Emergency Information:

1. **Medical Emergencies: Contact 911 and McGill Security 514.398.3000**
2. **Leave TEM as is. Do NOT shut down the vacuum system.**
3. **If possible, turn off High Tension and Close Column Valve.**
4. **Exit the Room/Building.**

Emergency Contact Information:

- **Jeannie Mui – EM Coordinator: Office 514.398.6329; Email: Jeannie.mui@mcgill.ca**
- **S. Kelly Sears – Facility Manager: Cell 514.576.1926; Email: kelly.sears@mcgill.ca**
- **Joaquin Ortega – Director: Office 514.398.6348; joaquin.ortega@mcgill.ca**

FEI Tecnai 12 ('T12') 120 kV TEM

Simplified Operating Manual

(Prepared by S Kelly Sears and Joaquin Ortega)

Safety

- Do NOT wander behind the microscope or step on cables.
- Do NOT touch the sample rod with bare hands below the black O-ring.
- There are two computers on the T12 TEM:
  1. Microscope PC (MPC) that controls the TEM
     - Note: Under no circumstances should a user insert a USB device into the MPC.
  2. Support PC (SPC) to store and retrieve images (on the desk to the left of the TEM).

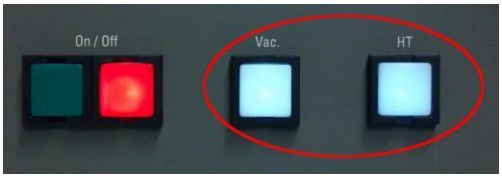
Protective Equipment

- Nitrile gloves for handling sample holder and safety glasses for filling liquid nitrogen dewar.

I. Microscope Start Up

Note: Your PC desktop may not appear exactly as shown in the images in this manual.

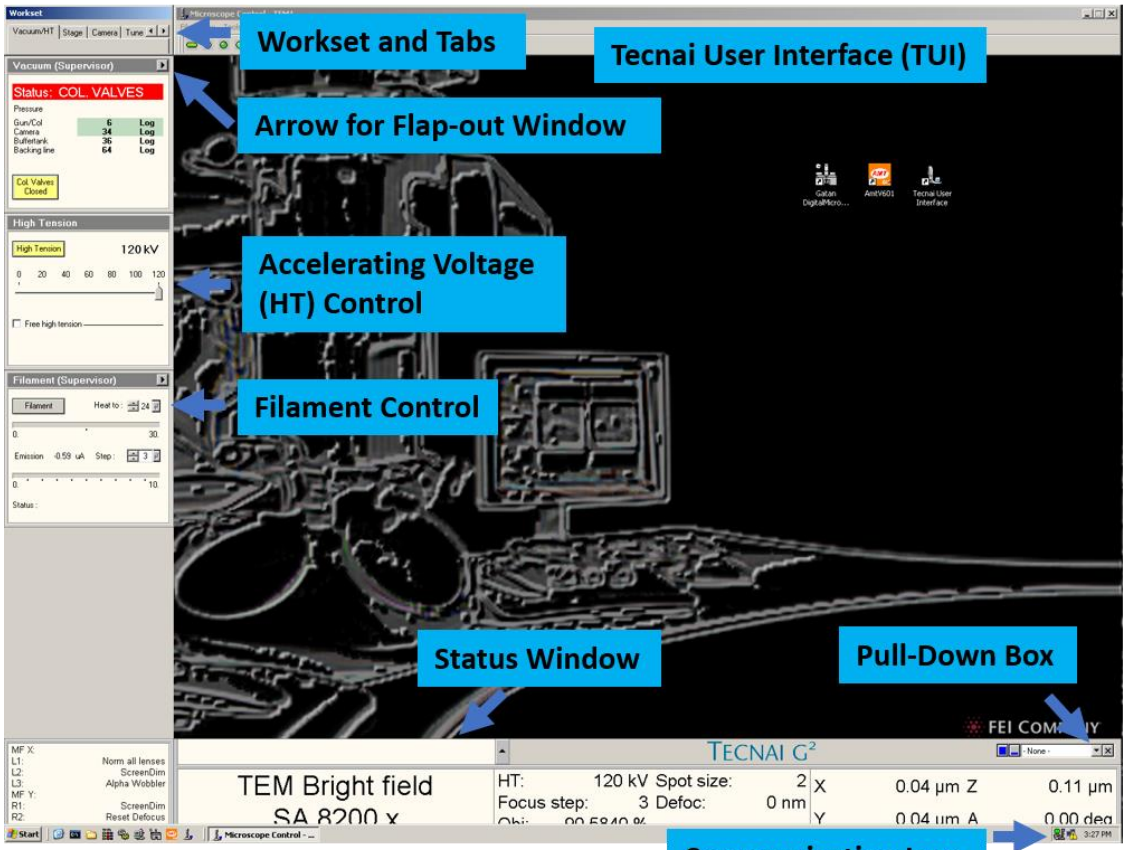
a. VERIFY the Red On/Off button and the White Vac. and HT buttons on the right console are lit (currently NOT WORKING).



b. LOG IN to the MPC with the username and password created during your training session. After logging in, VERIFY the microscope icon on the right side of the bottom task bar is present and green. If not, contact FEMR staff before proceeding further. Do NOT attempt to reboot the MPC as it will turn off the microscope.

