

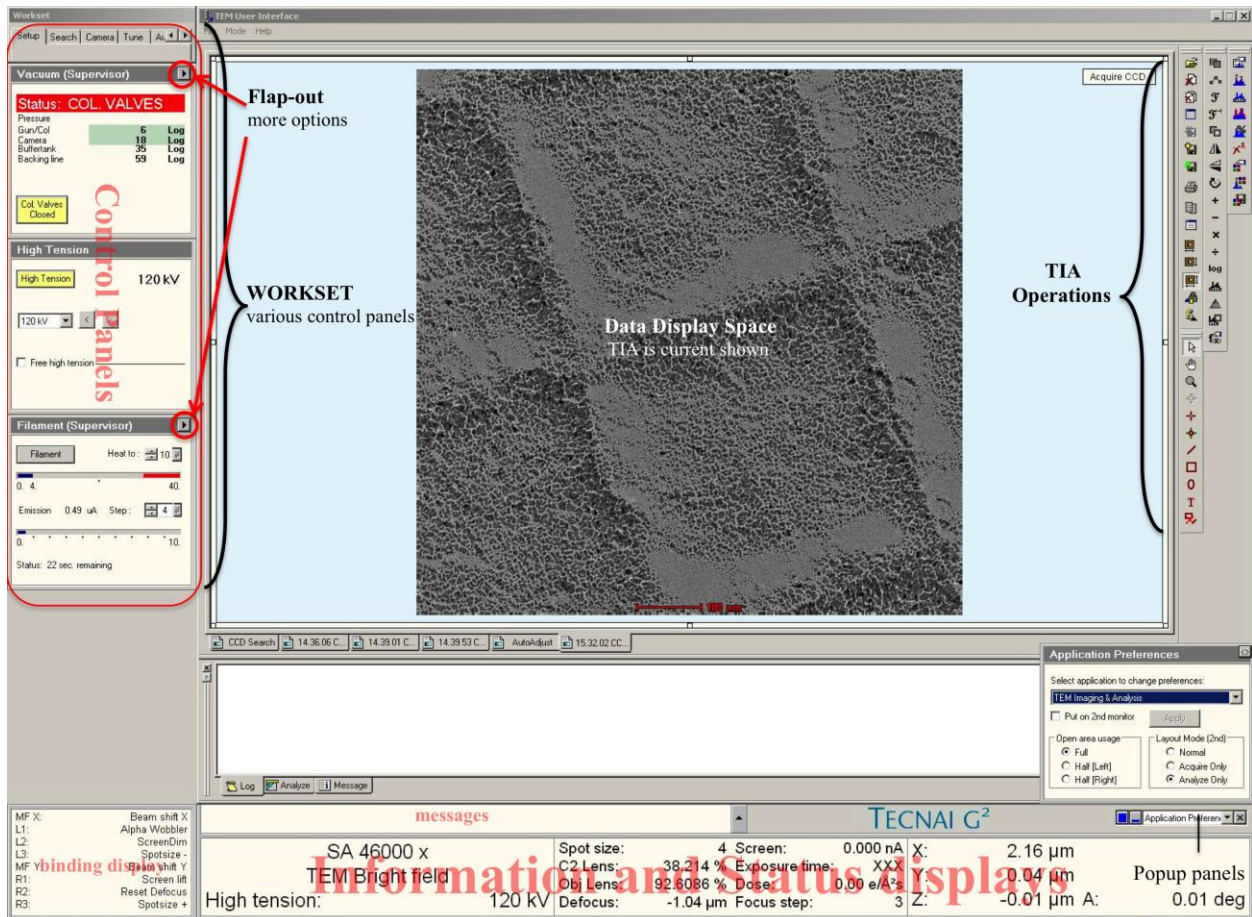


# 120 KV Tecnai Spirit TWIN TEM SOP

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 NOTE BOOK AS YOU PROGRESS THROUGH PRACTISE SESSIONS. **PLEASE ALWAYS BRING THIS MANUAL AND YOUR NOTE BOOK 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 show 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.



