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In the Event of an Emergency:
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FEI Tecnai G^2 F20 200 kV Cryo-S/TEM
Simplified Operating Manual

Safety

- **In the event of an audible alarm from the SF_6 or O_2 detector, you must EXIT the room IMMEDIATELY. Do NOT re-enter the room until a manual sweep of the room has been performed by FEMR staff.**
- Do not wander behind the microscope or step on cables.

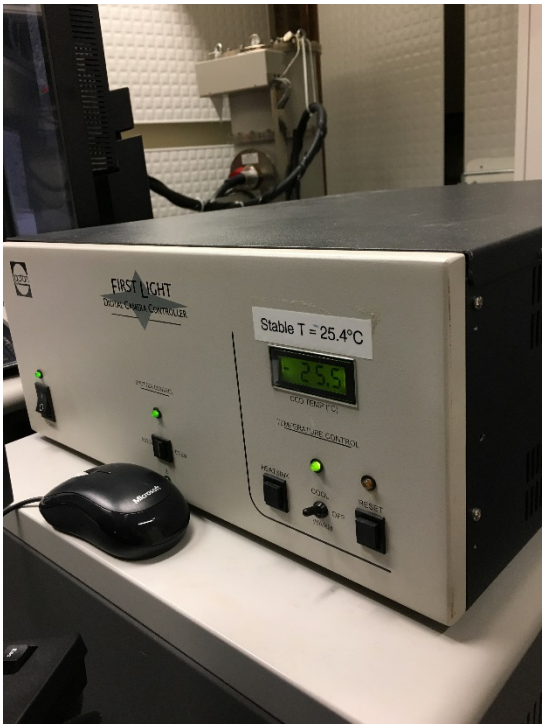
I. Microscope start up and visual inspection

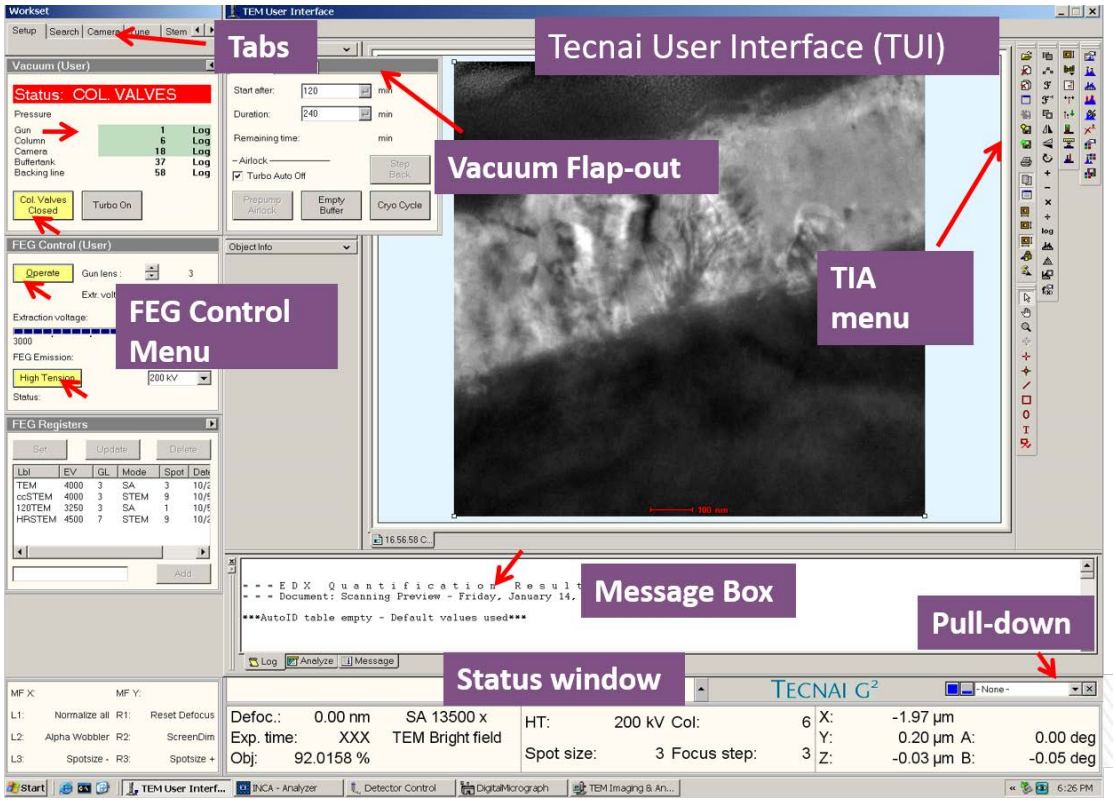
a. SIGN the LOGBOOK

b. On the control panel to the right of the column, VERIFY the RED OFF, WHITE VAC. and HT buttons are lit. If not, request assistance of staff.

c. On the Gatan Digital Camera Controller, VERIFY the temperature of the CCD camera is -25.4 ˚C or lower. If not, do NOT use the CCD camera and request assistance of FEMR staff.

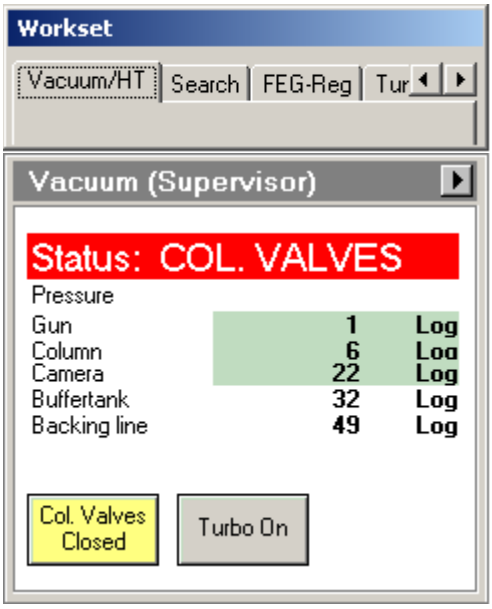
d. Log into the PC with the username and password created during your training session. After logging in, confirm the microscope icon on the right side of task bar (bottom) is present and green. If not, contact FEMR staff before proceeding further. Do NOT attempt to reboot the microscope PC.