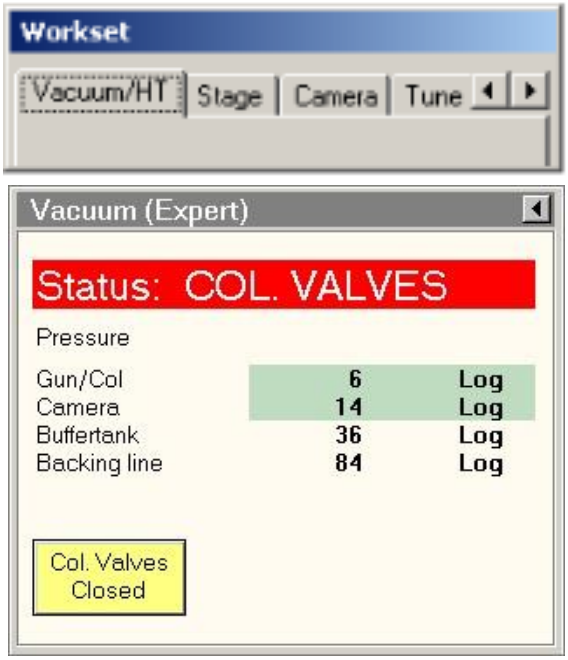
c. PRESS the icons to LAUNCH the Tecnai User Interface (TUI) and AMTV601 (CCD Camera) programs. The TUI will open in the left monitor and the AMTV601 will open on right

monitor. The icons are located on the upper right corner of the desktop or on the left side of the bottom taskbar.



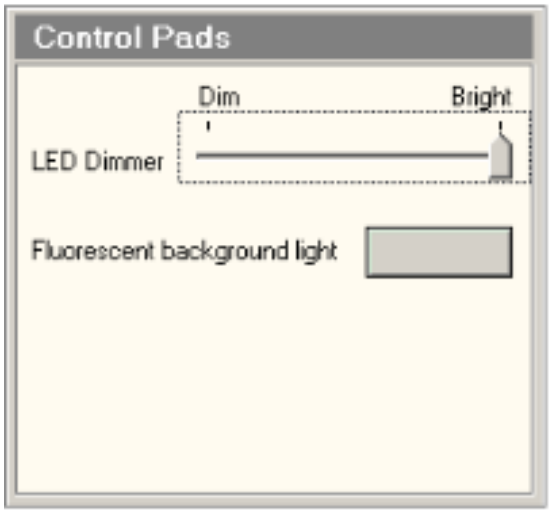
d. On the AMTV601 window, CHANGE the setting from Speedlive (button in red) to Qualitylive by pressing the button once.

e. In the Workset, CHOOSE the Vacuum/HT tab (or Setup) and on the Vacuum (Expert) window, VERIFY the microscope the reads Status: COL. VALVES and the Col. Valves Closed button is Yellow (buttons appear yellow when active).



f. Carefully FILL the anti-contaminator cold finger dewar flask (cold trap) with liquid nitrogen. VERIFY the black rubber mat is covering the viewing window. The first dewar flask of the day should last ~30 to 40 minutes. Later dewars will each last 2 to 3 hours. Do NOT allow the cold finger to fall below 50% or to warm up as the column vacuum will deteriorate significantly.

g. In the Workset, CHOOSE the Vacuum/HT tab and on the Control Pads window, PRESS the Grey button to TURN ON the lights of the Left and Right Control Pads.



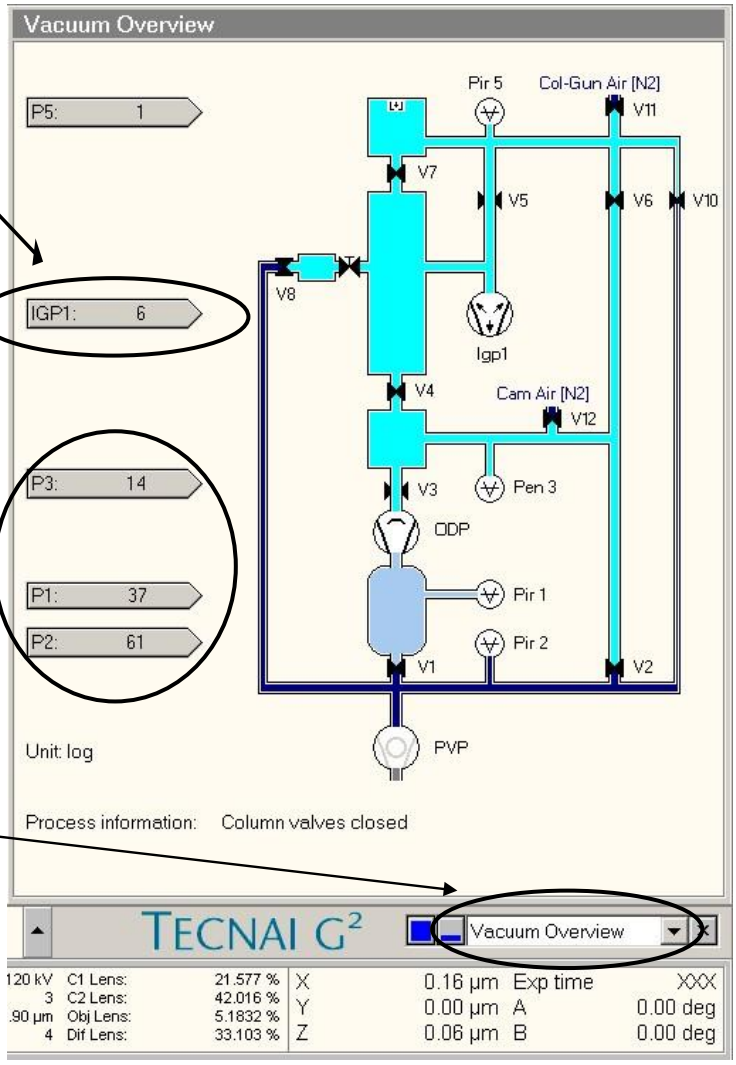
h. OPEN the Dialog Box window by PRESSING the drop-down Arrow from the bottom right corner of the screen and SELECT Vacuum Overview. VERIFY IGP1 is 20 Log or better (normally 6 Log).