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- Information panels (Bottom): binding, messages, status displays.

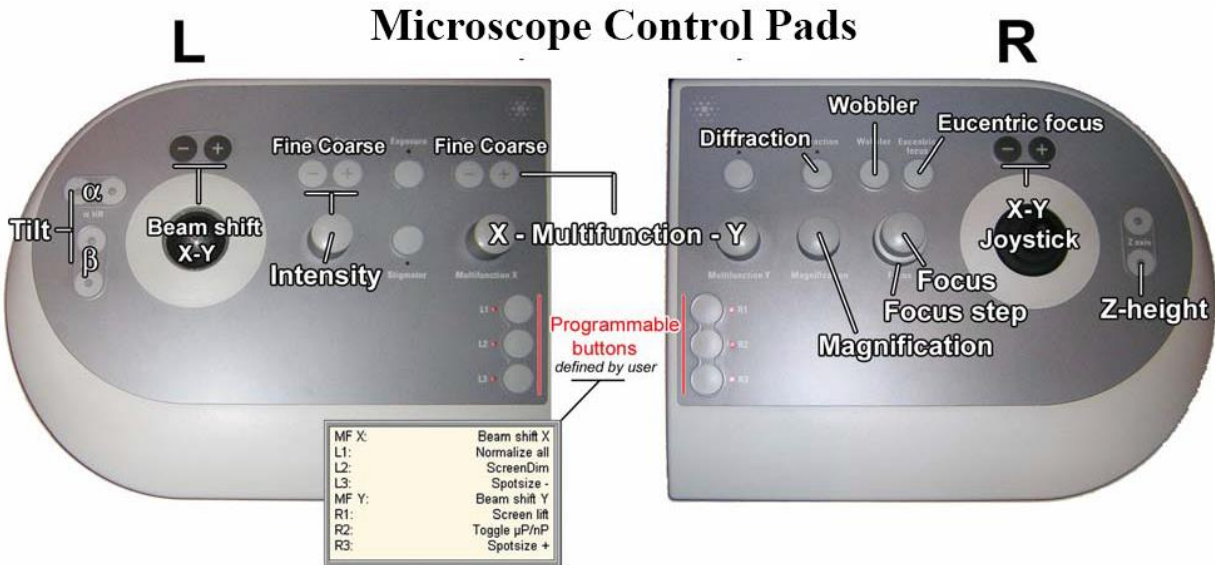
The central space is reserved for data display (e.g. TIA).

Notes: Position the mouse cursor over a particular panel and press **F1** on keyboard to invoke the TUI online 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 at lower right side of the column (right behind computer screen) (Figure below).

Note: check with the room lights on since the two buttons automatically dim when the room is dark. If **Vac.** is not lit, please contact the facility staff immediately. If **HT** is not lit, press the button to turn it on and proceed normally but make a note in the paper logbook.





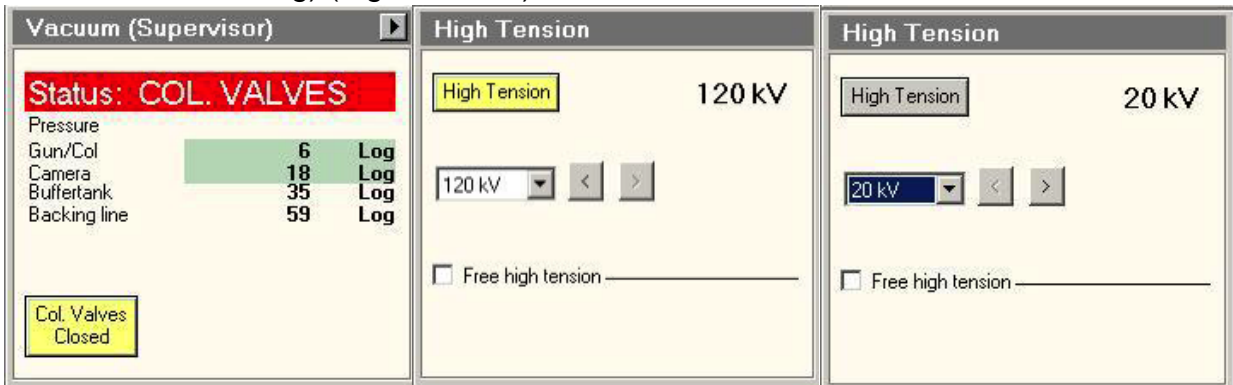
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2. Sign in Badger, choose facility "IS" and enable **FEI Spirit 120kV TEM** in Equipment Actions.
3. Tecnai User Interface (TUI) and TEM Imaging & Analysis (TIA) should be running. If not, launch TUI and TIA from quick launch bar at bottom left of computer screen.
4. Install the LN<sub>2</sub> dewar (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 LN<sub>2</sub>. Put the foam cap on.

