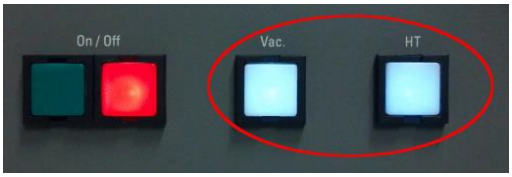
Tecnai G2 BioTwin Operating Procedures

I. Start-of-Session Steps

1.) Check that the “Vac.” and “HT” buttons are lit on the microscope control panel

If “Vac.” is not lit, contact the EM staff. **If “HT” is not lit**, press the button to light. DO NOT touch the On/Off buttons.



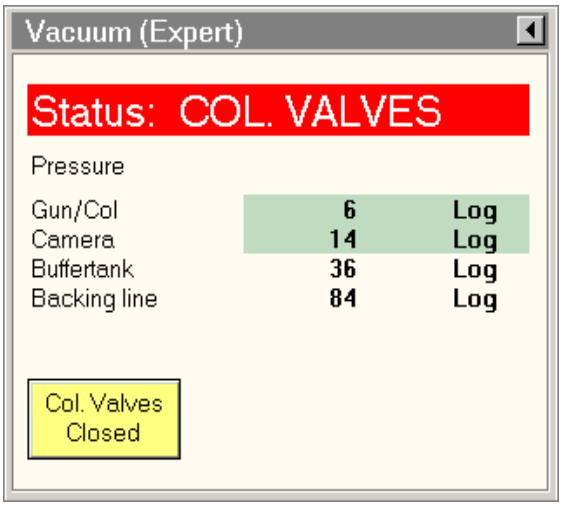
2.) Log in with the username and password created during training.

3.) Launch the Tecnai User Interface and AMT Camera software.

Drag the AMT window over to the right monitor.

4.) Check that the microscope status reads “**COL. VALVES**” and that the “**Col. Valves Closed**” button is pressed (buttons appear yellow when active).

5.) Fill the cold trap with LN2. The first Dewar flask of the day will last ~30–40 minutes. Later dewars will each last 2–3 hours. **Top off LN2 at the start of your session**. If the cold finger warms up the vacuum will deteriorate.



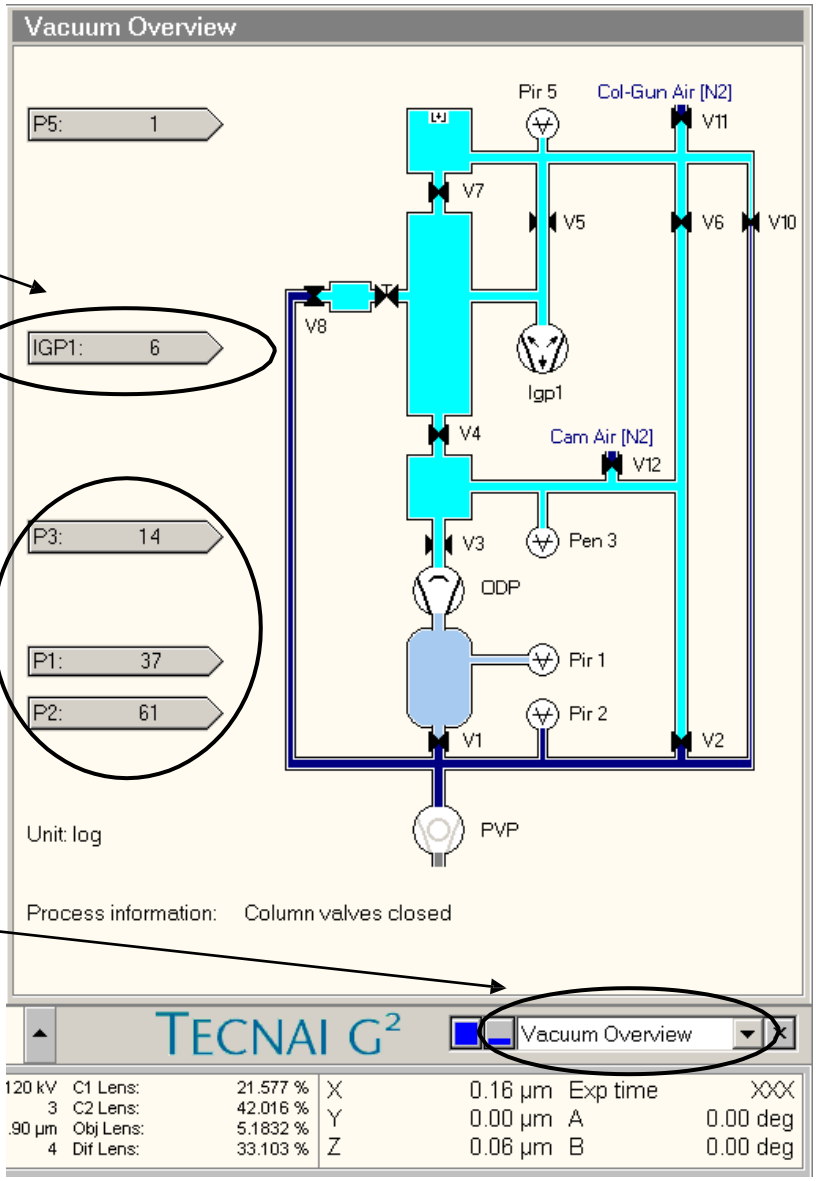
6.) Open the “Vacuum Overview” screen by selecting it from the pop-up menu in the bottom right corner of the screen. Check that **IGP1 (Gun/Col) reads ~6** (log units).