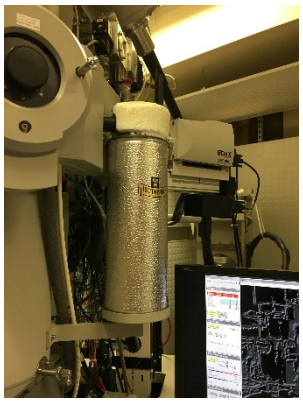
e. Launch the Digital Micrograph (DM), Tecnai Imaging and Analysis (TIA), and Tecnai User Interface (TUI) programs by clicking on the icons located either on the bottom left of taskbar or upper right corner of the left monitor.

f. Under the Workset, SELECT Vacuum/HT tab. On the Vacuum (Supervisor) window, VERIFY the microscope states STATUS: COL. VALVES" and that the "Col. Valves Closed" button is yellow (buttons appear yellow when active).



g. Fill the dewar flask (anti-contaminator cold trap on right side of column) with liquid nitrogen. Ensure the black rubber mat covers the viewing screen when pouring the liquid nitrogen. The first dewar flask of the day should last ~30–40 minutes. Later dewars will each last 2–3 hours. **Note:** Do NOT allow the cold finger to fall below 50% or to warm up as the column vacuum will deteriorate significantly.

h. If performing cryo-EM, also fill the anti-contaminator dewar on the retractable liquid-nitrogen cooled Gatan cryo-blades. It shields the specimen from residual gases so the rate of ice growth rate is reduced on cold specimens inside the microscope.



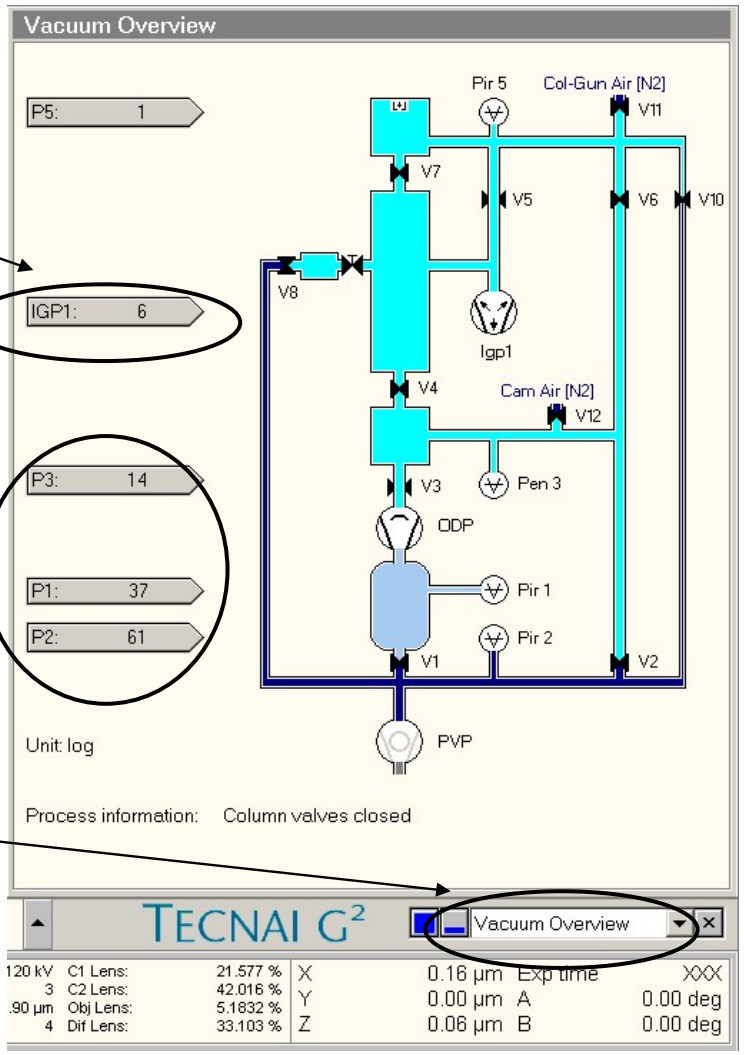
i. OPEN the Vacuum Overview window by SELECTING it from the Pull-Down box in the bottom right corner of the screen. VERIFY that IGP1 (Gun) reads ~15 Log or better (normally is 6 Log).

IGP1 indicates the vacuum level in the column and gun chamber. It should read 6 when the cold finger is chilled.

P3 indicates the camera vacuum level. It should read 25.

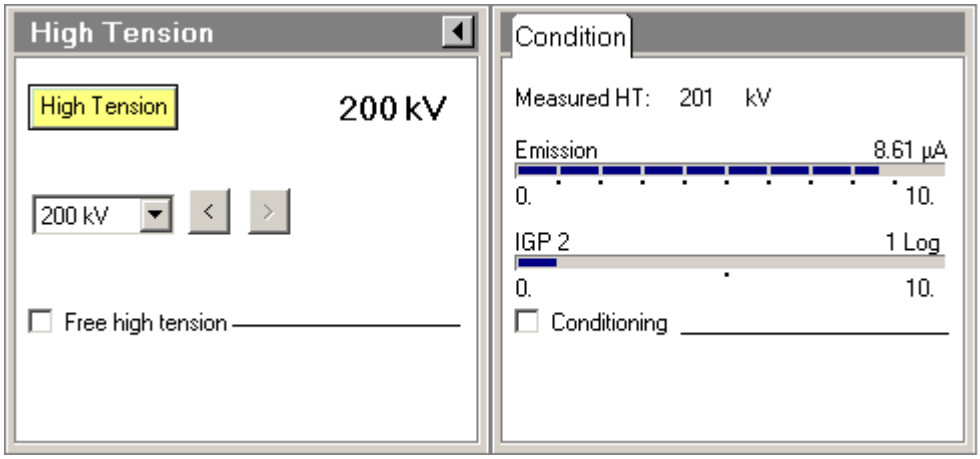
P1 indicates the pressure in the buffer tank, and P2 the pressure in the backing pump line.

This dialog box is used to select the vacuum overview (or any of several other information or setup tabs).

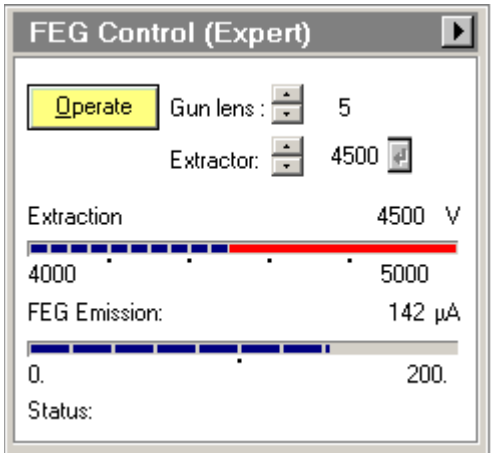


II. Accelerating Voltage

a. On the High-Tension window, VERIFY the HIGH TENSION button is yellow and the Measured Voltage is reading 201 kV.



b. On the FEG Control (Expert) window, VERIFY the OPERATE button is yellow and the EXTRACTION VOLTAGE is 4500 V.