IGP1 indicates the vacuum level in the column and gun chamber. It normally reads 6 Log.

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

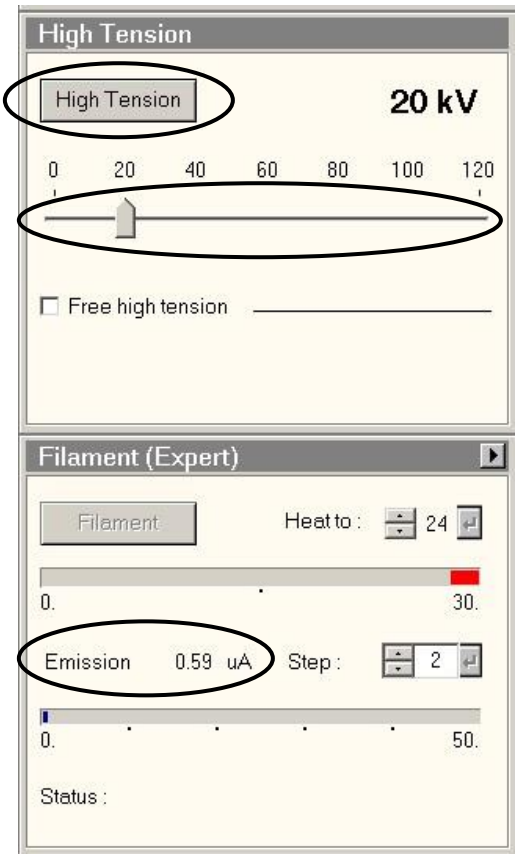
P1 indicates the pressure in the buffer tank, and P2 the pressure in the backing pump line.

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



II. Accelerating Voltage (High Tension)

a. If the High Tension (accelerating voltage) button is Yellow and the displayed value reads 120 kV, proceed to Section III.



b. The High Tension will be turned off (grey) for the first user each day. IGP1 must read <20 before proceeding (see Section I).

c. If the High Tension button is unavailable (grey with faded text), it is likely the HT on the microscope console panel is off. PRESS it once. If the HT button does not come on, request assistance of staff.

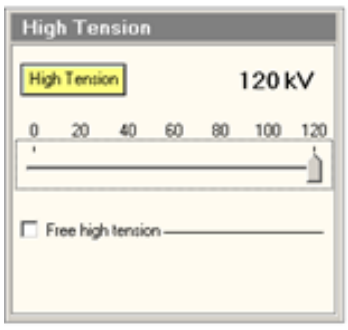
d. SELECT 40 kV with the slider in the High Tension control, then PRESS the High Tension button (turns yellow). The emission current (Emission in the Filament control) will spike and then stabilize to ~0.5–1 µA.

e. WAIT 1–2 minutes before proceeding, and then raise the slider to 60 kV. CONTINUE in 20 kV increments, waiting 1–2 min at each. If the emission current remains high (>1–2 µA), return to the previous step for several minutes.

Note: The HT may switch off and become disabled at 40 kV. If this occurs, simply PRESS the HT button on the console panel to re-enable it, PRESS the High Tension again at 40 kV, and continue.

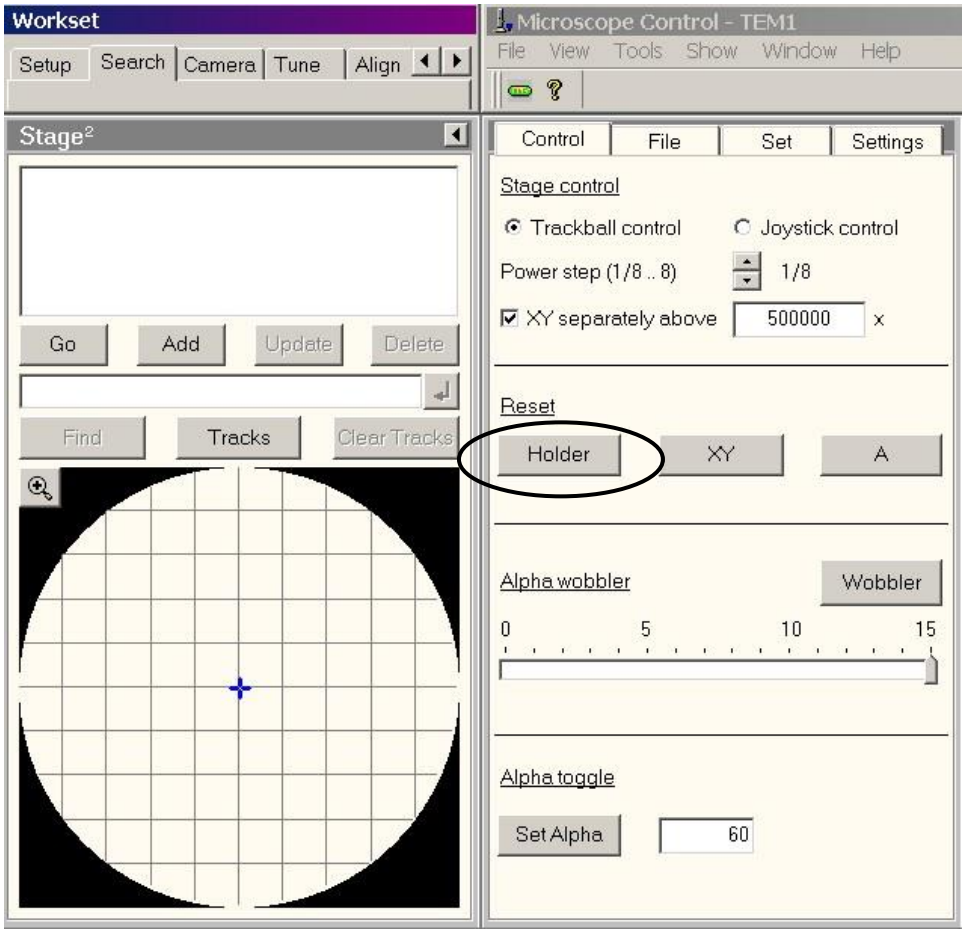
III. Specimen Loading and Holder Insertion/Removal

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. VERIFY the High Tension button is yellow and the slider is at 120 kV.

b. Before inserting or removing the sample holder, VERIFY the column valves are closed, and the holder is RESET. In the Workset, CHOOSE the Search tab, CLICK the Arrow to OPEN the Stage flap-out window, and PRESS Holder button below Reset.



c. Removing the the Sample Holder

i. RESET the sample stage.