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 < 35.

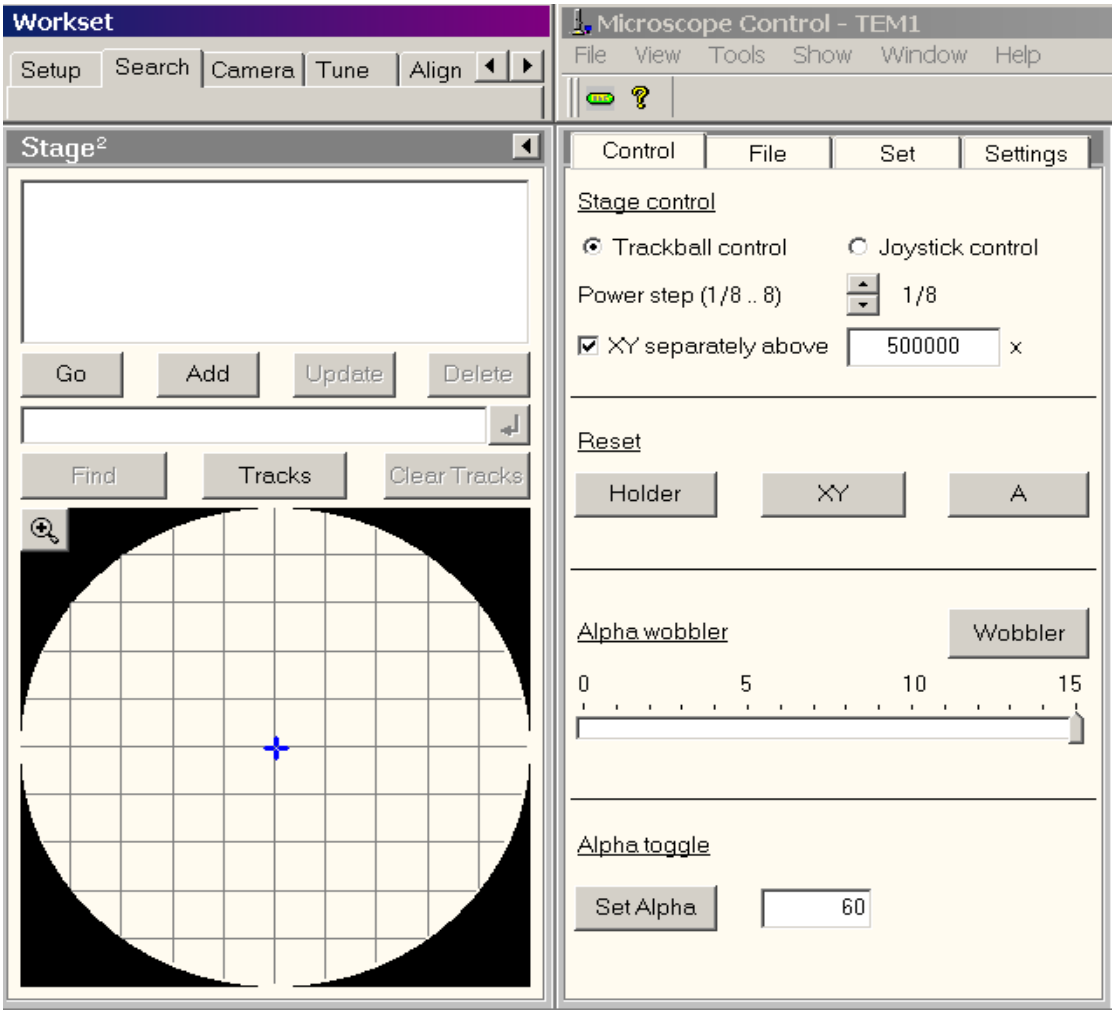
P1 indicates the pressure in the buffer tank, and P2 the pressure in the backing pump line.