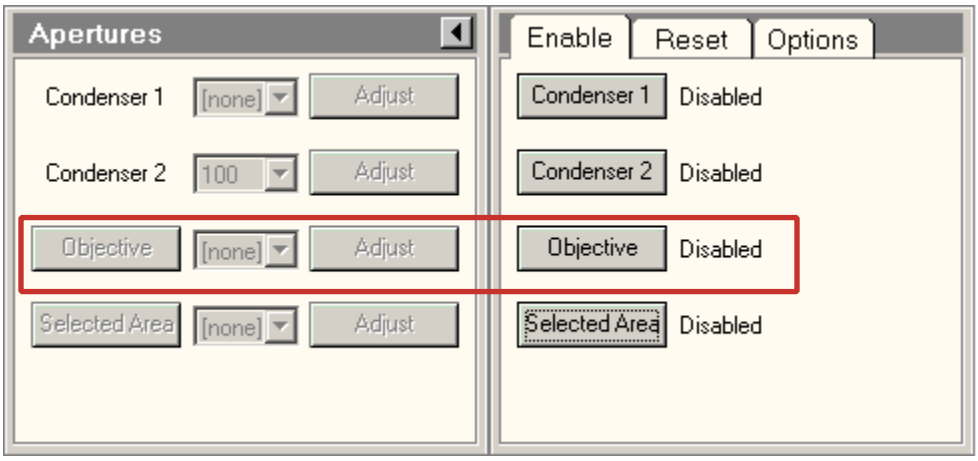


III. Specimen Loading and Holder Insertion/Removal

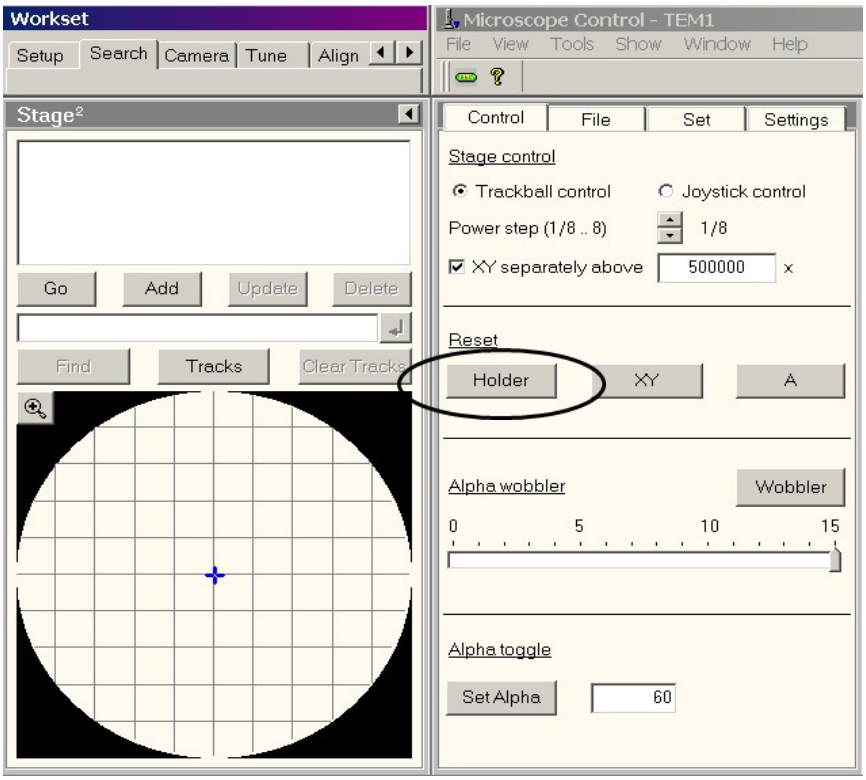
Note: the specimen holder, airlock, and CompuStage are delicate, precisely machined components. N**ever exert significant force during any step of this procedure. Doing so may result in serious damage to the instrument or holder.**

a. Before inserting or removing the sample holder, on the Workset, VERIFY the microscope reads STATUS: COL. VALVES and that the Col. Valves Closed button is yellow (buttons appear yellow when active).

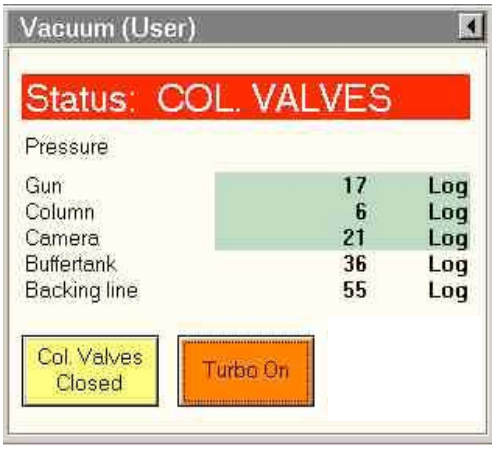
b. Under the Workset, CHOOSE the Tune tab and on the Aperture window, VERIFY the Objective Aperture is retracted (i.e. disabled).



c. CHOOSE the SEARCH tab on the Workset, CLICK the Arrow on the Stage flap-out window and PRESS the Holder button below Reset.



d. PRESS the TURBO ON button (turns fron gray to orange).



e. Always keep light pressure on the purple goniometer surface when removing the sample holder. PULL the sample holder straight back without rotating until it stops moving.

f. ROTATE the sample holder CLOCKWISE until it stops. This rotation moves the AIRLOCK PIN approximately from the 12 o'clock position to 5 o'clock.