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

iii. ROTATE the holder clockwise until it stops. This rotation moves the guide pin approximately from the 12 o'clock position to 5 o'clock.

iv. 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.

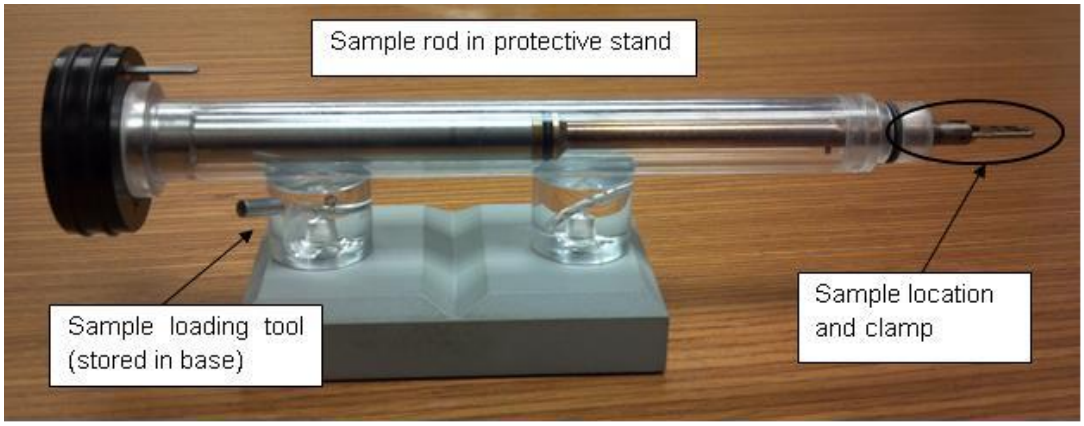
v. Remove the holder straight back out of the column while being careful not to scrape it along the inside of the airlock. Be careful not to touch the holder O-ring or any part past it with bare hands.

d. Loading a Specimen

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. NEVER touch from the O-ring to the tip of the sample holder without wearing nitrile gloves.

i. PLACE the sample holder in the protective stand.

ii. REMOVE the sample loading tool from the base of the stand.

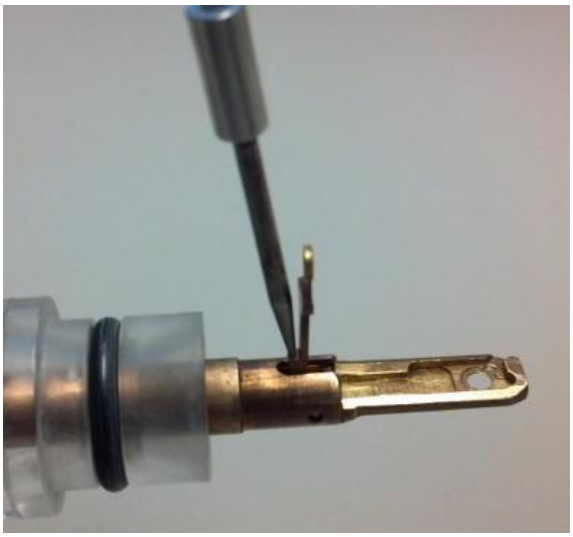


iii. Using one hand to prevent the holder from slipping out of the stand, INSERT the tool into the hole in the specimen clamp and GENTLY RAISE the clamp straight up until it stops.

iv. PLACE the specimen grid into the recess at the end of the holder.

v. Gently LOWER the clamp straight down to hold the grid securely. RETURN the tool to the base of the holder stand.

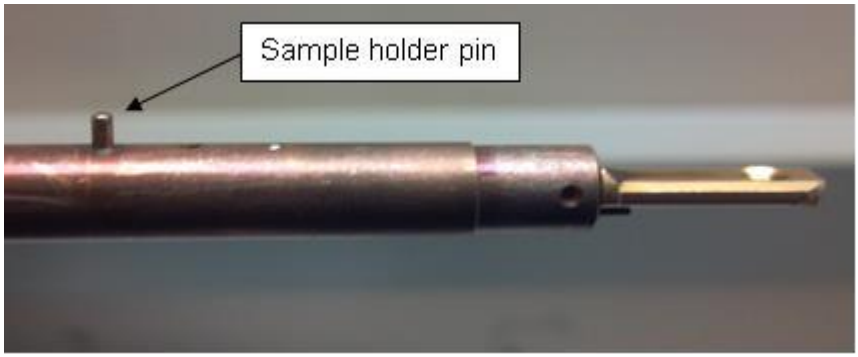
vi. RETRACT the holder slightly and turn it upside down. TAP the back end several times, then turn the holder upright and check that the grid has not moved (movement suggests the grid is not properly secured).



