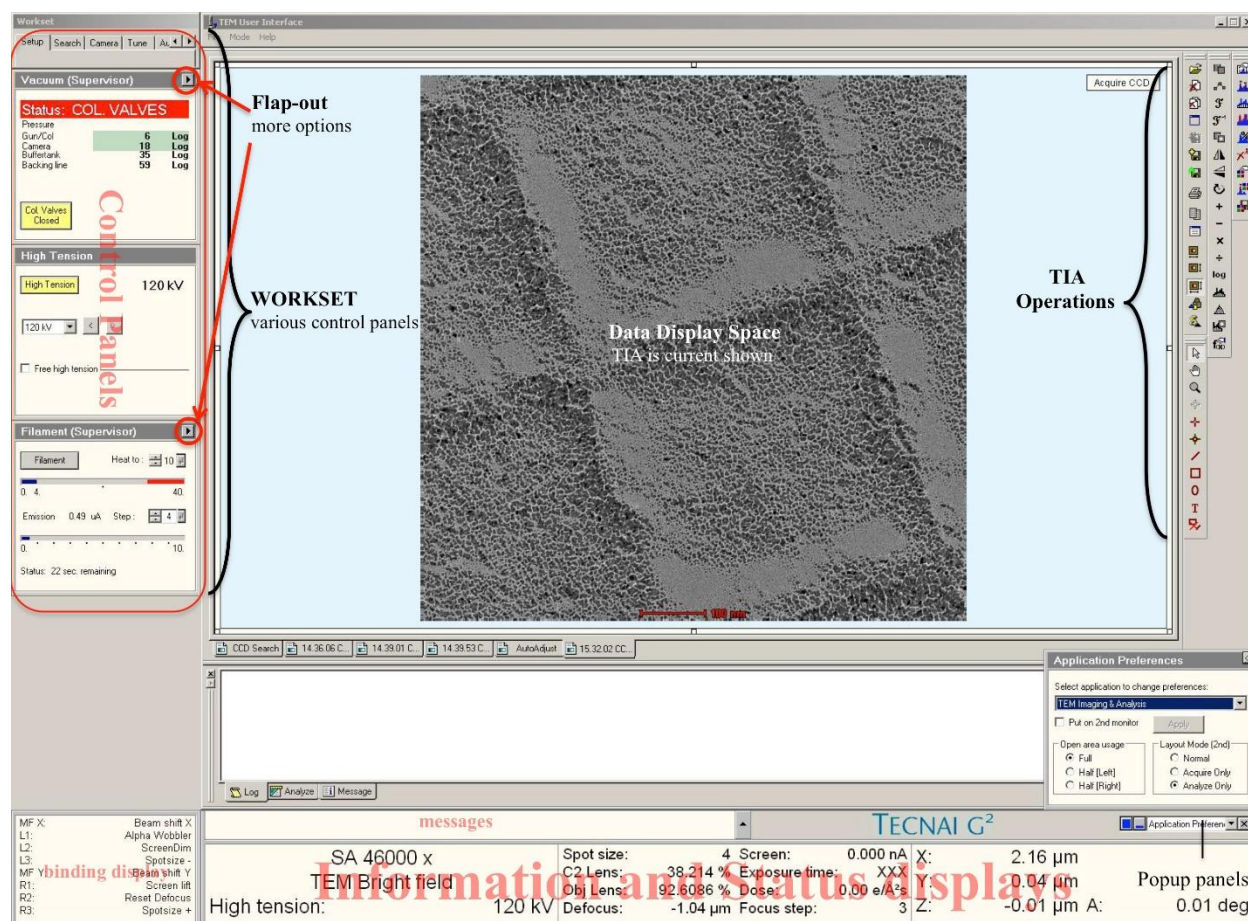


120 KV Tecnai Spirit TWIN TEM SOP

This manual is to be served as a detailed guideline for general use of the Tecnai Spirit 120kV TEM, including cryo-imaging. **You are expected to take notes during training session** and keep updating the notebook as you progress through practice sessions. **Please always bring this manual and your notebook with you each time you use Tecnai Spirit 120kv TEM.**

Please always remember to **enable** and **disable** FEI Spirit 120kV TEM in the Badger system before and after you use the instrument.

TEM User Interface Overview



An overview of Tecnai User Interface (TUI) is shown above. TUI contains elements that can be divided into three categories:

- Main program (Top): program title and menu bar.
- Control panels (Left): control panels are grouped in workset that can be selected via a tab at the top of the area with control panels.
- Information panels (Bottom): binding, messages, status displays.

The central space is reserved for data display (e.g. TIA).

Notes: Position the mouse cursor over a particular panel and press **F1** on keyboard to invoke the TUI help pages for that particular panel.

Microscope Control Pads Overview

Left and right control pads (LCP & RCP) consist of a set of standard TEM controls:

- Left: Tilt, user beam shift trackball, Intensity (Brightness), Stigmator, Multifunction X (MF X), programmable buttons (L1, L2, L3).
- Right: Diffraction, Wobbler, Eucentric Focus, Multifunction Y (MF Y), Magnification, Focus, Focus Step, Joystick, Z-axis (Z height), programmable buttons (R1, R2, R3).

MF X/Y knobs can be assigned (bind) to other functions. Programmable buttons are user-defined buttons and can be assigned (bind) to frequently used functions.



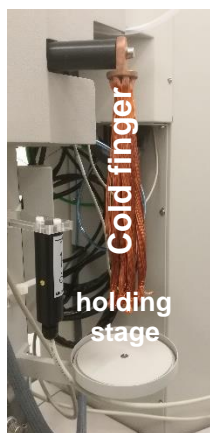
I. Preparation

1. Check and make sure that the **Vac.** and **HT** buttons are lit on the microscope control panel when the room lights are on (lower right side of the column, right behind computer screen) (Figure below).

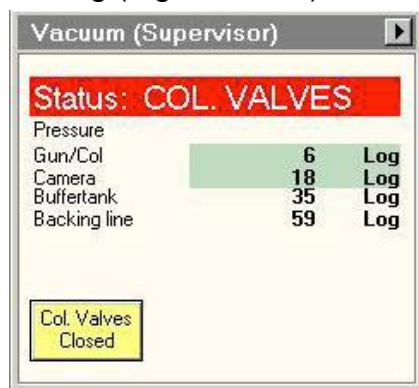


2. Sign in Badger and enable **FEI Spirit 120kV TEM** in Equipment Actions.
3. Install the cold trap dewar to its holding stage and make sure the cold finger (copper wires) is placed inside the dewar (Figure below), fill the dewar with LN2. Put the foam cap on.

Note: first dewar of LN2 should last 1–2 hours. Later dewars will each last 2–3 hours.
Remember to refill the dewars or the GUN/Col vacuum will deteriorate significantly.