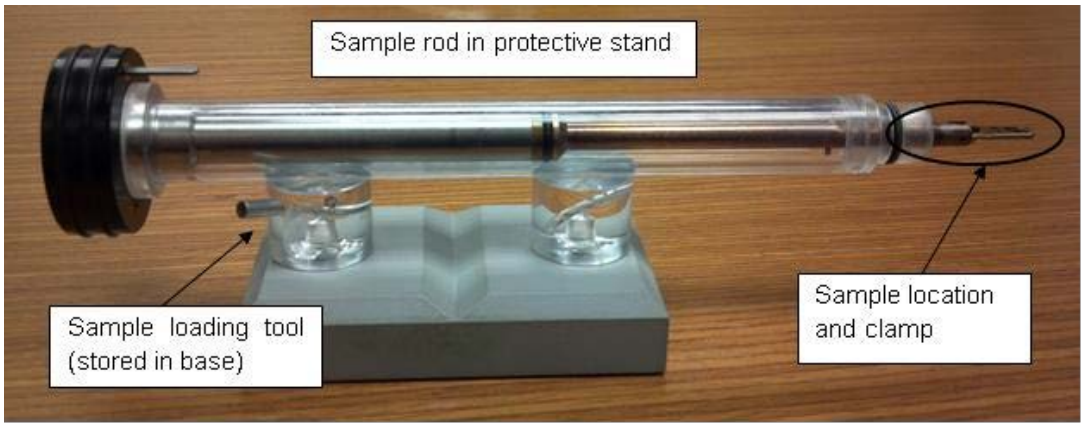
g. Gently, while keeping pressure on the goniometer, PULL the sample holder back to break the airlock vacuum. This will require a small amount of force.

h. REMOVE the sample holder straight back and out of the column while being careful NOT to scrape it along the inside of the airlock. Be careful NOT to touch the holder O-ring or any part beyond it with bare hands.

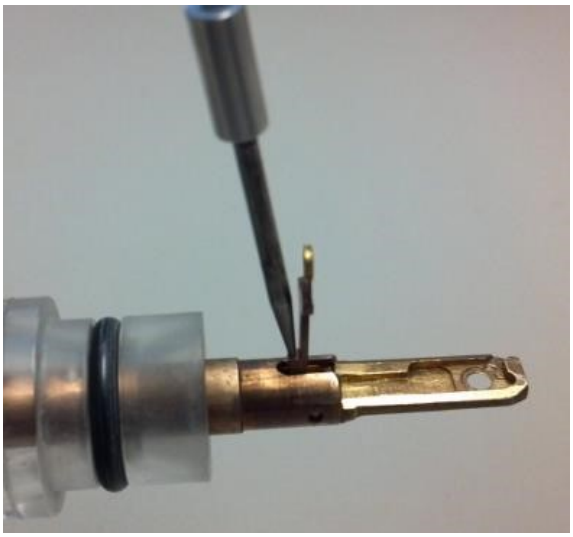
IV. Loading Specimens onto the Room Temperature Sample Rod (See Protocol for Loading the Gatan Cryo-holder Model 626).

Note: Never mount magnetic specimen discs in the clamp holder. The clamp spring is not strong enough to prevent the specimen from attaching to the objective lens polepiece.

a) Place the sample holder in the protective stand.
b) Remove the sample loading tool from the base of the stand.



a. Using one hand to prevent the holder from slipping out of the stand, INSERT the loading tool into the hole in the specimen clamp and GENTLY RAISE the clamp straight up until it stops. NEVER use tweezers.



b. Place the specimen grid into the recess at the end of the holder.

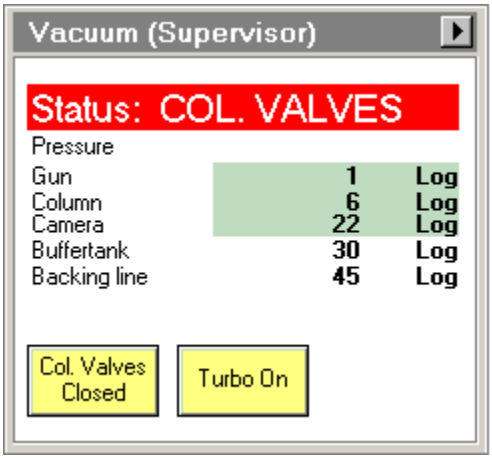
c. GENTLY LOWER the clamp straight down to hold the grid securely. Return the tool to the base of the holder stand.

d. Retract the holder slightly and turn it upside down. Tap the back end several times, then turn the holder upright and check that the grid has not moved (movement suggests the grid is not properly secured).

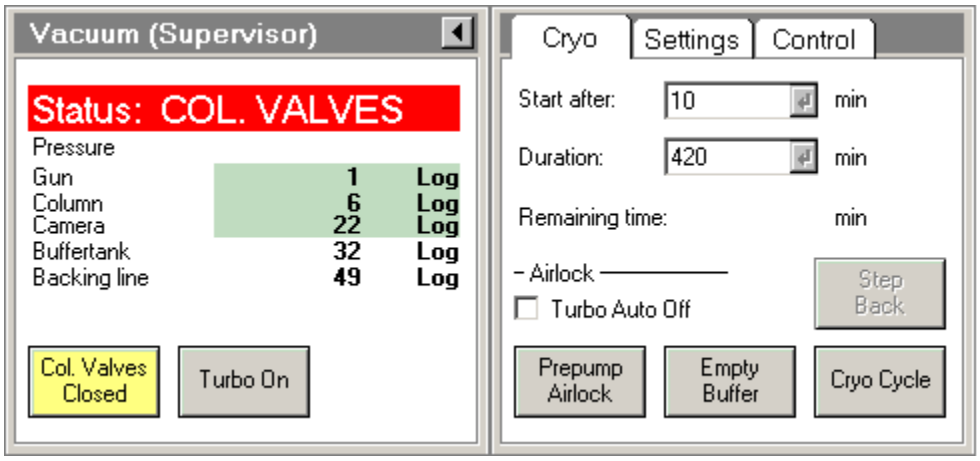
e. Use the microscope to inspect the holder O-ring for debris. Gently remove any debris using a sheet of Kimwipe.

V. Inserting the Sample Holder

a. WAIT until the Turbo On button has turned from orange to yellow before attempting to insert the sample rod



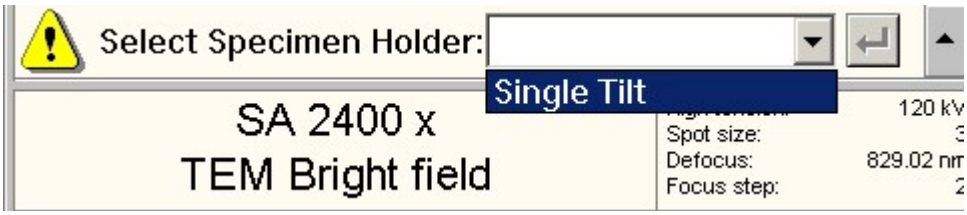
b. Just BEFORE inserting sample, on the Cryo tab, PRESS the Prepump Airlock button and wait for the time countdown to reach zero.



c. Carefully ALIGN the Airlock (Sample Holder) Pin on the sample holder with the 5 o'clock position on the goniometer and gently INSERT the sample holder until it stops. Be careful not to scrape the tip on the inner mechanisms of the goniometer. You should feel some resistance as the holder O-ring seats in the airlock chamber.

d. Once the sample holder has been inserted, the Red LED on the Goniometer will light and the pumping Countdown will begin for 60 seconds in the Vacuum overview window. Do NOT move the holder during this period.

e. The Message Window will pop-up to Select Specimen Holder. Using the dropdown arrow, SELECT the Single Tilt holder. PRESS the Enter button to confirm selection.