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



II. Specimen Loading and Holder Insertion/Removal

1.) **Before inserting or removing the sample holder**, make sure that the column valves are closed and the holder has been reset (centered). The stage is reset by using the “Search” tab, “Stage” (flapout), “Reset: Holder” button. See below:



2.) **Sample Holder Removal:**

a.) Reset the sample stage. See previous page.

b.) **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.

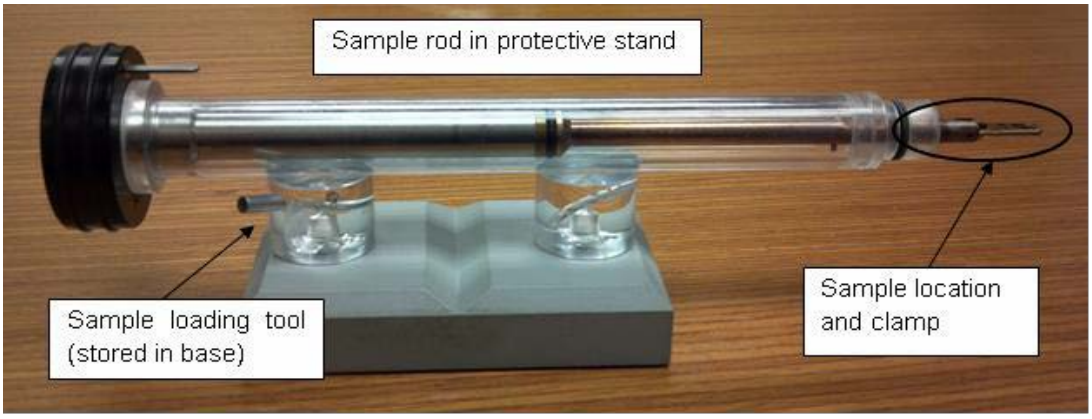
c.) **Rotate** the holder **clockwise** until it stops. This rotation moves the guide pin (see steps 3 and 4) approximately from the 12 o'clock position to 5 o'clock.

d.) **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.

e.) Remove the holder **straight back** out of the column while being careful not to scrape it along the inside of the airlock

f.) Be careful not to touch any part past the holder o-ring with bare hands.

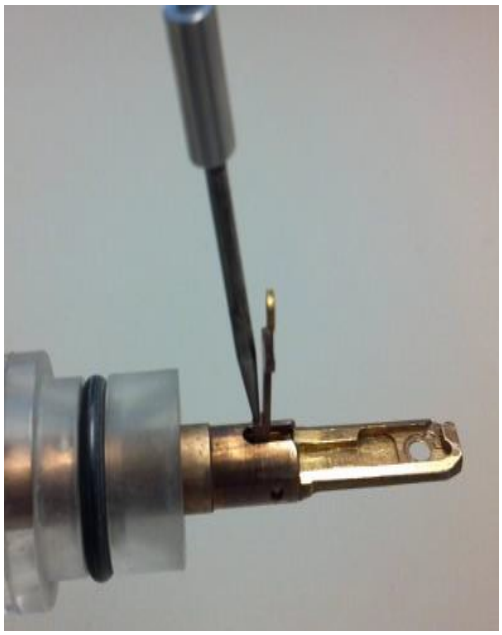
3.) **Specimen Loading:**



a.) Place the sample holder in the protective stand.

b.) Remove the sample loading tool from the base of the stand.

c.) 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.



d.) Place the specimen grid into the recess at the end of the holder.