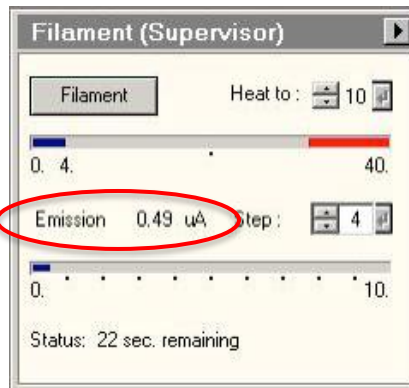
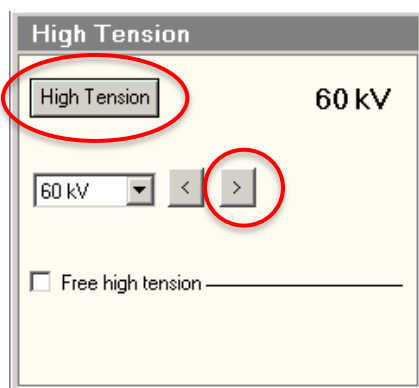


4. Go to **Setup** tab on the left side of TUI, On **Vacuum** control panel check that the **Gun/Col** and **Camera** vacuum readings in log unit: Gun/Col should be ~ 6 log; Camera should be < 25 log (Figure below).



II. High Tension (Accelerating Voltage)

- III. Select 60 kV in the **High Tension** control tab (Figure below), then click **High Tension** button once to turn it on (button color changes from grey to yellow). The **Emission** in the **Filament** control tab will spike and then stabilize at ~0.5 -1.5 μA .



1. Wait 30 - 60 seconds before proceeding; raise High Tension to 80 kV. Continue in

20 kV increments, waiting 30-60 seconds after each increasing.

2. Repeat 20 kV increments until the final High Tension 120 kV is reached.

III. Grids Loading and Holder Insertion

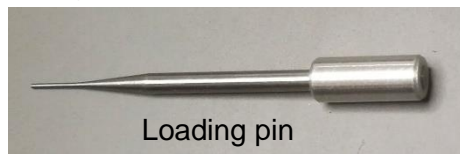
1. Specimen Loading:

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

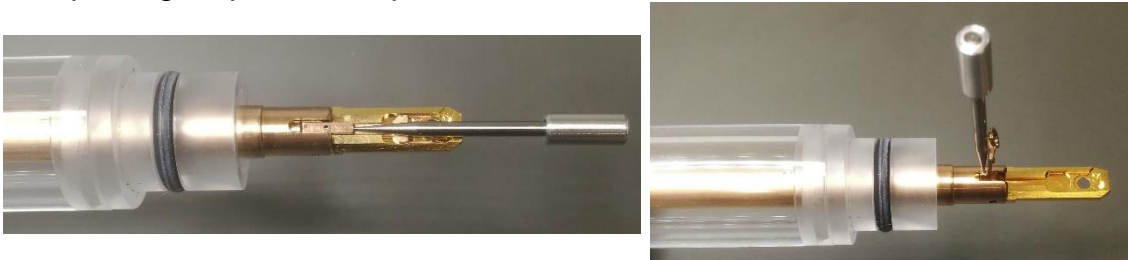
- 1) Place the single-tilt holder in the grid loading station.



- 2) Remove the sample-loading pin from station base.



- 3) Using one hand to prevent the holder from slipping out of the station, insert the pin into the pinhole in the grid clamp from the right side and gently raise the clamp straight up until it stops.



- 4) Place the grid into the recess at the end of the holder and make sure the grid and the bottom hole are aligned concentrically.



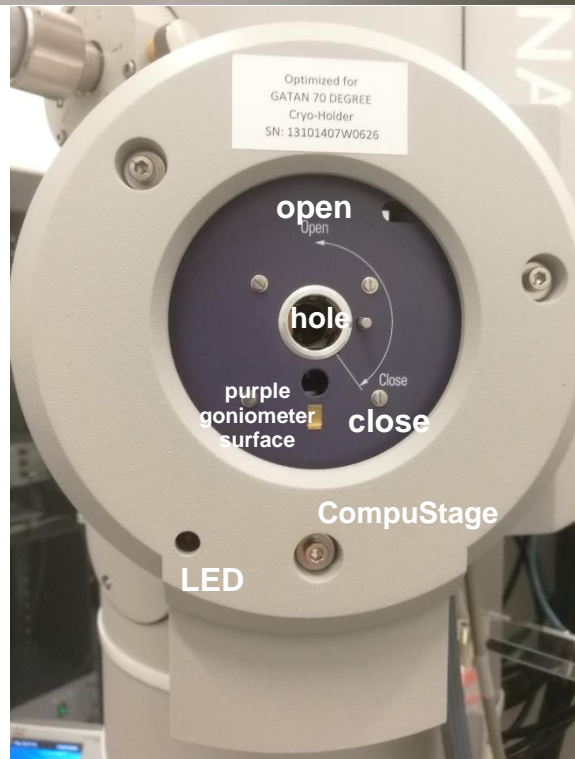
- 5) Gently lower the clamp straight down with sample-loading pin to hold the grid securely. Return the pin to station base.
- 6) Retract the holder slightly and turn it $\sim 180^\circ$. Tap the back end several times to check that the grid does not move in the holder.

2. Sample Holder Insertion:

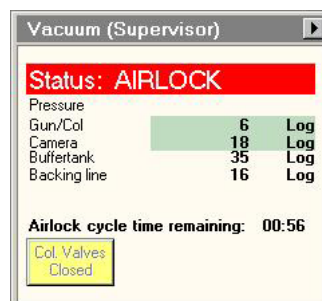
- 1) Before inserting the sample holder, make sure that the column valves are closed

(**Col. Valves Closed** button is yellow), the objective aperture is not inserted (aperture lever is switched to Out), and the stage has been reset (stage is reset by using **Search>Stage** (flapout)>**Reset: Holder** button).

- 2) Align holder well with stage hole and point holder guide pin to 5 o'clock position on the goniometer (**Close** position on the purple surface), gently insert the holder until you feel some resistance. Push the holder further in for 8 mm with slightly increased force, sometimes need to twist the holder a little to find the right angle to push further inside. The holder should be firmly inside the hole.

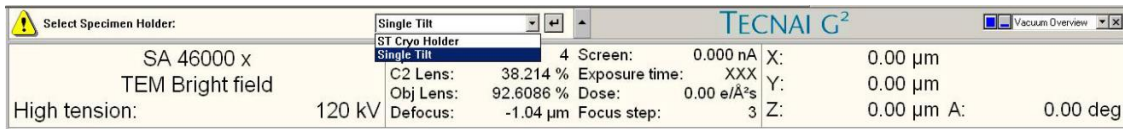


- 3) The airlock begins pumping and the red light on the CompuStage turns on. The airlock cycle time counting-down is visible in the **Vacuum** control panel.



- 4) Select the specimen holder type (Single Tilt) from the box in TUI message

window. Be sure to click the ↵ button to confirm the selection.