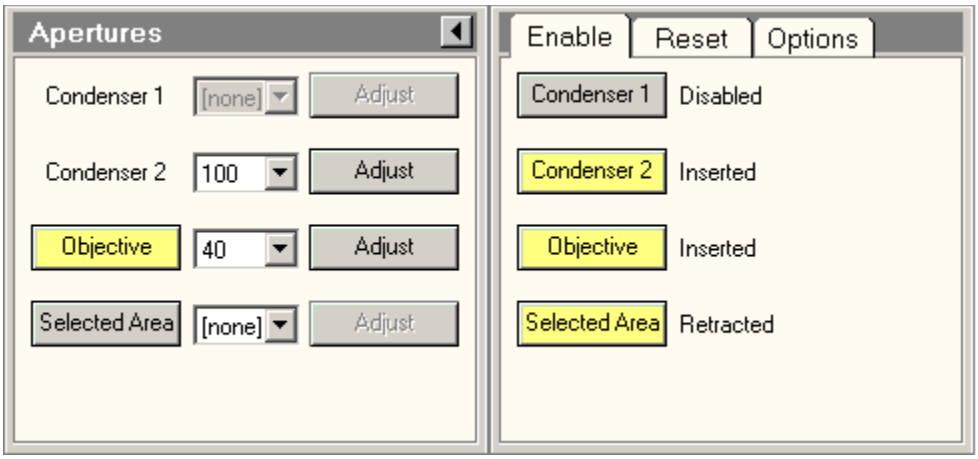


f. When the timer reaches zero (status reads “COL. VALVES”) and the RED STAGE LED goes out, support the purple goniometer surface with one hand and GRIP the SAMPLE HOLDER securely with the other. Slowly ROTATE the sample holder COUNTERCLOCKWISE from the 5 o’clock to 12 o’clock position.

g. GENTLY allow the sample holder to SLIDE into the microscope column until it stops. TAP the end of the sample holder to VERIFY it is securely seated.

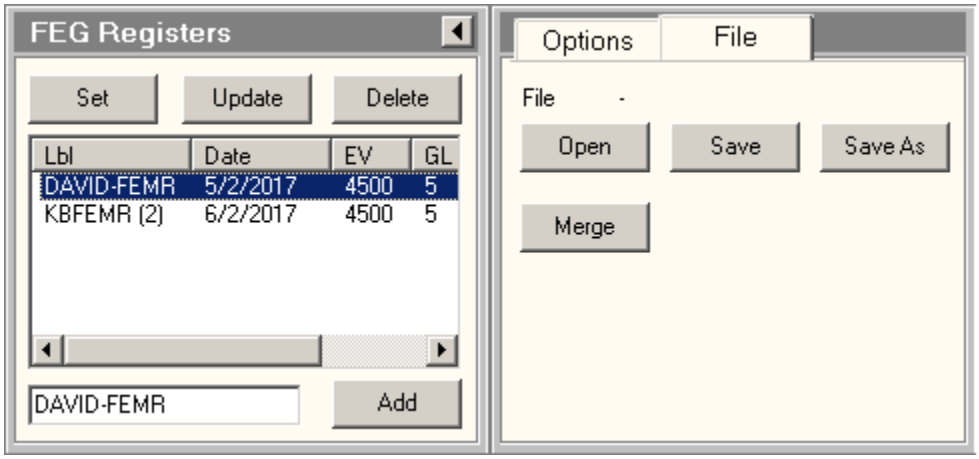
h. PRESS the yellow Turbo On button to turn it off (button turns grey).

i. VERIFY the apertures inserted are correctly inserted. Condenser aperture 1 is DISABLED. Condenser 2 aperture size should normally be 100 µm unless using EDAX EDS. Unless you are carrying out diffraction, the Selected Area Electron Diffraction (SAED) aperture (lower) should be retracted. **Note:** Always INSERT apertures one at a time with the beam ON in case an aperture blocks the beam (see below).