**Note: first dewar of LN<sub>2</sub> should last 1–2 hours. Later dewars will each last 2–3 hours. Do not allow the cold finger to warm up or the GUN/Col vacuum will deteriorate significantly.**



5. Go to **Setup** tab on the left side of TUI, On **Vacuum** control panel check that the microscope status **Status: COL. VALVES** and that **Col. Valves Closed** button is yellow. On the same **Vacuum** panel check Gun/Col and Camera vacuum readings in log unit: Gun/Col should be ~6 log; Camera should be < 25 log (normally it should be ~18 log) (Figure below).



Note: Vacuum can also be read on **Vacuum Overview** screen by selecting it from the box in the bottom right corner of the screen.

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

P1 indicates the pressure in the buffer tank;

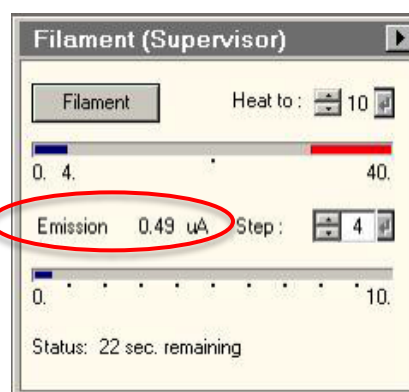
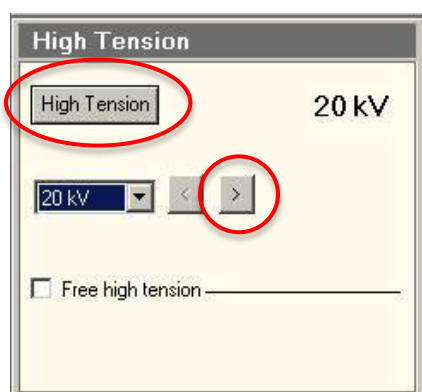
P2 the pressure in the backing pump line;

IGP1 indicates the vacuum level in the column and gun chamber



## II. High Tension (Accelerating Voltage)

1. If **High Tension** button on **High Tension** control panel is yellow and the displayed value is 120 kV (Figure above), proceed to next section “Holder Insertion/Removal”. If the bottom is grey, please continue to step 2 below.
2. The **High Tension** button will be grey (off) for the first user of the day (Figure above). IGP1 (GUN/Col) must be < 10 log before proceeding.
3. If the **High Tension** button is unavailable (grey with grey text), check that **HT** is lit on the microscope control panel (Section I). If not, press **HT** once to light it.
4. Select 20 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 current (**Emission** in the **Filament** control tab) will spike and then stabilize at ~0.5-1  $\mu\text{A}$ .



5. Wait 1-2 minutes before proceeding; raise High Tension to 40 kV and wait for 1-2 min; Continue in 20 kV increments and wait for 1-2 min after each increasing. If the emission current remains high (>1-2  $\mu\text{A}$ ), return to the previous High Tension step and wait for several minutes before proceeding.

Note: The High Tension may switch off and become disabled when increasing happens too fast. If it occurs, simply press the main **HT** button on microscope control panel to re-enable it (see Section I), repeat step 4 and 5.