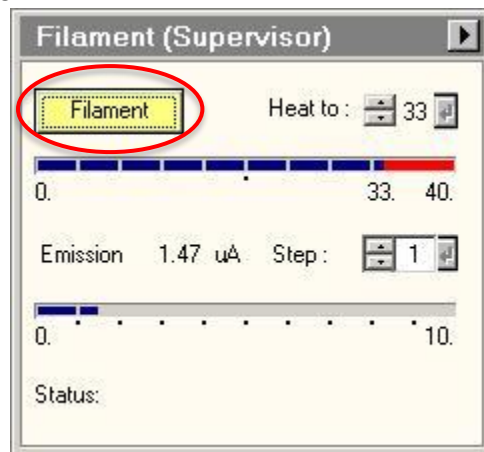


- When the pumping time counting-down finishes (**Status: COL. VALVES**) and the red stage LED is off, slowly turn the holder **counterclockwise** (~150°). Now you feel the column vacuum is pulling the holder in. Guide the holder slowly into the microscope column until it stops, gently tap the end of the holder to make sure it is securely seated.

Note: Description in this section (III) may not describe every action in details; please pay full attention to the trainer during the training, trainer will emphasize several critical points.

IV. Filament Heating

- After insert sample holder, wait for GUN/Col vacuum drops < 10 log, click the **Filament** button in **Filament** control panel. Its color changes from grey to yellow and the filament begins automatically heating to the selected temperature. Required time (~ 500s) to reach target temperature is visible at the bottom of **Filament** control panel.



Note: Do not change the **Heat To** value and **Step** value in **Filament** control panel.

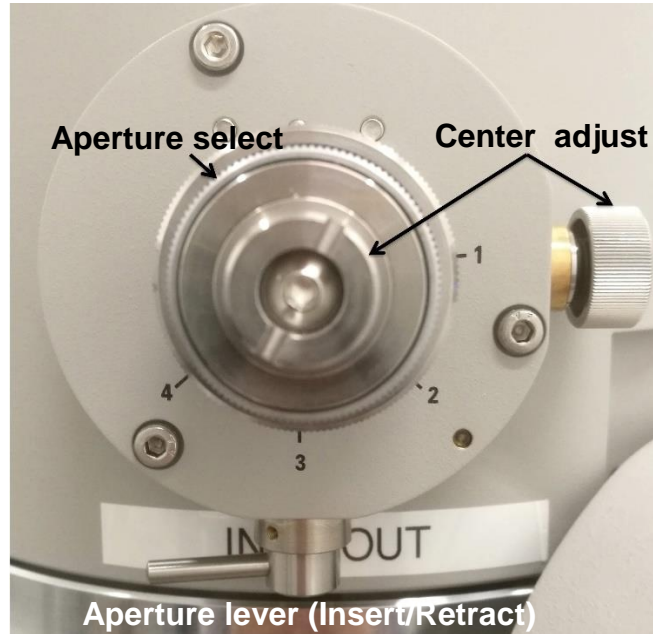
V. Alignment

- Finding the Beam
 - Wait until the Filament heating finishes and GUN/Col vacuum drops to 10 log.**
 - Click the **Col. Valves Closed** button to open the column valves (button color changes from yellow to grey and **Status: Ready**). Use fluorescence screen to find the beam. If no beam is visible, try decreasing the magnification, in case a grid bar is blocking the beam path. After you see the beam (partial or full) you can move the specimen stage to center one grid window.

Note: Spot size 3 is suggested for room temperature sample imaging.

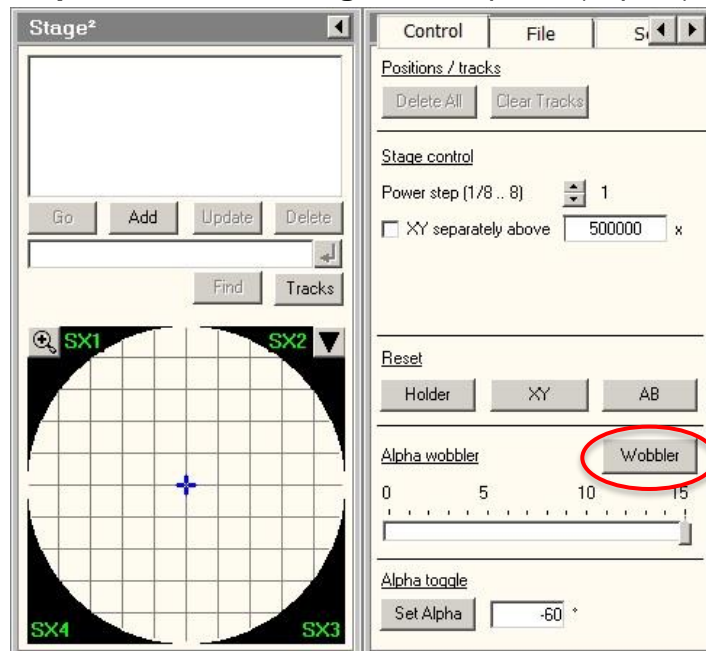
Note: Never move the Track Ball or the Intensity knob if not seeing any beam.

2. Check the Condenser Aperture at magnification range 5000x – 10000x
 - 1) Select the desired condenser aperture (3 or 4) using the large outer knob on the aperture.
 - 2) Converge the beam to crossover (**Intensity**) and center the beam (**Trackball**).
 - 3) Spread the beam (**Intensity**) to check if the beam spreads concentrically.



Note: Refer to **Appendix I** to center the Condenser Aperture if it's not concentrically or adjust the Condenser Astigmatism if the beam shape is not circular.

3. Specimen Eucentric Height Adjustment
 - 1) Find a visible feature on the specimen using the stage **Joystick** (RCP).
 - 2) Activate the **Alpha wobbler** in **Stage** control panel (flapout). The stage will begin



tilting through a range of $\pm 15^\circ$.

- 3) Minimize the specimen movement by adjusting the **Z-axis** control buttons (RCP).
- 4) Deactivate the **Alpha wobbler**.
- 5) Click **Eucentric focus** button (RCP) to reset objective lens on eucentric plane.


4. Check the Objective Aperture at magnification range 5000x – 10000x (skip if no objective aperture is desired).

- 1) Select and insert the desired objective aperture.
- 2) Check if the beam is blocked or partially blocked by the aperture.

Note: Refer to **Appendix I** to center the Objective Aperture if beam is blocked or partially blocked.

VI. Camera Control and Imaging

1. Camera Operation

- 1) In **Camera>CCD/TV Camera**, click **Search** button. Adjust **Intensity** to spread the beam to cover the full fluorescence screen. Lift the fluorescence screen with **R1** button. In TIA, click  to show a real-time **FFT** (fast Fourier transform) of the image.
- 2) Find areas of interest, adjust Focus to bring the sample close to in-focus (no rings on FFT at magnification > 20 kx or minimum contrast seen). Adjust defocus (suggested range -1 ~ -1.5 μm) in counterclockwise direction (under-focus) and take images by clicking **Acquire** in **Camera>CCD/TV Camera**.

Note: press **R2** button to reset focus value when it's in focus.

Note: Refer to **Appendix I** to adjust Objective Astigmatism if FFT Thon rings are not circular.