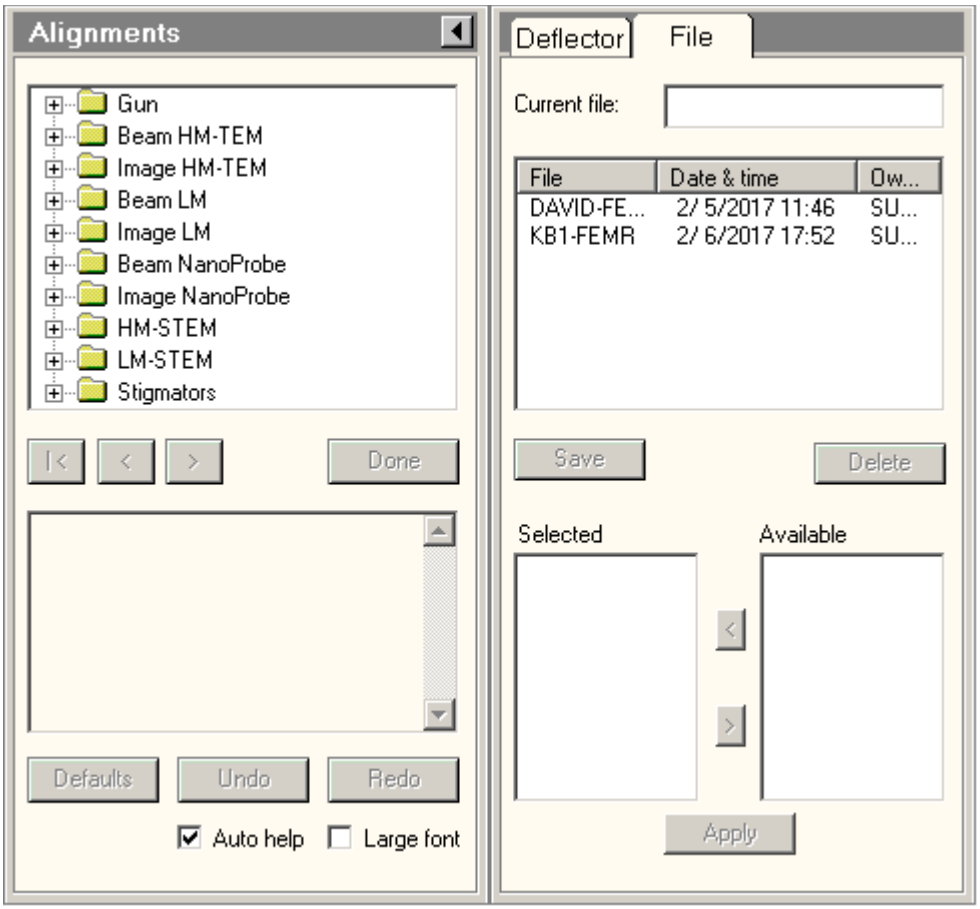


VI. Load the Alignment and FEG Register

a. In the Workset, SELECT the FEG_REG tab. PRESS the Arrow to open the FEG Register flap-out window. HIGHLIGHT the KBFEMR (2) file, PRESS the Open button and then PRESS the Set button.



b. SELECT the Alignments tb, PRESS the Arrow to open flapout window, and SELECT the File tab. CHOOSE the File named KB1-FEMR, SELECT and HIGHLIGHT all the options in the Available Box, PRESS the Arrow to bring all the options to the Selected Box, and PRESS Apply.



VII. Adjust Eucentric Height

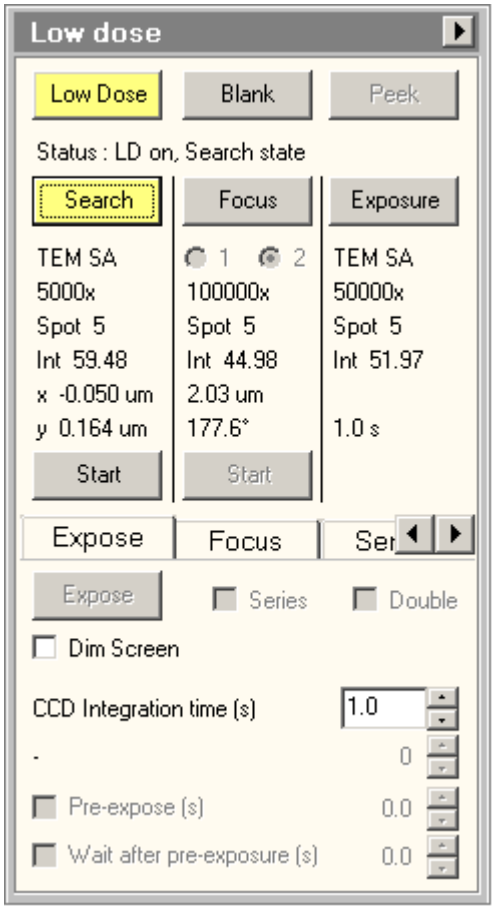
a. At a low magnification setting (, find a recognizable feature in your samples such as a particle fragment or small hole and bring it to the centre. PRESS the Eucentric Focus button on the Right-Hand Control Pad.

b. PRESS the L3 button on Left Hand Control Pad to START the Alpha Wobbler. The CompuStage will start to rock +/- 15° automatically. Press the + or – Z-axis buttons on the Right-Hand Control Panel to minimize the movement of the feature.

VIII. Low Dose (Option)

a. VERIFY IPG1 is 15 Log or less (normally is 6 Log).

b. In the Workset, SELECT the Low Dose tab and PRESS the Low Dose button (turns yellow) to use this option.



c. SET UP the different modes in Low Dose. VERIFY the settings for Search, Focus and Exposure as shown in the image. The CCD integration time should be one second.

d. CYCLE through Search, Focus and Exposure and VERIFY the electron beam is present. It is necessary to WAIT ~ 20 seconds between modes to observe the beam.

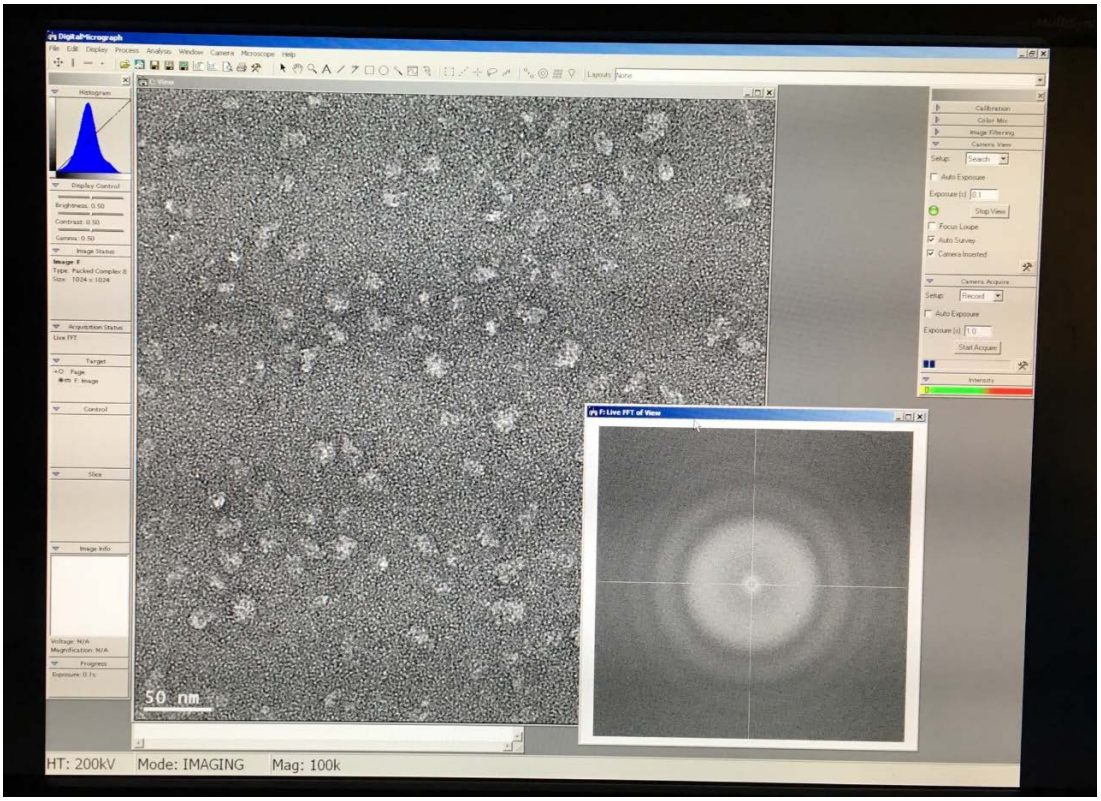
e. If the beam is not visible in Focus or Exposure modes, DECREASE magnification until the beam is observed. CENTRE the beam with the tracking ball on left hand panel, then return to previous magnification.