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

e. Inserting the Sample Holder into the CompuStage/Column

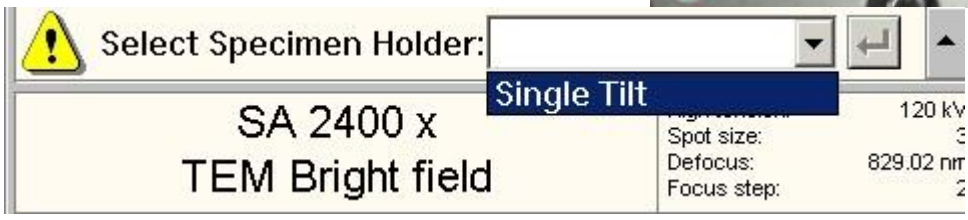
i. Carefully ORIENT the Airlock (Sample Holder) Pin on the sample holder with the 5 o'clock position on the goniometer and gently INSERT the holder until it stops. Be careful NOT to scrape the tip on the inner mechanism of the goniometer. You should feel some resistance as the sample holder O-ring seats in the Airlock Chamber.



ii. The airlock will begin pumping, and the red light on the CompuStage will go on. *Do not move the holder while the red stage LED* is lit.

iii. The pumping time remaining will be visible in the Vacuum Overview window.

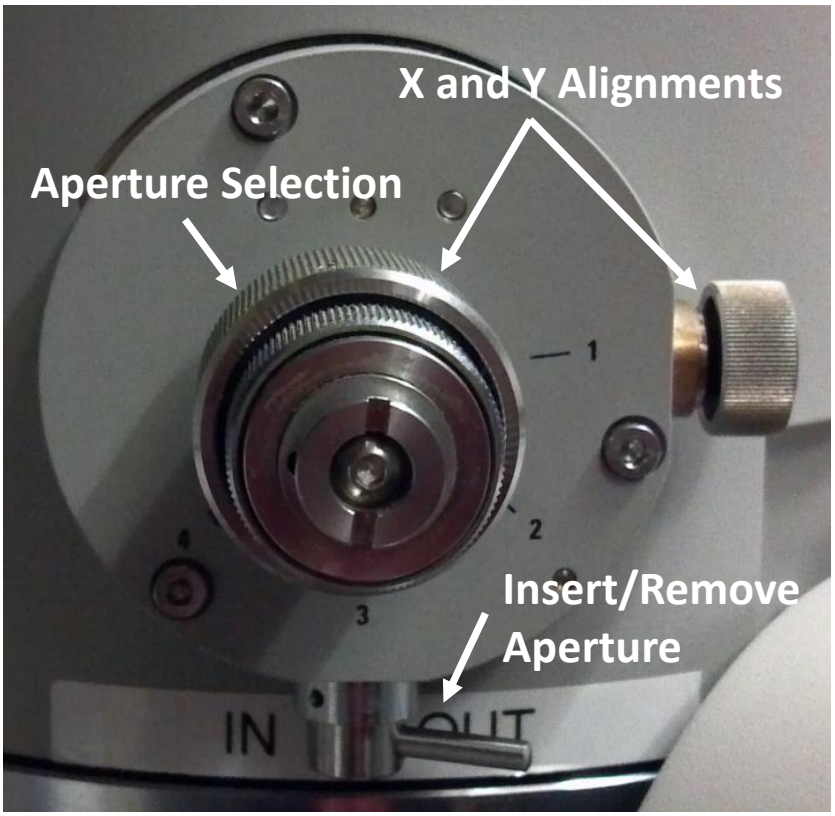
iv. SELECT the specimen holder type (Single Tilt) from the box in the interface. Be sure to to PRESS the Enter button to confirm the selection.



v. After the pumping countdown reaches zero and the red stage LED goes out, SUPPORT the purple goniometer surface with one hand and GRIP the holder securely with the other. Slowly ROTATE the holder counterclockwise from 5 o'clock to 12 o'clock position.

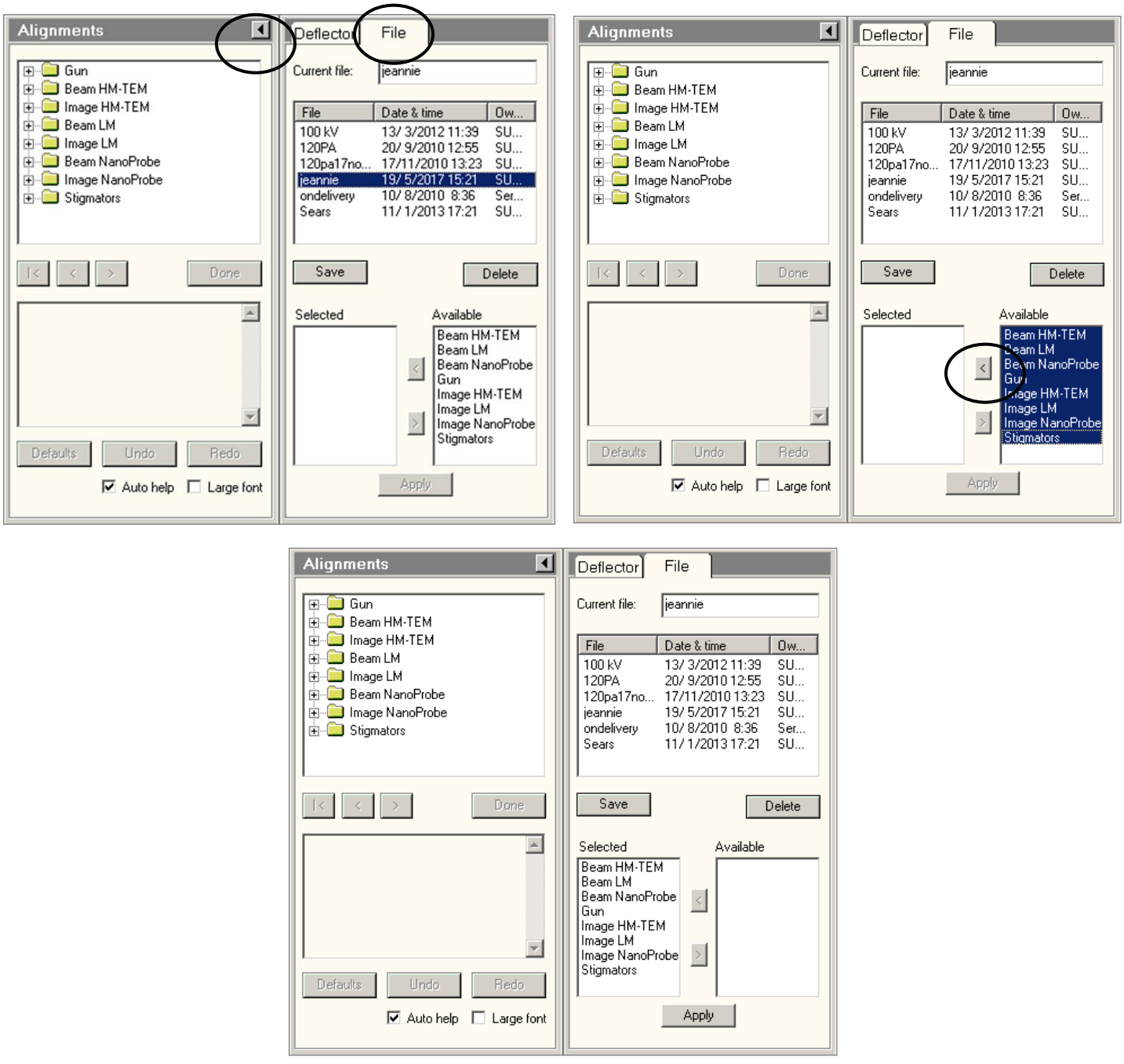
vi. Gently SLIDE the holder to into column of the microscope until it stops. TAP the end of the holder to make sure it is securely seated.