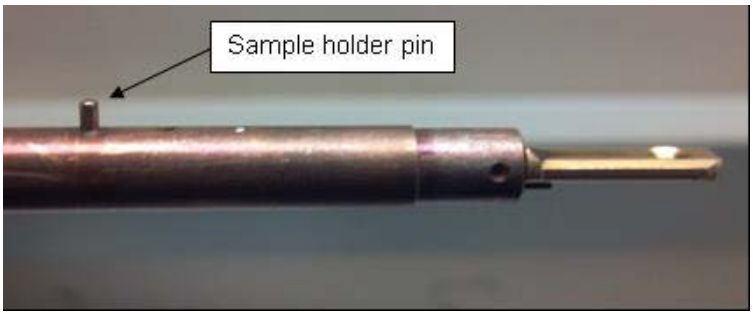
e.) **Gently** lower the clamp **straight down** to hold the grid securely. Return the tool to the base of the holder stand.

f.) 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).

g.) Inspect the holder o-ring for debris. Remove any debris using compressed air.

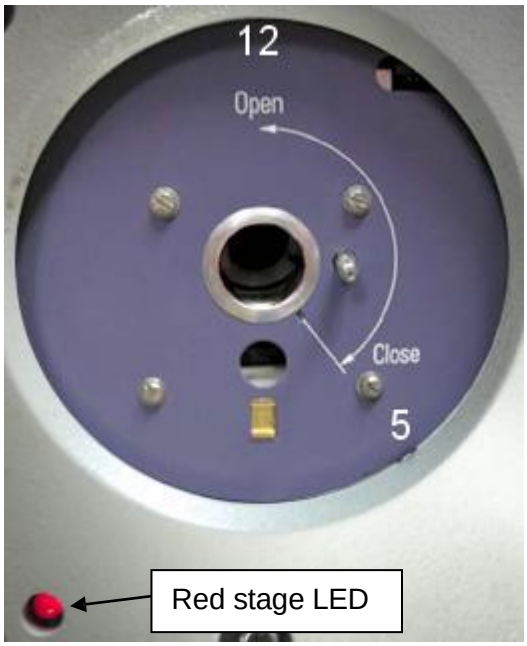
4.) **<u>Sample Holder Insertion</u>:**

a.) Carefully line the **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. You should feel some resistance as the holder o-ring seats in the airlock chamber.



b.) The airlock will begin pumping, and the red light on the compu-stage will go on. **Do not move the holder while the red stage LED is lit.**

c.) The pumping time remaining will be visible in the Vacuum Overview window. (30-60 seconds)



e.) When the **pump times ends** (status reads “COL. VALVES”) 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**.

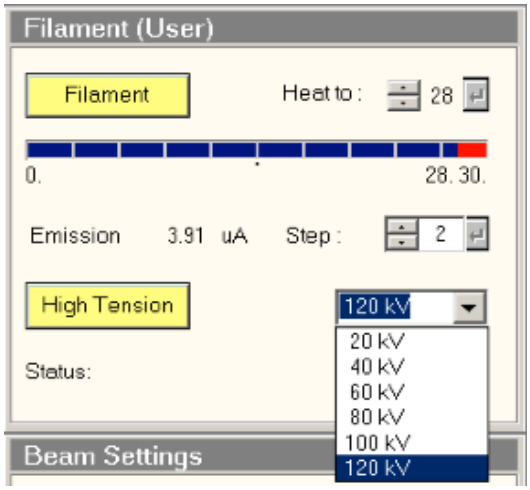
f.) Gently allow the holder to slide into the microscope column until it stops. Tap the end of the holder to make sure it is securely seated.