f. SELECT Exposure and PRESS the Eucentric Focus button.

g. SELECT the Search position and Enter a magnification of 5000x. In the Workset, CHOOSE the Search tab, PRESS the Arrow button to open the flap-out window, PRESS the Wobbler button (turns yellow), and adjust the Eucentric Height with the + and – buttons of the Z-Axis on the Right-Hand Control Pad.

h. PRESS the R2 button on the Right-Hand Control Pad to RESET the defocus value. SELECT the Focus mode and locate the zero defocus by using the binoculars or onscreen with Digital Micrograph (DM).

i. If using DM, VERIFY in Focus mode and SELECT Camera Inserted. ENTER Exposure Time as 0.1 second. MAXIMIZE the DM window and PRESS Start View. SELECT the PROCESS TAB on the DM window and SELECT Live FFT to observe the Thon Rings.



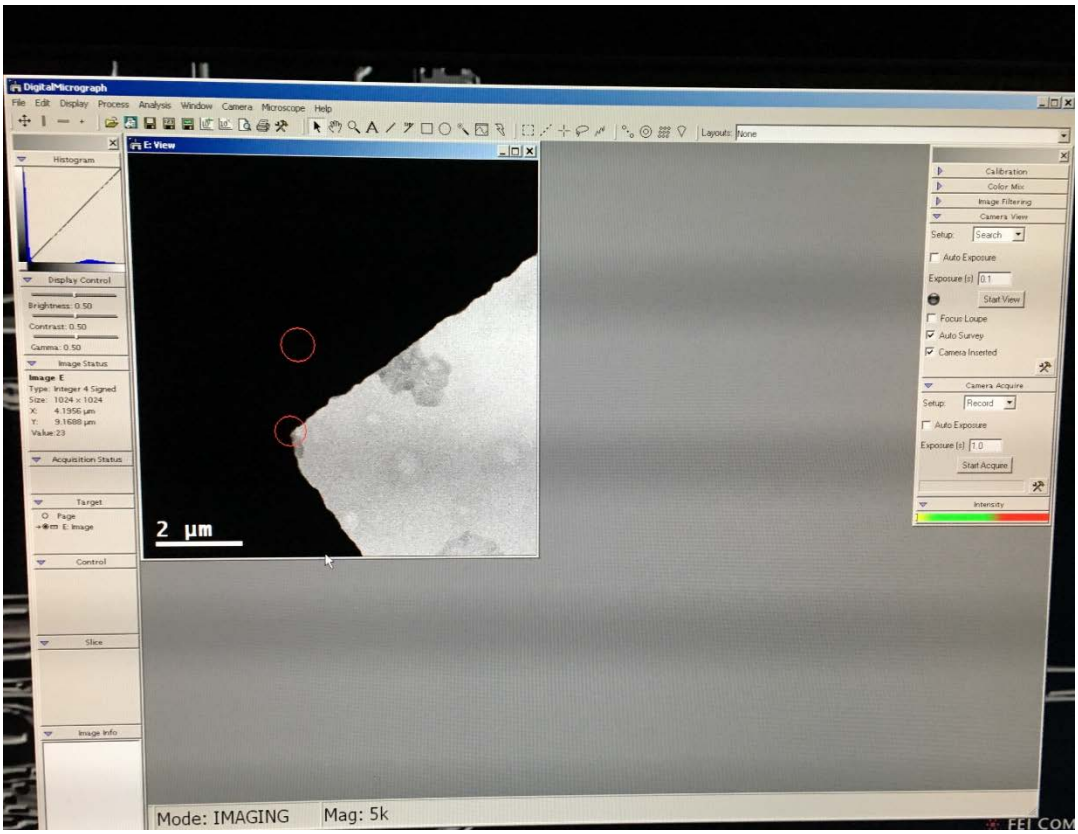
IX. Aligned exposure area with search area (Overlapping areas)

a. SELECT the Low Dose tab, go to Exposure and place the field of view in the corner of a square.

b. Go to Search and using the Multifunction X and Y buttons, move the corner of the square to the center of the screen and make a circle with the circle tool.

c. Go to Focus and move the stage until you find the same corner of the square and place it in the centre of the field of view. You may need to lower the magnification until you see the corner. Move the stage to the corner and bring the magnification back to 100,000x.

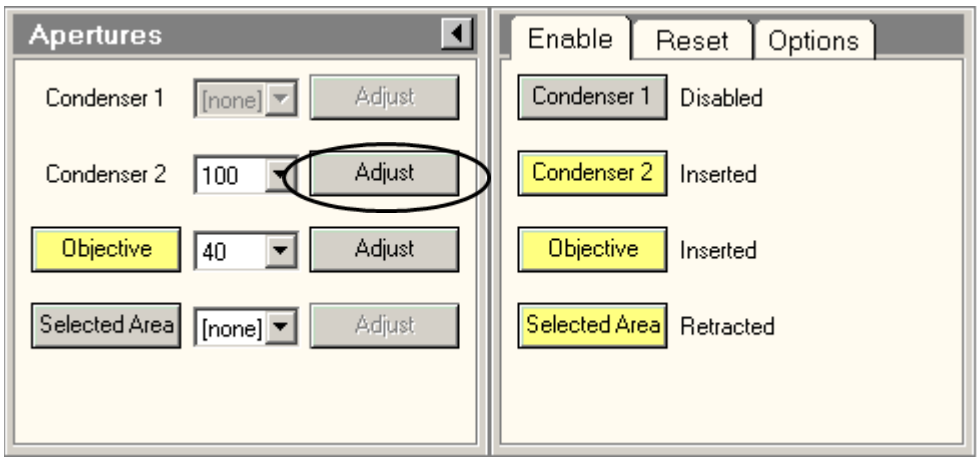
d. Go to search and without moving the stage make a second circle with the circle tool where the corner of the grid is located now.



X. Center condenser aperture

a. VERIFY you are in Low Dose Exposure mode.

b. To center the condenser aperture, use the Intensity button to confirm that both sides of the beam crossover illuminate the same area. If they don't, then under the Workset Tune tab /Apertures window and in the Apertures window, PRESS the Adjust button (turns yellow) on the Condenser 2 aperture, and centre the beam using the Multifunction X and Y knobs.