vii. VERIFY the apertures inserted are correctly inserted. Condenser (Upper) and Objective (Middle) apertures are inserted (lever to the left) and the dial at number 3. The Selected Area Electron Diffraction (SAED) aperture (lower) should be retracted (lever to the right).

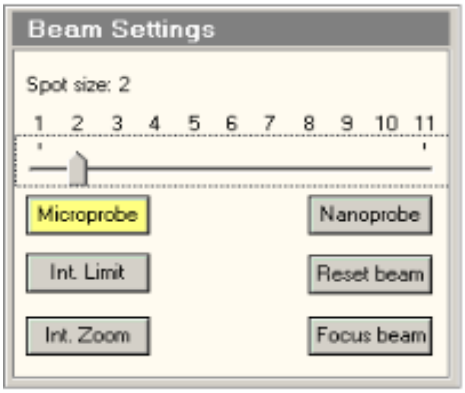


IV. Loading the Main Alignment

a. In the Workset, SELECT the Alignments tab, PRESS the Arrow to open the flap-out window, and SELECT the File tab. Chose the File named Jeannie, 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.

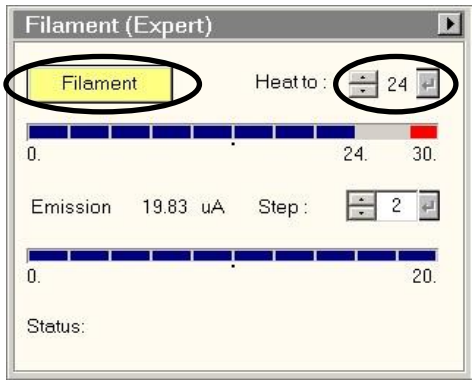


b. In the Workset, CHOOSE the Tune tab and on the Beam Settings window, VERIFY or SELECT Spot Size 2.



V. Turning on the Emission Current

a) PRESS the Filament button. It will turn yellow and the filament will begin automatically heating to the selected temperature.



VI. Control Pads

Left Control Pad (LC)
Controls beam shift (trackball), beam intensity, stigmators, sample tilt, and multifunction (MF) X.

Right Control Pad (RC)
Controls stage position (trackball), Z-height, focus, magnification, multifunction (MF) Y, and selecting diffraction/imaging mode