6. Repeat step 5 until the final High Tension (80 kV or 120 kV) is reached.

Note: Default operation HT for Spirit is 120kV. However it is aligned at both 80 kV and 120 kV, do not operate the TEM at High Tension other than 80 kV and 120 kV.

## III. Grids Loading/unloading and Holder Insertion/Removal

**Note:** 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.

1. Specimen Loading:

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



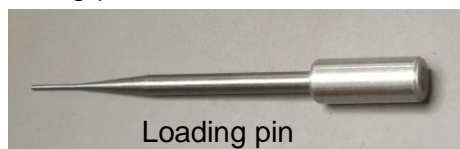


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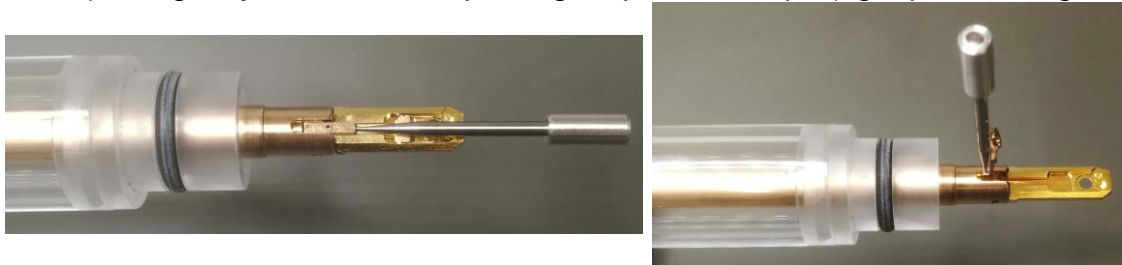
- 1) Place the sample holder in the grid loading station and remove station cap.



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



- 3) Use one hand to hold back end of holder to prevent the holder from slipping out of the station, insert the pin into the pinhole in the grid clamp (left panel in Figure below) and gently raise the clamp straight up until it stops (right panel in Figure



below).

- 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 (Figure below).

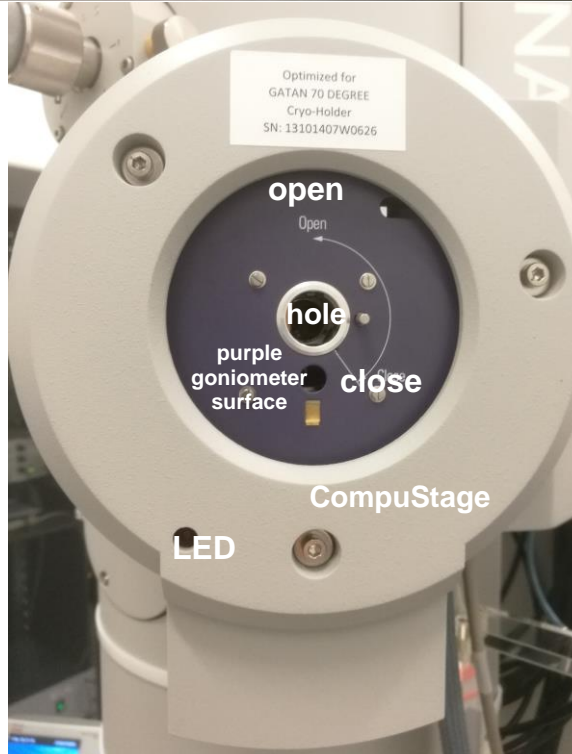


- 5) Gently lower the clamp straight down with sample-loading pin to hold the grid securely. Return the pin to station base immediately after use.
  - 6) Retract the holder slightly and turn  $\sim 180^\circ$ . Tap the back end several times to check that the grid does not move in the holder.
  - 7) Inspect the holder o-ring and remove any debris using Kim Wipes.
2. 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 (X,Y,Z, A are all close to zero)stage is reset by using **Search>Stage (flapout)>Reset: Holder** button).
  3. Sample Holder Insertion:

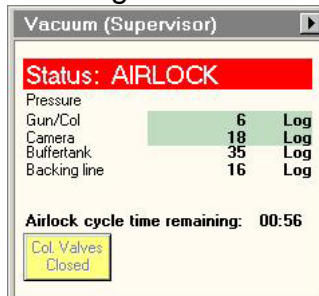


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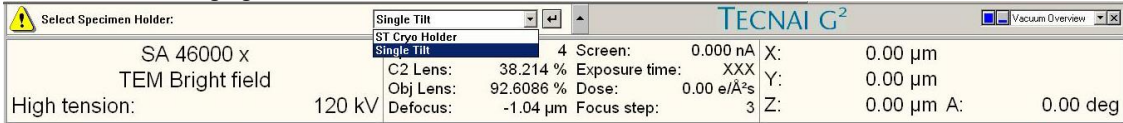
- 1) Align holder rod well with stage tube and point holder guide pin to 5 o'clock position on the goniometer (**Close** position on the purple surface), gently insert the holder until you feel some resistance. Be careful not to scrape the tip inside stage tube. Push the holder further in for 8 mm with slightly increased force.



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



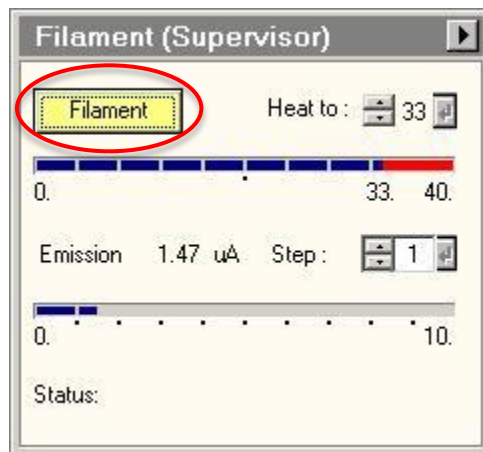
- 4) Select the specimen holder type (Single Tilt) from the box in TUI message window. Be sure to click the ↵ button to confirm the selection.



- 5) When the pumping time counting-down finishes (**Status: COL. VALVES**) and the red stage LED is off, grip the holder securely and slowly turn the holder counterclockwise (~150°). This rotation moves the guide pin approximately from 5 o'clock (Close) to 12 o'clock (Open).
  - 6) 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 14-20 log and slowly goes down.
4. Sample Holder Removal:
- 1) Remove any objective aperture (out); leave condenser aperture inserted (In).
  - 2) Set the column valves closed (**Col. Valves Closed** is yellow).
  - 3) Place the viewing screen down; cover the window with the rubber mat. Reset the **Holder** in **Stage** flap-out panel of **Search**.
  - 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. Note: experienced user can skip this step.
  - 5) 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.
  - 6) Rotate the holder clockwise (~150°) until it stops. This rotation moves the guide pin approximately from the 12 o'clock position to 5 o'clock.
  - 7) While keeping pressure on the goniometer surface with left hand, slowly pull the sample holder out to break the airlock vacuum. This will require a small amount of force.
  - 8) 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.
  - 9) Be careful not to touch the holder o-ring or any part beyond it with bare hands.

## IV. Emission Current

1. After insert sample holder, wait for GUN/Col vacuum drops < 10 log, make sure the **Heat To** value to the desired cathode temperature (**33** or check the logbook for recently used settings) and **Step to 1** in **Filament** control panel. If a change was





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made, click the ↵ button to confirm.

2. 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 (~7 mins) to reach target temperature is visible at the bottom of **Filament** control panel. Note: experienced user only need to do this step for first sample.

Note: The emission “Step” setting controls the bias voltage on the Wehnelt cylinder. A higher step value decreases the bias. This produces more emission current but increases the energy spread and source size of the beam.