III. Generate Beam

1. On SETUP tab, find the FILAMENT window. If HIGH TENSION and FILAMENT are ON (both buttons Yellow) skip to Step 6

2. Select desired kV from pull down list. **Usually we keep it set at 80kV.**

3.) Click the “Light” button. It will turn yellow and the filament will begin automatically heating to the selected temperature.



Finding the Beam

a.) **Click the “Col. Valves Closed” button** to open the column valves (button turns grey and status becomes “Ready”)

i.) If no beam is visible, try decreasing the magnification (RC “Magnification”) or moving the specimen stage (RC trackball), in case a grid bar is blocking the beam path.

IV. Camera Control and Imaging

1. **TEM Setup**. Find the beam, align the TEM and center the beam. Insert your sample and find a region of interest. Get your sample roughly into focus using the TEM viewing screen. Final focus will be done using the camera.

2. Spread Beam. Turn the condenser knob clockwise (from crossover), spreading the beam past the edges of the TEM viewing screen, until it appears somewhat dark.

3. Open AMT software. The AMT icon is on the desktop. Give the program a few seconds to open completely.

4. **Click for Live Image**. On the right side of the AMT interface, click the button “Click For Live Image”. This will start live imaging and insert the camera.

5. Center the Histogram. Once Live and inserted, check the light meter. That is the histogram on the right side of your display. If the red curve is in the left of the box, the beam intensity is too low. If it is on the right intensity is too high. Use the TEM condenser knob to move the red curve near the center of the box. If the brightness goes 'off scale', take the camera out to readjust.

6. **Acquire a new Background. This will be done every morning by EM Facility staff for 80kV.** If you change the kV you will need to do it again. For this either pull the sample rod out slightly and put a pencil in the gap to keep sample out of the beam. This should be at the KV you will be working at, somewhere near the anticipated mag and with whichever apertures you will be using for imaging. Cover the TEM viewing window to block ambient light. Click the "Corrections" item on the upper menu and choose "Acquire a Background". The Background Control window will open, giving you the choice to "Proceed" or "Cancel". If you haven't already done it, center the Histogram. Place the histogram's red curve in the center of the box using the Condenser 2 (brightness) knob. Then click "Proceed" and wait while backgrounds are collected for all four imaging modes. Then you can put your sample back in and go Live.

7. Scan and Focus. Using "Live Imaging", navigate to your best region of interest, adjust the TEM mag and focus. "Focus" mode, the second radio button next to "Click for Live Image", is a tool to aid focusing. It zooms in and enhances the resolution.

8. Final Image. When you have the image set up, hit **"Click for Final Image"** to collect it. It takes a few seconds to integrate.

9. **Save the Image**. After collecting a final image save it to your disk or network. If you are saving to a case which is already created, click the File Cabinet icon at the bottom of the left toolbar. In the "Camera Information" window make sure the mag and voltage are correct and click "Save With Caption". To create a new case, click on the upper menu, "File -> Case Study". If not saving to a case, but directly to a folder, click, on the upper menu, "File - Save As". Then navigate to your folder and type in a file name.

10. Measurements. For linear measurement have a final image displayed. Click the Ruler icon on the left toolbar to open the measurement window. Make sure the mag and voltage are correct and then click once on each side of a feature (do not drag). A list of your measurements appears in the measurement window, along with the calculated Mean and Standard Deviation.

11. **Camera Out**. When you are done imaging take the camera out for safety. Close the AMT software by clicking the upper right "X" or "File -> Exit"

V. End of Session

Leave the **microscope in the standard condition** for the next user:

a.) **Leave** condenser and Objective **apertures inserted.**

b.) Leave the **column valves closed**.

c.) Place the v**iewing screen down**; cover the window with the rubber mat.

d.) Switch the **filament off** ("Filament" button in the "Filament" panel).

e.) Leave the **magnification** in the **SA range** (preferably 2400×).

f.) **Remove** your sample, and **return the holder to the microscope**.

g.) Close AMT and Tecnai softwares

h.) Log Out

i.) If you are **not the last user** of the day, **fill the LN2 dewar**.