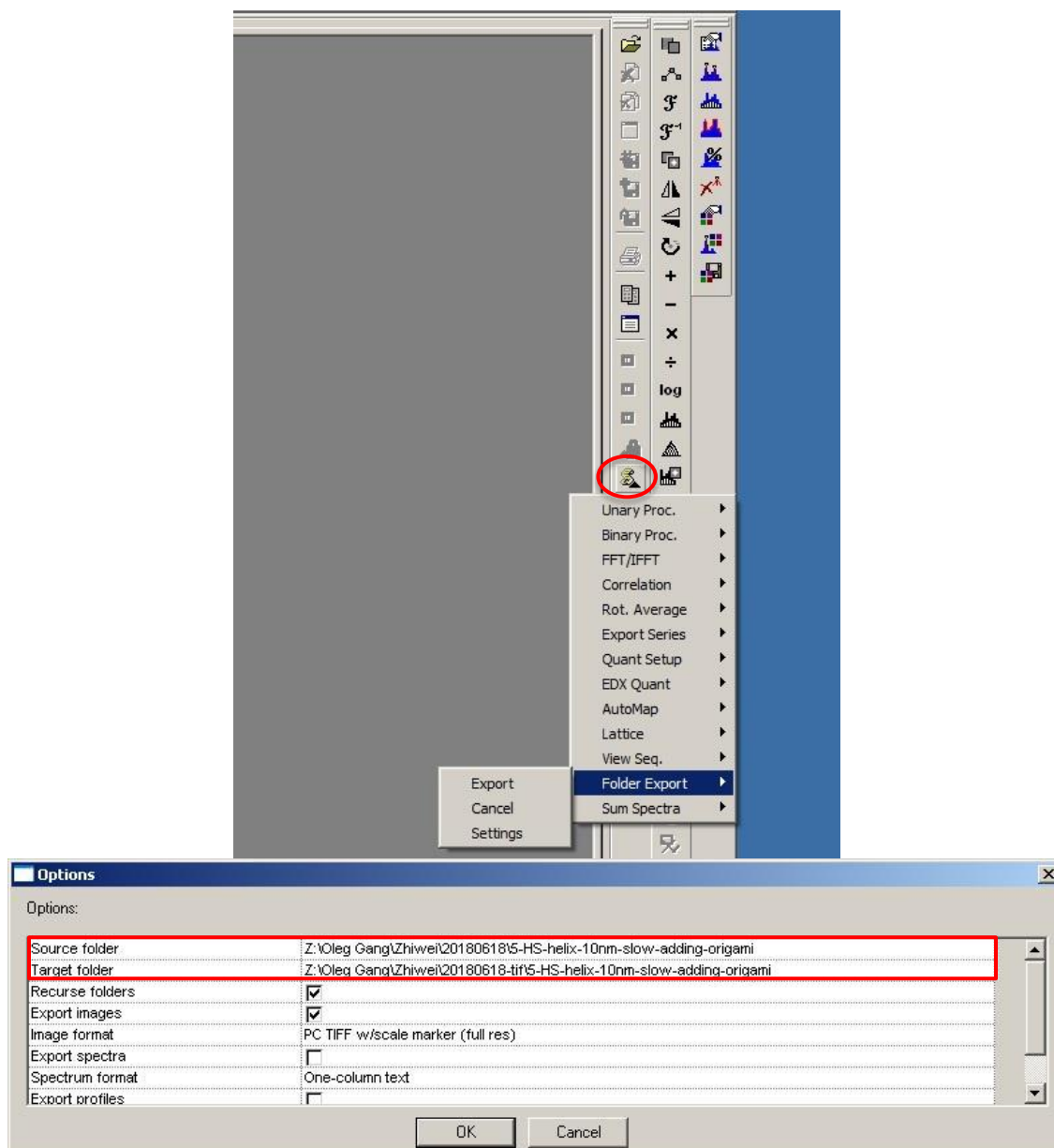
Note: Only activate the Search function of Camera when you need it, like searching for good imaging area and working on focus/defocus. You **MUST** turn off Search function of Camera when you are not using it.

2. Saving Images

- 1) Save files to the location **Z:\<advisor directory>\<user directory>**. Images should be saved to the support PC folder, never to the microscope PC.
- 2) Click **Save as** or **Auto Save** to save the images in *.emi format. Alternatively, right click on the image and select **Export**, save the image as tif file with scale marker and full resolution.

Note: You need to wait until auto save finishes (**Acquire** button becomes white and image saved message pop up) before activate **Search** again.

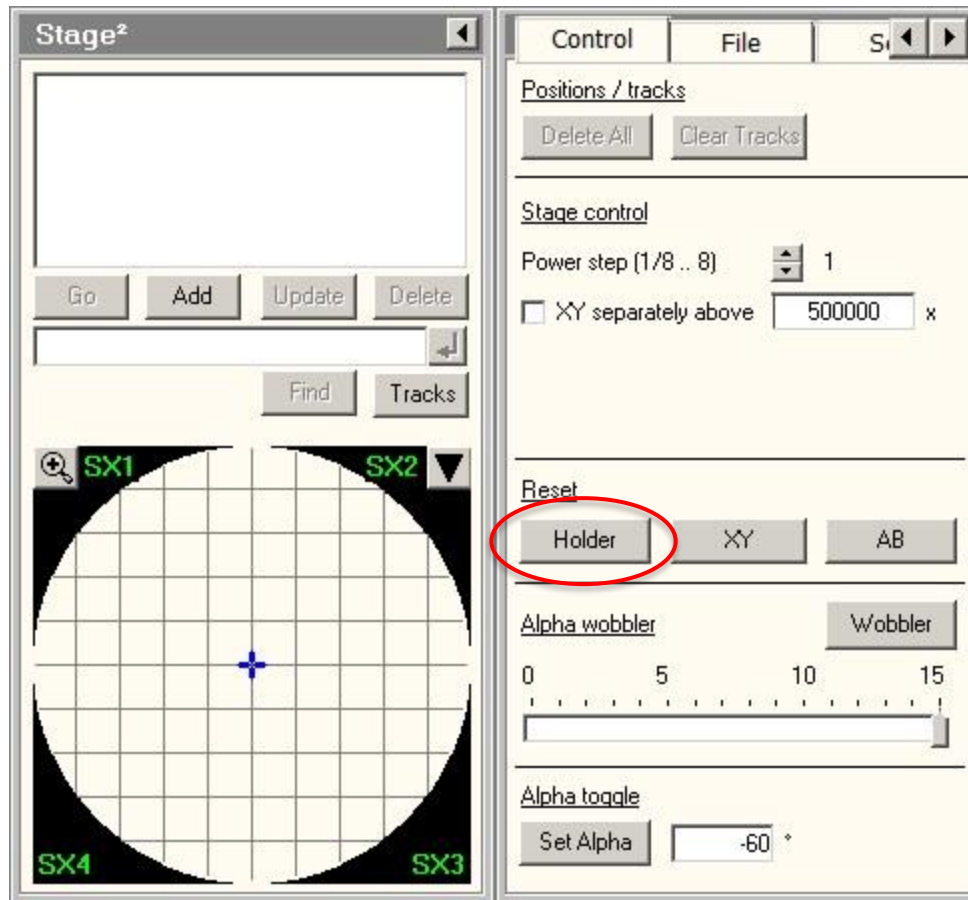
- 3) Folder Export all *.emi files: after capturing all the images on the sample, locate the **Component** icon in the **TIA** (see picture below). Select **Folder Export > Settings**, change both Source folder and Target folder to where you saved *.emi files (**Z:\<user directory>\.....**). Then click **Export** in **Component > Folder Export** to export all the emi files to tif files.



