i. Use the "Fast Scan" with the bar to the left (faster) or right (slower)



        ii. Use the "Run/Freeze" to start/stop image acquisition 
2. In very low magnification (20x), identify the location of your specimen using the stage controls
3. Click the **Auto Brightness and Contrast Button** (Alternatively can use knobs on handpanel)

4. Focus
    a. Roughly focus using coarse knob on hand panel.

    *<u>*rule of thumb for focusing*</u>: Double the magnification, focus with "Fine" focus knob, and return to the magnification of interest. The SEM is <u>*parafocal*</u>, whatever is in focus at higher magnifications will also be in focus at lower magnifications unless there is a great difference in topography
5. Select High Magnification Mode with **H/L Button**
    Change to desired magnification (1000x)
6. Adjust focus using knobs
7. Adjust Astigmatism (X and then Y) using the Stig knobs on the control panel
8. Slow scan speed to Slow 1 setting
9. Capture image using 

    To adjust imaging parameters 

    Image may be saved by selecting the 

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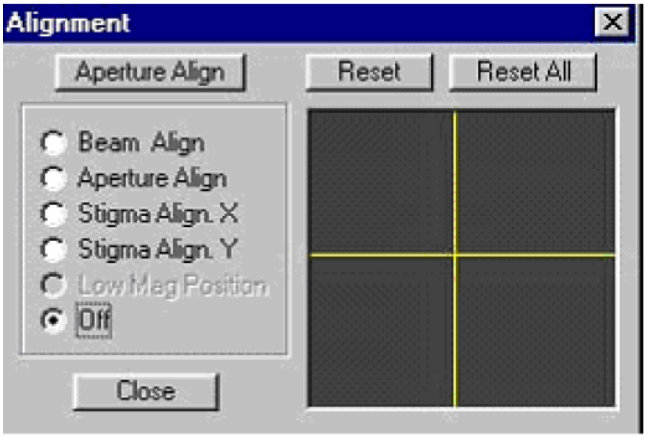
General Operations: **Alignment and Focusing (Iterative process)**

1. Once an image is well-focused and astigmated, alignments should be performed **** any time a working parameter (ie. specimen, working mode, V_{acc}, I_e or working distance) is changed, the alignment sequence should be completed.*

2. Click on the **ALIGNMENT** icon 

3. Select **Beam Align,** a target will Appear, using the Stig/Align knobs Move the circular beam to the center of the "bulls-eye". If beam does not appear, adjust contrast



4. Center and focus a unique point (like an edge that has variations in contrast)

5. Increase magnification to > 50KX. Select **Aperture Align**. Minimize the movement by using X/YStig/Align knobs (you won't be able to completely stop this movement).

**** having minimal movement at this step is CRITICAL for high resolution imaging*

6. Perform again for Stig/Align X & Y, you should be able to completely stop the movement by using X/Y Stig/Align knobs.

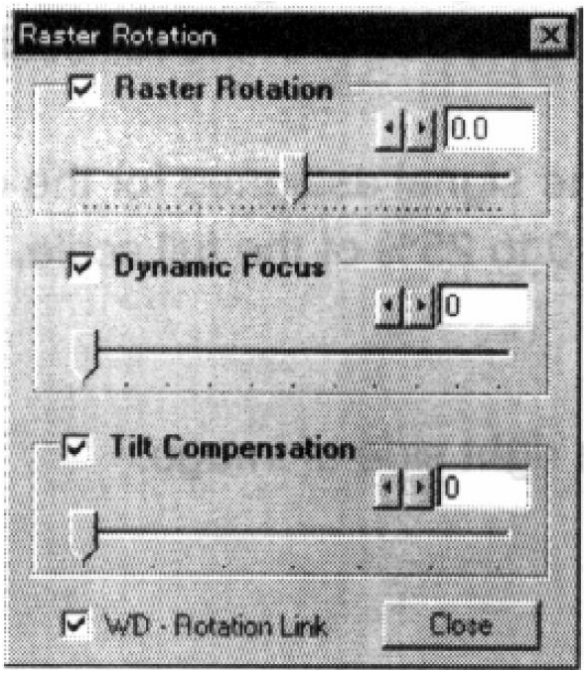
7. Click off and close Alignment window.

8. Refocus and restigmate using X/Y Stig/Align knobs

General Operations: **Mechanical Rotation, Raster Rotation and Tilting/Tilt Compensation**

1. **Mechanically rotate** your stage using the knob on the front-left of the microscope. To reset, place both counters back to zero (*necessary to take specimen out!).*
   **** how does the secondary electron signal coming from the sample change upon rotation?*

2. **Raster rotation** is available under the OPERATE menu
   Enable raster rotation by checking the box, and change the rotation angle with the slider.
   **** you can use this to change the orientation of the image.*
   Uncheck raster rotation when you are finished



3. **Tilt** your sample by turning the knob "(/)" at the front right of the instrument. The angle of tilt is shown on the top scale of the knurled knob. Watch your sample in the chamber monitor as you turn the knob

4. **Tilt compensation** compensates for changes in focus across the surface of the tilted specimen. Enable tilt compensation by checking the box, and change the angle with the slider. Uncheck tilt compensation when you are done

***Imaging processing and analysis*: Calibration and Measurements**

ImageJ is a free shareware program developed at NIH. It is now a JAVA based program and can be downloaded at http://rsb.info.nih.gov. Then go to the Image J page, then Downloads, and then select your platform. (for Windows w/Java). This is an excellent all-around image processing and analysis program that is widely used and supported.

1. Calibration using Image J.
    1. Open ImageJ.
    2. Open one of your images
    3. Calibrate the image by
        i. Select the STRAIGHT LINE in the TOOLS window
            1. **Note:** hold the shift key down to draw straight horizontal or vertical lines
        ii. Draw a line over the scale bar in the information field.
        iii. Go to SET SCALE under the ANALYZE menu
            1. In the UNITS window, type in the units, i.e. micrometers
            2. Type in KNOWN Distance (the number below the scale bar) i.e., 100
    4. Repeat for each image.
2. Linear Measurements
    1. Select the SOLID LINE in the TOOLS window.
    2. Start at one point and while holding the mouse, drag it to the end point. **Do not release the mouse!**
    3. The information (line length) appears in the TOOL Window
    4. Repeat for more measurements
3. Area Measurements
    1. Select the RECTANGLE tool
    2. Draw a rectangle over the area of interest.
    3. Select SET MEASUREMENTS under the ANALYZE menu.
        i. Select AREA; deselect others.
    4. Select MEASURE under the ANALYZE menu.
        i. A RESULTS box will appear with the measurements.
4. Percentage Measurements
    1. With the RECTANGULAR tool, select the entire image except the information field
    2. Make a Binary image by
        i. Select BINARY -> MAKE BINARY under the PROCESS menu.
    3. Select SET MEASUREMENTS under the ANALYZE menu.
        i. Select AREA FRACTION; deselect others.
    4. Select MEASURE under the ANALYZE menu.
    5. A RESULTS box will appear with the measurements, (% black).