VII. Operating the Microscope

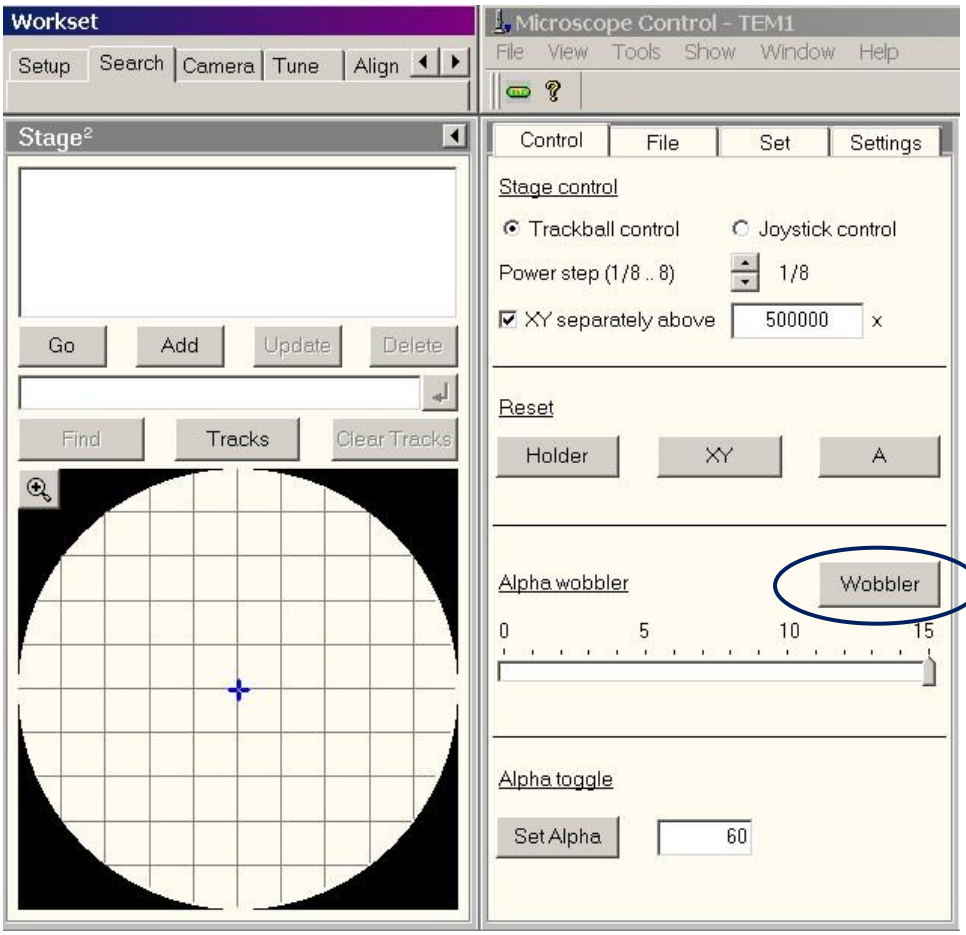
a. In the Workset, on the Vacuum/HT window, PRESS the yellow Col. Valves Closed button (changes from Yellow to Grey) to enable the Electron Beam to be displayed on the viewing screen.
b. FIND the e-beam. If you don't see the beam, try to DECREASE the magnification or MOVE the specimen stage with the trackball on the Right Control Panel in case a grid bar is blocking the beam. Once you see the beam, CENTRE it with the Tracking Ball on the Left Control Panel.

VIII. Eucentric Height Adjustment

Note: to ensure proper alignment of the beam, both the specimen and objective lens focus must lie on the eucentric plane of the microscope

a. To adjust the Eucentric Height, CHOOSE a low magnification setting (<10000x) and FIND a point of interest, i.e. particle or small hole on the TEM grid, using the stage joystick. PRESS the Eucentric Focus button (blue box on Image) on the Right-Hand Control Pad. ACTIVATE the Alpha Wobbler by PRESSING the L3 button (green box on image) on the Left-Hand Control Pad. Alternatively, you may PRESS the Wobbler button on the Stage control window. The stage will automatically rock through a tilt range of +/- 15°.

b. PRESS the (+) and/or (–) Z-axis control buttons (red box on image) on the Right-Hand Control Pad, and minimize the specimen movement.



c. Once the Eucentric Height is completed, DEVACTIVATE the Alpha Wobbler by PRESSING the L3 button or the Wobbler button on the Stage flap-out window.

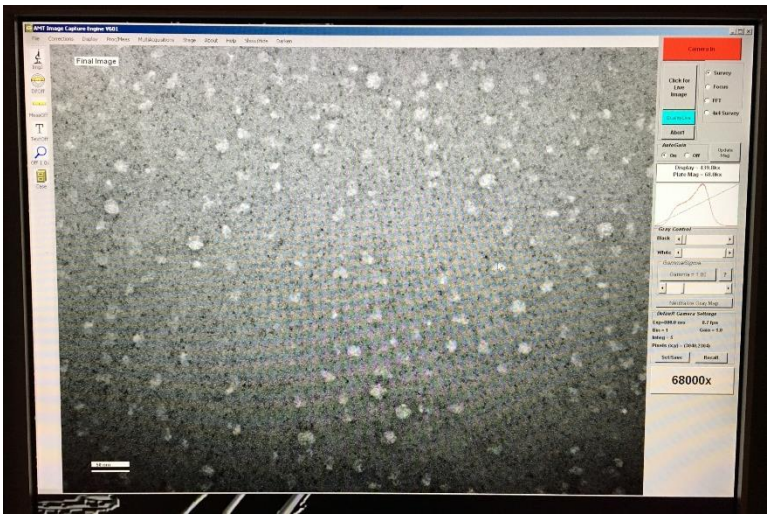
d. ALWAYS Focus the sample using the Z height buttons. If you move to other areas or tilt, refocus by PRESSING the Eucentric Focus and then use Z buttons. Only use the Focus knobs sparingly at very high magnification and reset Eucentric focus often.

IX. Camera Control and Imaging

a. CREATE a new Background Image. Note: The background image compensates for phosphor inhomogeneity and optical imperfection.
   i. VIEW the electron beam on the TEM viewing screen.
   ii. CENTRE the beam.
   iii. REMOVE specimen from column or locate a hole on TEM grid
   iv. SET the magnification to roughly the magnification you will take the images.
   v. SPREAD the beam with C2 control clockwise so that the current is about 1.0 second on the exposure meter.
   vi. LIFT the large screen and cover the viewing glass window with the black rubber mat.
   vii. On the AMT software, SELECT the menu item Background --> Acquire Background.
   viii. ADJUST the intensity knob (C2) so the histogram on the AMT software is approximately centred in the box. When this is correct, PRESS on the orange command button.
   ix. It will take ~60 seconds for the background correction to be completed. WAIT until the Click Live Imaging button is re-enabled.

b. On the AMTv601 program, PRESS Live Image.

c. LOCATE a region of interest using the stage joystick on the Right-Hand Control Pad.