- 4) Files can be copied from the support PC.

VII. Switch Samples

- 1) Stop **Search** in Camera. Place the fluorescence screen down (R1 button); cover the window with the rubber mat.
- 2) Set the column valves closed (**Col. Valves Closed** in yellow).
- 3) Remove any objective aperture (out); leave condenser aperture inserted (In).
- 4) Switch the filament off by clicking **Filament** button (its color changes from yellow to grey) in the **Filament** control panel. This step takes a few minutes to finish.
- 5) Center the stage: click **Holder** in **Reset** panel of **Search> Stage>Control** page to move stage to center, watch the TEM status window at the lower right corner of UI and make sure that values for X, Y, Z, and Alpha are zero or close to zero.

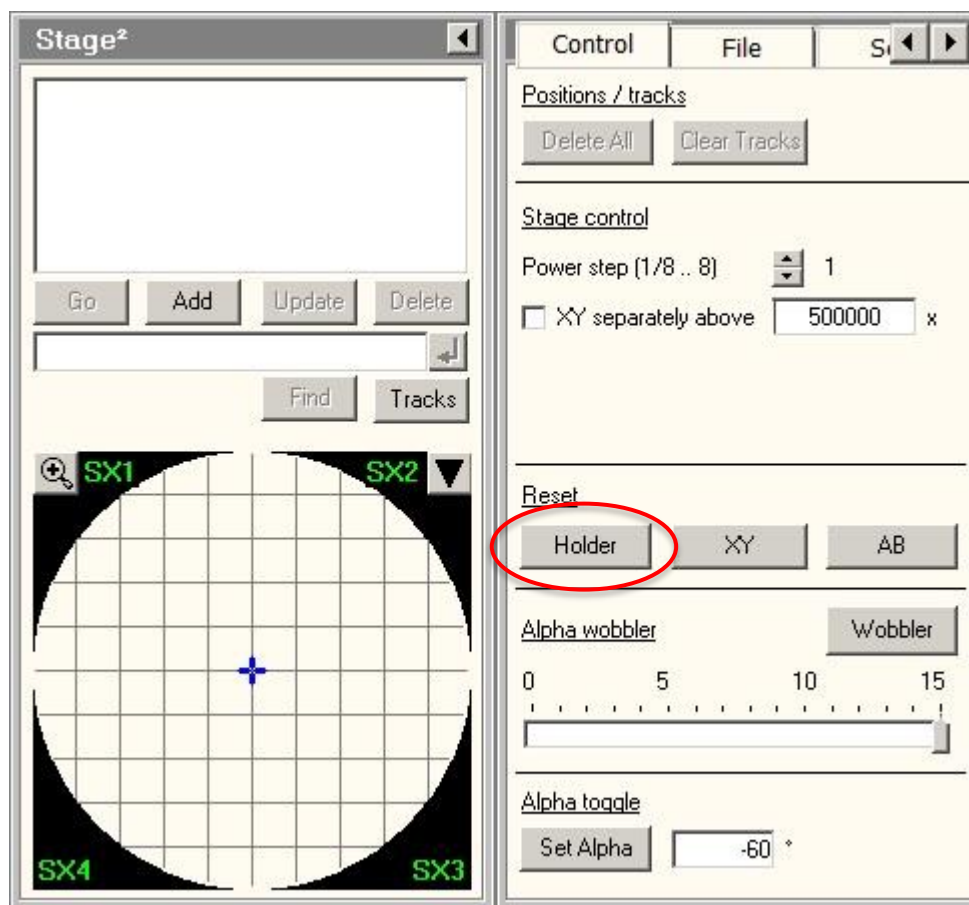


- 6) Wait until the **Filament** reading below 10, remove the sample holder: Pull the holder straight back without rotating until it stops, rotate the holder clockwise (~150°) until it stops, while keeping some force on the stage purple surface with one hand, slowly pull the sample holder out to break the airlock vacuum.
- 7) Unload the sample, load another sample, and insert the holder back to the stage following **Section III Grids Loading and Holder Insertion**.
- 8) Switch the filament on by clicking **Filament** button (its color changes to yellow) in the **Filament** control panel. Required time (~ 500s) to reach target temperature is visible at the bottom of **Filament** control panel.
- 9) When **Filament** finishes heating and GUN/Col vacuum drops to 10 log, open column valves, move sample to eucentric height, insert objective aperture if needed and use Camera for imaging.

VIII. End of Session

1. Leave the microscope in the standard condition for the next user:
 - 1) Stop **Search** in Camera. Place the fluorescence screen down (R1 button); cover the window with the rubber mat.
 - 2) Set the column valves closed (**Col. Valves Closed** in yellow).
 - 3) Remove any objective aperture (out); leave condenser aperture inserted (In).

- 4) Switch the filament off by clicking **Filament** button (its color changes from yellow to grey) in the **Filament** control panel. This step takes a few minutes to finish.
- 5) Leave the magnification in the lower SA range (2.5kx - 5kx). This is essential to maintain stable objective lens current and prevent thermal drift for the next user.
- 6) Center the stage: click **Holder** in **Reset** panel of **Search> Stage>Control** page to move stage to center, watch the TEM status window at the lower right corner of UI and make sure that values for X, Y, Z, and Alpha are zero or close to zero.

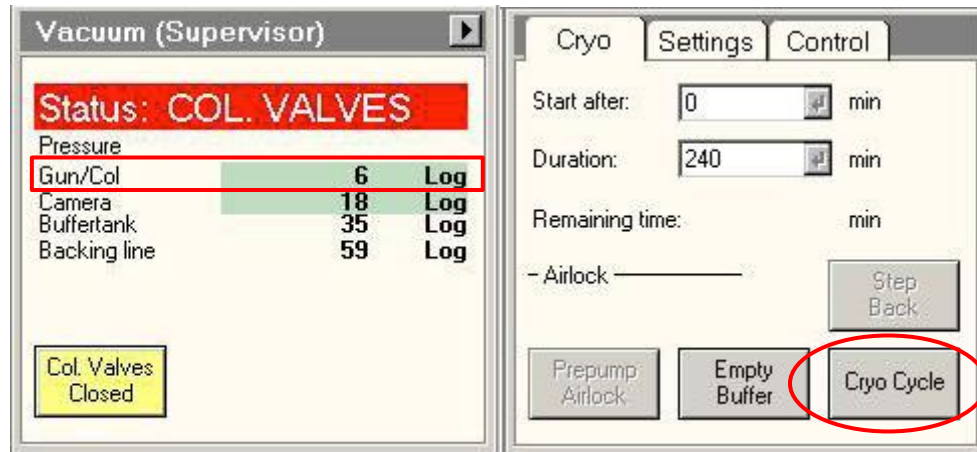


- 7) Wait until the **Filament** reading below 10, remove the sample holder: Pull the holder straight back without rotating until it stops, rotate the holder clockwise (~150°) until it stops, while keeping some force on the stage purple surface with one hand, slowly pull the sample holder out to break the airlock vacuum.
 - 8) Unload the sample from the holder.
2. Check the Badger Reservation Calendar to see if another user has scheduled after you on the same day. If you are not the last user of the day:
 - 1) Fill the LN2 dewar of the cold trap.
 - 2) Disable **FEI Spirit 120kV TEM** in Badger.
 3. If you are the last user of the day:
 - 1) Remove the LN2 dewar from the stage, empty the LN2 in the dewar, and place

the dewar upside-down in the styrofoam box. Place a paper towel below the cold finger on the microscope to collect condensing moisture.