XI. Adjust Condenser Astigmatism

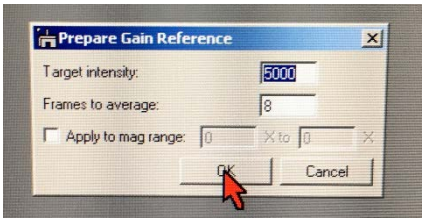
a. Use the Intensity button on the Left-Hand Control Panel to focus C2 and observe the spot shape. If the spot becomes oval when out of focus, under the Workset Tune tab/Stigmators, PRESS the Condenser button (turns yellow), and use the Multifunction X and Y buttons to make the illumination spot round while checking the focus. PRESS the None button to DESELECT the Condenser button.

XII. Adjust Objective Astigmatism

a. Verify Low Dose Exposure mode.
b. MOVE to an area on the TEM grid with a thin layer of amorphous carbon film at around 100,000x magnification and using the Focus Knob adjust to slightly UNDERFOCUS or near focus (one or two Thon Rings).
c. SELECT Objective Stigmator button (turns yellow).
d. SPREAD the beam using the Intensity Knob on the Left-Hand Control Pad. PRESS R3 to Lift Screen and PRESS Start View on DM to starting imaging with the CCD camera.
e. On the DM window, CHOOSE the Process tab and SELECT Live FFT. USE the Multifunction X and Y buttons to make the Thon Rings in the FFT image appear circular. It is imperative that the original image is square otherwise the rings will appear elliptical, even when astigmatism is not present.
f. PRESS the None button to DESELECT the Objective button.

XIII. Prepare a Gain Reference Image with the CCD Camera

a. SELECT the Low Dose tab, GO to Search and move the sample to an empty square.
b. SELECT Exposure, spread the beam with the Intensity Knob until it fills the phosphorus screen.
c. PRESS R3 to lift the screen.
d. In the DM program, CHOOSE the Camera tab and SELECT Prepare Gain Reference. CLICK On and follow on the on-screen instructions with the settings in the image.



XIV. Collecting Images with the CCD Camera in Low Dose Mode

a. SELECT the Low Dose tab, GO to Search and PLACE the circle aligned with the exposure area in the region of interest.

b. SWITCH to Focus, find your zero focus. PRESS R2 to reset the defocus value and APPLY the desired defocus with the defocus knob.
c. PRESS the Exposure button on the Low Dose interface and the CCD will take the image.

XV. Saving Images

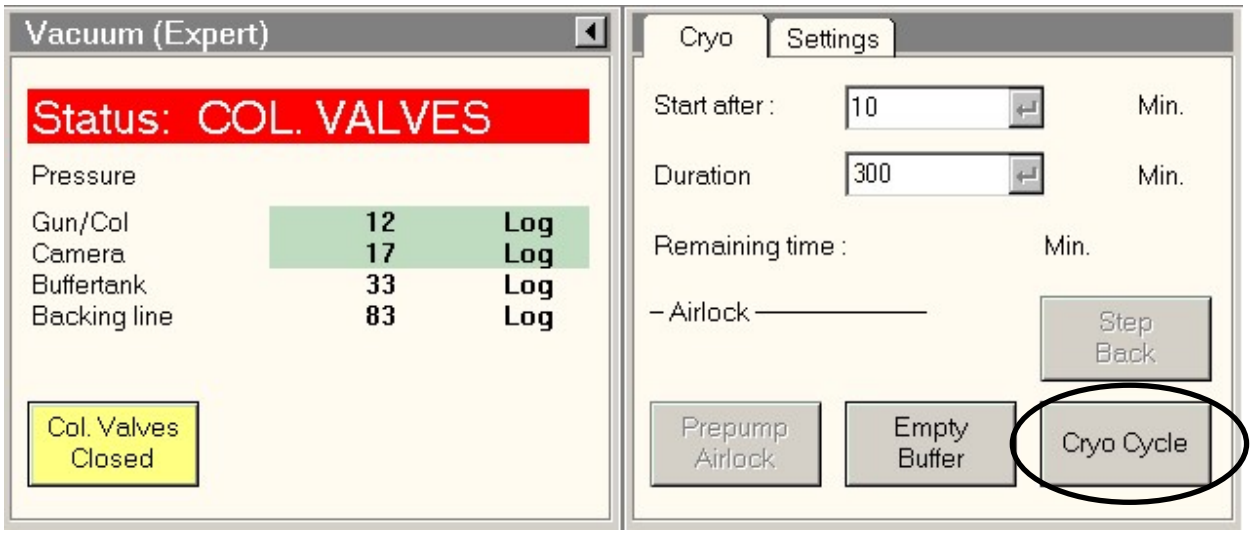
a. In DM, CHOOSE the File tab and SELECT Save As.
b. CHOOSE the Z: drive (192.168.1.2) as the location for saving images.
c. CREATE a folder under you name. NAVIGATE to your folder and SAVE your images with the preferred name. Note: Folders/Files are deleted after three months.

XVI. Shutting Down the Microscope

a. EXIT Low Dose and return magnification to 4400x.
b. SPREAD the beam.
c. RESET the stage.
d. CLOSE the column valves and holder shutter (if you are using the cryo-holder).
e. PRESS the Turbo On button (turns orange) and until the button turns yellow.
f. RETRACT the CCD camera.
g. REMOVE the RT holder or cryoholder.
h. Follow the protocol for the cryo-holder the rod of the cryoholder to pump and do the warm up cycle.
i. If you are using the RT holder, remove the grid from holder.
j. INSERT the room temperature sample holder into the column of the microscope.
k. Check the Scheduler to determine if you are the last person operating the microscope for the day. If so, you MUST start the cryo cycle. Otherwise, proceed to the next step.
l. Cryo cycle procedure
    i. REMOVE the liquid nitrogen dewar from the anti-contaminator cold trap.
    ii. POUR the remaining LN2 into the white dewar.

iii. CHOOSE the Vacuum/HT tab and on the VACUUM window, CLICK the Arrow to open the flap-out window. VERIFY the Settings for the cryo-cycle are Start After **10** Minutes and Duration of **420** minutes.

iv. PRESS the Cryo Cycle On button (turns yellow). Do not leave the room until the cryo-cycle has started.



XVII. End of Session

a. CLOSE Digital Micrograph and Tecnai User Interface.
b. LOGOUT from your account.
c. SIGN the Logbook.