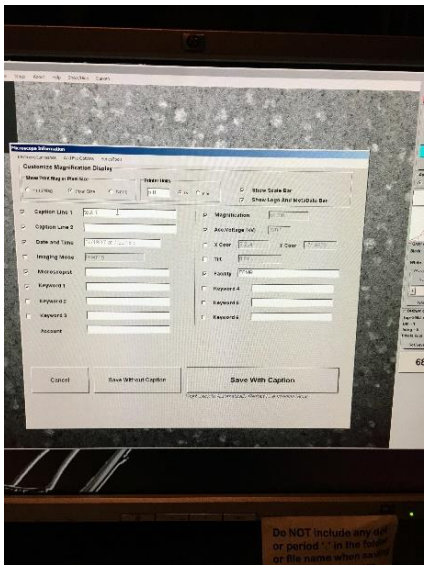


d. To accurately focus, PRESS Focus. The image will magnify four times and enable the imaging of the carbon grain and fine tune your focus.

e. The Focus knob has two parts. The smaller, Inner Knob changes the Focus. The larger, Outer Knob (i.e. the step size adjustment) modifies the amount of Focus Change per click of the Outer Knob. i.e., the degree of focus change with each movement of the inner knob.

f. Go to higher magnification. Reset defocus with R2. Find the correct defocus and image your sample
g. To save your image, PRESS Go to File/Save As. In the Caption Line 1, enter the filename.
h. PRESS Save with Caption.

i. Save images in the Shared Document folder in a folder under your own name.
j. NEVER INSERT a USB device into the MPC. Retrieve your images via the SPC (see below).



X. End of Session

1. Leave the microscope in the standard condition for the next user:

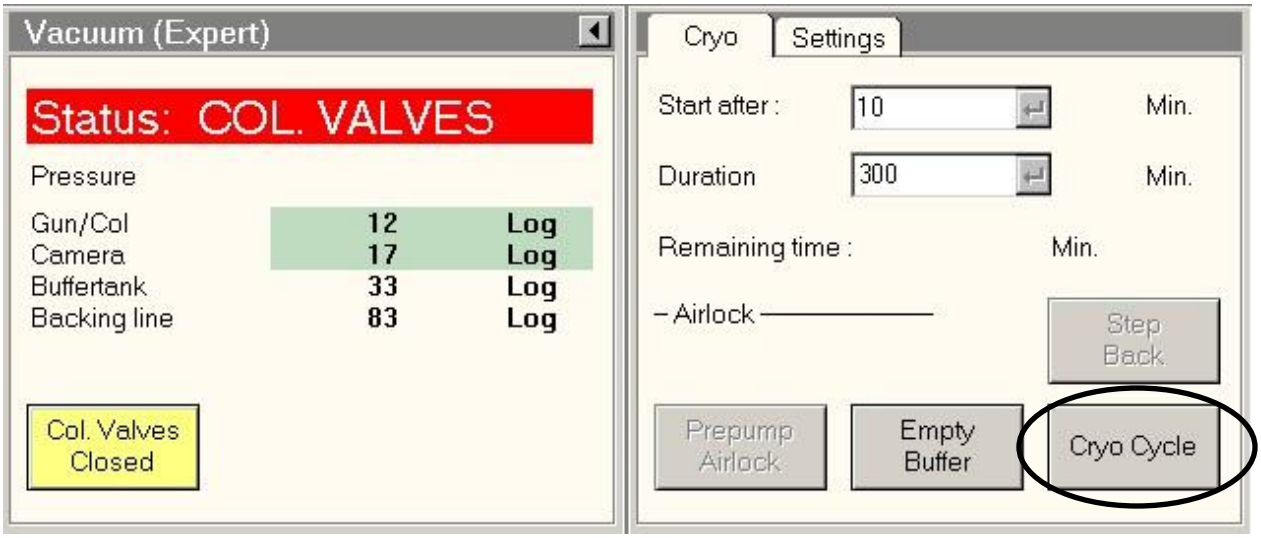
a. VERIFY the column valves are CLOSED.
b. VERFIFY the viewing screen down; COVER the window with the rubber mat.
c. PRESS the yellow filament button (turns grey) to turn off the emission current.
d. Leave the magnification in the SA range (preferably 8200×). This is essential to maintain stable objective lens current and prevent thermal drift for the next user.
e. RESET the stage (see Section IIIb). REMOVE the sample holder. RETRIEVE your TEM grid. REINSERT the holder into the CompuStage/column of the microscope.
f. CHECK the Scheduler to determine if there is another user after you.
g. If you are NOT the last user of the day, refill the LN_2 dewar.

h. CLOSE the AMTv601 program. CLOSE the Tecnai User Interface (TUI). LOG OUT from your account. GO to Start button and SELECT log off User. NEVER Shut Down or Restart of the computer.

2. If you are the last user of the day:

a. REMOVE the LN_2 anti-contaminator dewar, pour the remaining LN_2 into the large dewar, and PLACE the microscope dewar on the counter. PLACE a towel below the dewar platform to collect condensing moisture.

b. On the Workset, CHOOSE Vacuum (Expert) window, PRESS the Arrow button to open the Cryo/Settings window, and SELECT the Cryo tab. VERIFY the settings are "Duration" = 420 min and "Start after" = 10 min, and then PRESS the Cryo Cycle button (turns yellow).



3. Retrieving Images from the Support PC (SPC). Note: Files are deleted on the SPC every three months.

a. LOG IN with the Username/Password: femr
b. On the Desktop, OPEN folder T12 MPC. FIND and COPY your folder containing your images to the D:\ drive on the SPC.
c. COPY the folder/files to your flash drive.
d. After RETRIEVING your images, LOG OFF the SPC and TURN OFF monitors.

4. Last Steps

a. TURN OFF the monitors.
b. SIGN the logbook and LEAVE any relevant comments. If you START the cryo cycle, please indicate this in the comments.

c. TURN OFF any lights.
d. EXIT the room and CLOSE the door.