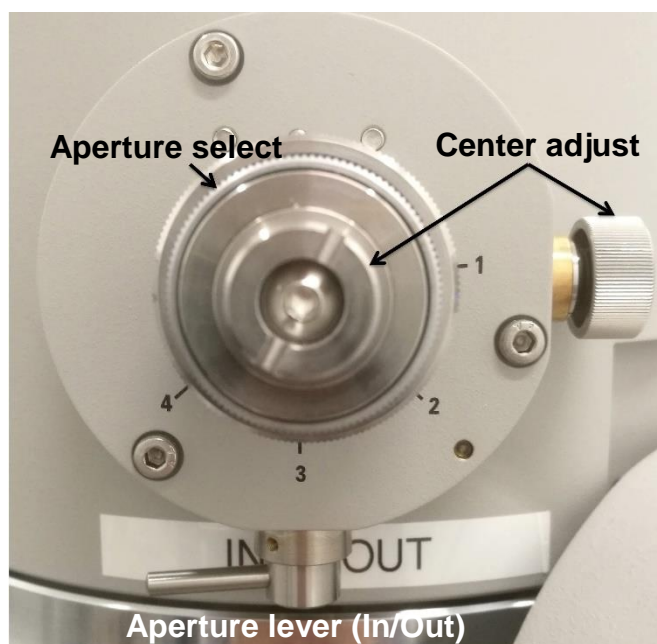
- 2) Switch HT off by clicking **High Tension** button (its color changes from yellow to grey). Choose 60 kV in the drop-down HT list.
- 3) Disable **FEI Spirit 120kV TEM** in Badger.
- 4) Watch Gun/Col vacuum reading on **Vacuum** tab of **Setup** panel, **after Gun/Col vacuum rises above 20 Log**, run the **Cryo Cycle** immediately. **Cryo Cycle** is located in the flap-out panel from the **Vacuum** tab.

Note: the last step may take up to 15 mins (generally ~11 mins), so please be patient and watch the Gun/Col vacuum reading, **please do not leave the room until you start Cryo Cycle**.

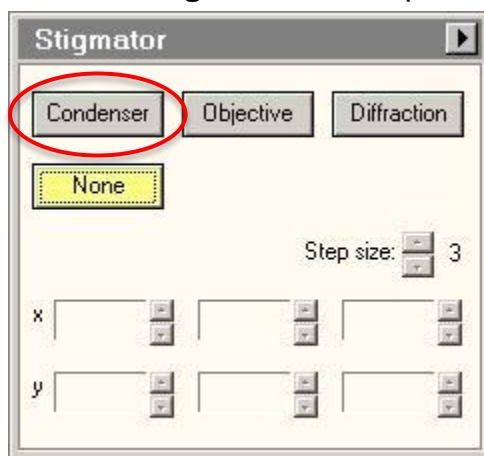


Appendix I

- **Centering the Condenser Aperture if Intensity not changing concentrically**
 - 1) Converge the beam to crossover (LCP **Intensity**) and center the beam using the beam shift (LCP **Trackball**) at magnification range 5000x – 10000x.
 - 2) Spread the beam (**Intensity** clockwise from crossover) and center the illuminated area adjusting the front and side knobs on the aperture.
 - 3) Repeat steps 1) and 2) until the beam spreads evenly across the screen.



- **Adjusting the Condenser Astigmatism if the beam shape is not circular**
 - 1) Converge the beam to crossover (LCP **Intensity**) and center it with the beam shift (LCP **Trackball**) at magnification range 5000x – 10000x.
 - 2) Select **Condenser** on the **Stigmator** control panel.




- 3) Spread the beam and use the **MF X/Y** knobs to make the beam as circular as possible.
- 4) Select **None** on the **Stigmator** control panel to end stigmator control.

- **Centering the Objective Aperture if beam is blocked or partially blocked**

- 1) Select and insert the desired objective aperture.
- 2) Enter diffraction mode (RCP **Diffraction**). The aperture should be visible in the diffraction plane with camera length ~ 500mm.
- 3) Center the aperture on the central spot by adjusting the front and side knobs on the aperture.
- 4) Deselect **Diffraction** (RCP) to return to imaging mode.

Note: If the aperture is not visible in the diffraction pattern, do not randomly adjust the aperture. Remove the aperture and return to imaging mode. Decrease magnification to low mag. ("LM" in magnification value) and reinsert the aperture. It should be visible in the image plane and can now be roughly centered by adjusting the aperture X and Y knobs. Return to the desired magnification and repeat steps 1) - 4).

- **Adjusting the Objective Astigmatism if FFT Thon rings are not circular**

- 1) Select an appropriate magnification (50-100kx is good) and an amorphous carbon film region.
- 2) In TIA, click  to show a real-time **FFT** (fast Fourier transform) of the image.
- 3) Click **Objective** in the **Stigmator** control panel.



- 4) Use the **MF X/Y** knobs to adjust the objective stigmator. The goal is for the rings in the FFT to appear circular, not elliptical or hyperbolic. The rings will grow larger and astigmatism will be more apparent when closer to focus. Adjust the focus so that the rings are large enough to be clearly visible but are not hyperbolic. As astigmatism is corrected, move closer to focus (larger rings) to fine tune the stigmator settings.
- 5) Click **None** in the **Stigmator** control panel or press LCP **Stigmator** button.

Note: Objective astigmatism should be checked periodically throughout a session. It can be changed by changes in magnification mode, spot size, Z-height of the sample, or objective aperture size or position.

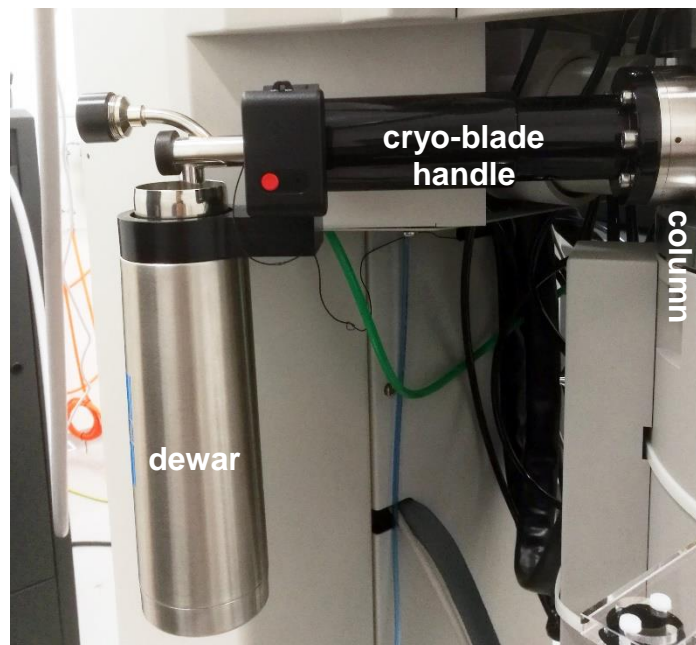
Note: Each stigmator has three registers available. Clicking on a register selects among them. These can be used to store stigmator settings for different conditions, such as different objective apertures. They can also be used to store a backup before adjusting the astigmatism. One register can be copied to another by right clicking.

Appendix II

- **Grids Loading/unloading and Holder Insertion/Removal (cryo)**

Note: the cryo-specimen holder, airlock, and Compustage are made up of delicate, precisely machined components. You should never have to apply significant force during any step of this procedure. Doing so may result in serious damage to the stage or holder. **You must attend the general Spirit TEM training session and at least one practice session before attend cryo-EM training session.**

1. Fill LN2 to the stainless steel dewar of Gatan cryo-blade and refill the dewar every two hours during cryo-imaging. The Gatan cryo-blade dewar locates on the left-back side of the TEM column.

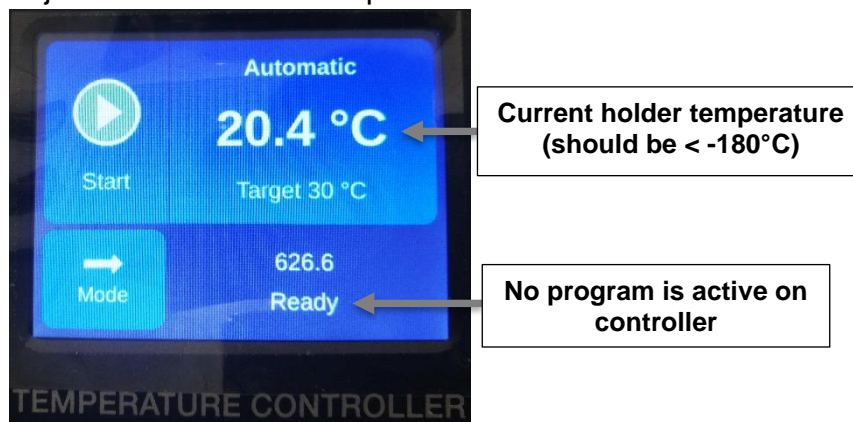


2. Specimen Loading:
 - 1) Place the sample holder in the cryo-workstation.



- 2) Keep cryo-holder connected to its controller, fill LN2 to the holder dewar and

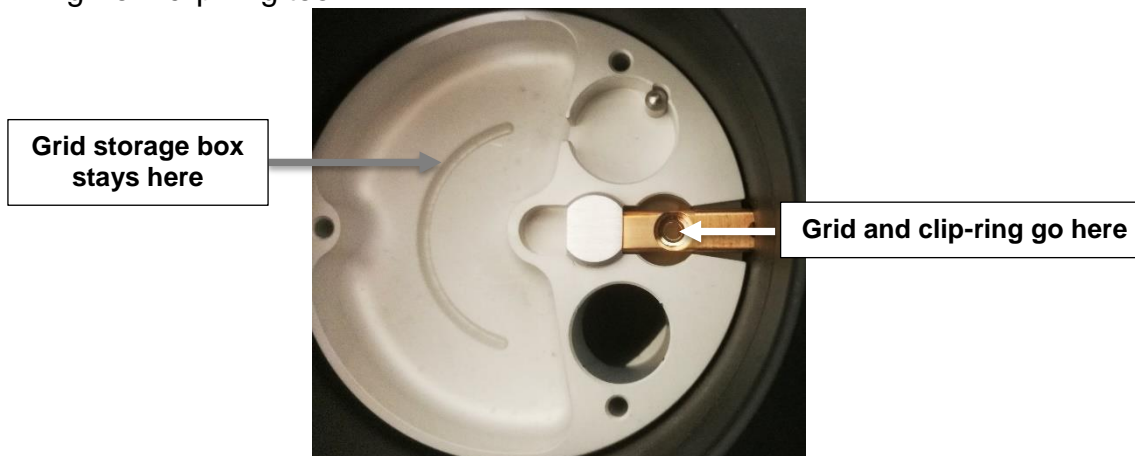
cryo-workstation, wait for the holder temperature to drop below -180°C (minimum) before loading grid to the holder.
 Note: keep surface of LN2 just above the holder tip.



- 3) Quickly transfer cryo grid storage box from storage tube to the cryo-workstation, keep the storage box submerged in LN2 while it is in cryo-workstation.

Note: any tools that will touch the storage box, sample grids, and holder tip must be precooled to LN2 temperature.

- 4) Unfasten the crew on storage box with precooled screw-driver by rotating it one full turn, gently grip the grid with precooled tweezer and move it from storage box to the holder tip, make sure the grid sit in the center of the holder tip.
- 5) Secure the clip-ring on the clip-ring tool, load clip-ring straight down to holder tip and push down straight down to secure clip-ring on the holder tip, release clip-ring from clip-ring tool.

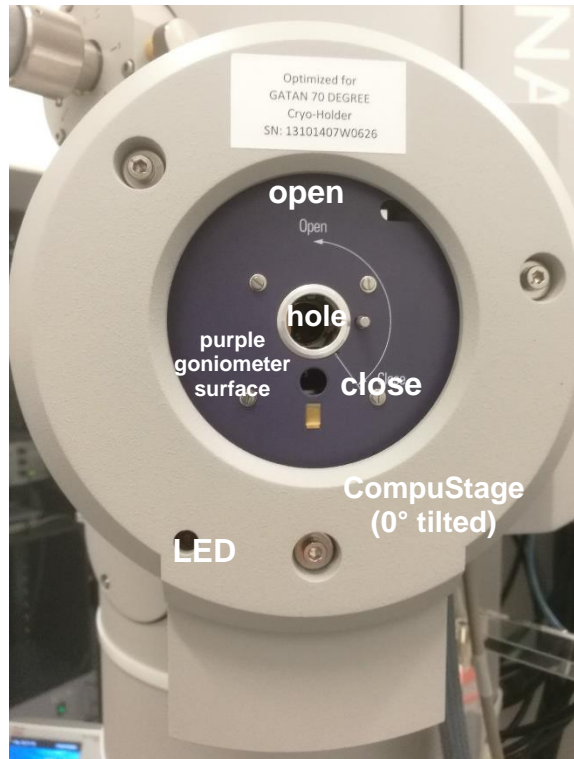


- 6) **Always make sure the clip-ring sits securely on the holder before inserting holder into the TEM column. You can use tweezer to gently touch the edge of clip-ring to check it out.**

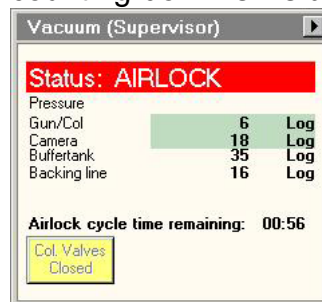
3. Sample Holder Insertion:

Note: Before inserting or removing the sample holder, make sure that the column valves are closed (**Col. Valves Closed** button is yellow), the objective aperture is not inserted (aperture lever is switched to right), and the stage has been reset (stage is reset by using **Search** control tab>**Stage** (flapout)>**Reset: Holder** button). Always put a Styrofoam box below holder dewar to collect LN2.

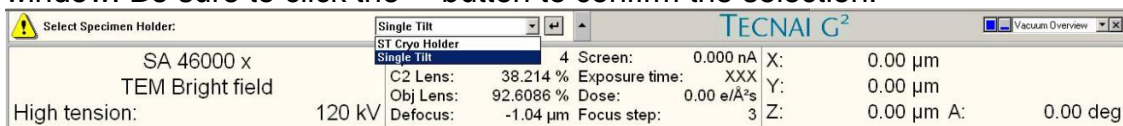
- 1) Tilt CompuStage -60° by clicking **Search** control tab>**Stage** (flapout)> **Set Alpha: -60°**.
- 2) Gently and quickly retract holder from cryo-workstation, align holder rod well with stage hole and point holder guide pin to 3 o'clock position on the goniometer (**Close** position on the purple surface), gently insert the holder until you feel some resistance. Be careful not to scrape the tip. Push the holder further in for 8 mm with slightly increased force.



- 3) The airlock begins pumping and the red light on the CompuStage is on. Do not move the holder while the red stage LED is on.
- 4) The airlock pumping time counting-down is visible in the **Vacuum** control panel.



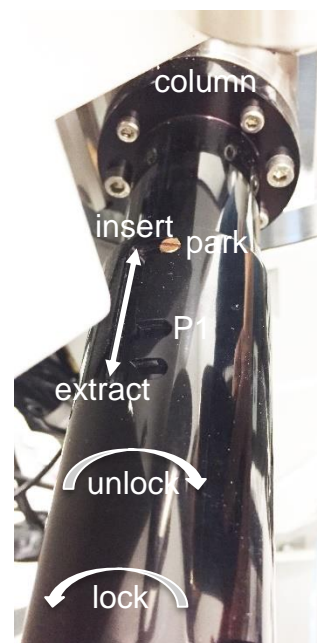
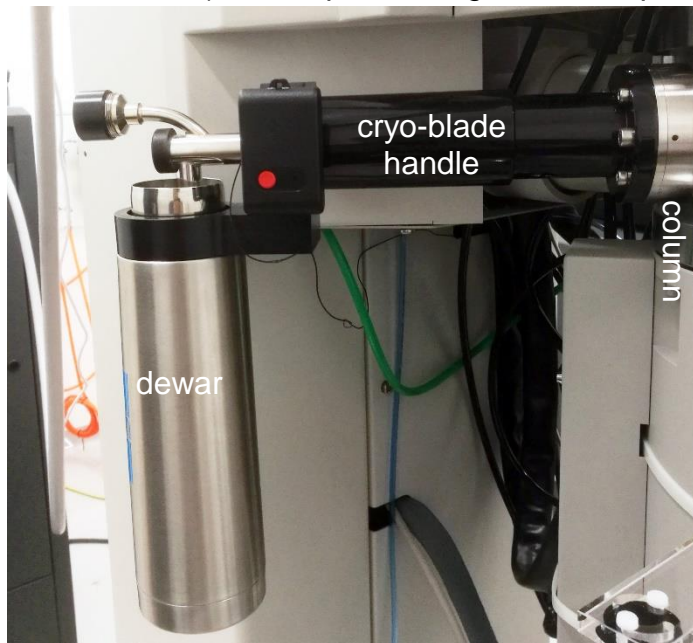
- 5) Select the specimen holder type (ST Cryo Holder) from the box in TUI message window. Be sure to click the ↵ button to confirm the selection.



- 6) When the pumping time counting-down finishes (**Status: COL. VALVES**) and the red stage LED is off, tilt CompuStage back to 0° by clicking **Search** control

tab>**Stage** (flapout)> **Set Alpha: -60°**. While the stage is rotating clockwise, grip the holder tightly and slowly turn holder counterclockwise (must rotate slower than stage rotation)

- 7) After the stage is back to 0° tilt, continue turning the holder counterclockwise. This rotation moves the guide pin to 12 o'clock (Open).
- 8) Now you feel the column vacuum is pulling the holder in. Continue gripping the holder securely and guide the holder slowly into the microscope column until it stops. Gently tap the end of the holder to make sure it is securely seated. GUN/Col vacuum may rise to 30-50 log and slowly goes down.
- 9) Insert Gatan cryo-blade from park position to P1 position and fill holder dewar with LN2. Wait for the GUN/Column to drop <15 log (<12 log is highly recommended) before proceeding to next step.



- 10) Open specimen shutter on cryo-holder and wait for 1 min before opening column valve.
- 11) Refill LN2 to holder dewar every three hours to guarantee holder temperature to be kept at ~ -180°C.

4. Sample Holder Removal:

Note: Before inserting or removing the sample holder, make sure that the column valves are closed (**Col. Valves Closed** button is yellow), the objective aperture is not inserted (aperture lever is switched to right), and the stage has been reset (stage is reset by using **Search** control tab>**Stage** (flapout)>**Reset: Holder** button). Always put a Styrofoam box below holder dewar to collect LN2.

- 1) Precool cryo-workstation to LN2 temperature if you plan to load another grid to the holder.
- 2) Close shutter on the holder and tilt CompuStage -60° by clicking **Search** control tab>**Stage** (flapout)> **Set Alpha: -60°**.
- 3) Always use left hand to keep light pressure on the purple goniometer surface

when removing the sample holder. Pull the holder straight back without rotating until it stops.

- 4) Rotate the holder clockwise ($\sim 150^\circ$) until it stops. This rotation moves the guide pin approximately from the 10 o'clock position to 3 o'clock.
- 5) While keeping pressure on the goniometer with left hand, slowly pull the sample holder out to break the airlock vacuum. This will require a small amount of force.
- 6) After the airlock vacuum breaks, gently remove the holder straight out of the stage while being careful not to scrape it along the inside of the stage.
- 7) Be careful not to touch the holder o-ring or any part beyond it with bare hands.
- 8) if you plan to load another grid, gently and quickly insert holder to precooled cryo-workstation if you plan to load another grid.
- 9) If you finished your cryoimaging session. Empty LN2 from the holder dewar after removing it from microscope and insert cryo-holder into the pumping station, open valve 1 and 2 on the pumping station, connect the cable, start Warm-up cycle with the old controller or Automastic with new controller.