## V. Alignment

**Note:** Always align the microscope from the top (gun) down. Press F1 on keyboard at any time for online help with an alignment. Begin with the objective apertures removed. Leave a condenser aperture inserted to avoid specimen damage from high beam intensity. Steps 2,3,7,8,9 are for experienced user only. Facility staffs will check those steps several times in a week to guarantee they are good for screening purpose.

1. Finding the Beam
  - 1) Wait for GUN/Col vacuum drops around 10 log.
  - 2) Click the **Col. Valves Closed** button to open the column valves (button color changes from yellow to grey and **Status: Ready**) lower fluoro-screen to find the beam. If no beam is visible, try decreasing the magnification to M range (or even to LM range) before moving the specimen stage, in case a grid bar is blocking the beam path.
2. Gun Tilt (experienced user)
  - 1) Set the microscope magnification in the 10-60 kx range and set spot size to 3.
  - 2) Center the beam using the beam shift (LCP **Trackball**) and spread the beam (LCP **Intensity**) clockwise from crossover to 2/3-3/4 the size of the fluoro-screen. Make sure Screen current and Exposure Time are in decent values

Note: If the beam is not circular, roughly adjust the condenser stigmatism (see step 5 below).

- 3) Select **Gun Tilt** from the **Direct Alignments** control panel in the **Tune** tab.
- 4) Adjust the gun tilt using the **Multifunction (MF) X/Y** knobs (LCP & RCP) to produce the brightest beam by minimizing the Exposure time. The +/- button above MF-X on LCP controls the sensitivities of MF knobs.
- 5) Press **Done** in **Direct Alignments**.
3. Gun Shift (experienced user)
  - 1) Select **Gun Shift** from the **Direct Alignments** control panel in **Tune** page.
  - 2) Set the Spot size to 9.
  - 3) Converge the beam to crossover using the **Intensity** (LCP) knob and center the beam using the beam shift (LCP **Trackball**)

Note: if the beam moves significantly when changing the intensity, roughly center the condenser aperture (see step 4.) before proceeding.

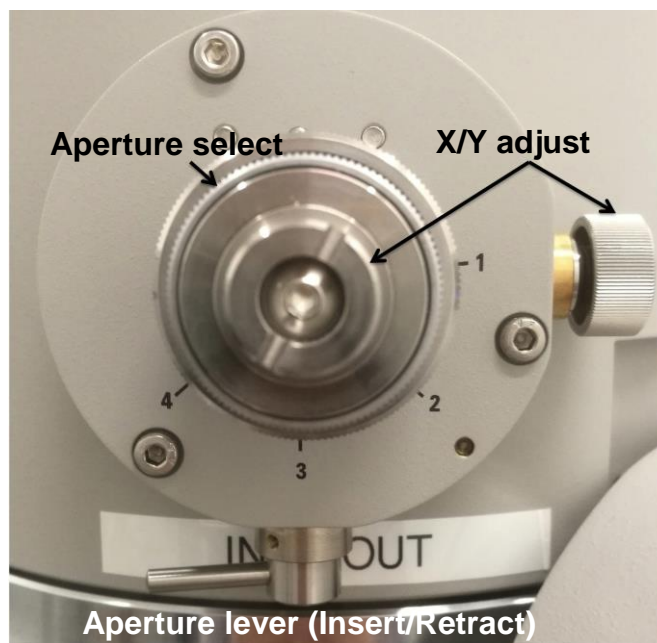
- 4) Set the Spot Size to 3.
- 5) Converge the beam to crossover and center the beam using the gun shift (**MF X/Y**).





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- 6) Repeat steps 2) - 5) until the beam does not move when changing between spot sizes 3 and 9.
  - 7) Press **Done** in **Direct Alignments**.
  - 8) If the gun shift must be adjusted a substantial amount and it results in a significant change in the measured exposure time at a given spot size, re-check the gun tilt alignment.
4. Centering the Condenser Aperture
- 1) Select the desired condenser aperture (4 is largest; 1 is smallest) using the large, outer knob on the aperture.
  - 2) Converge the beam to crossover (LCP **Intensity**) and center the beam using the beam shift (LCP **Trackball**).
  - 3) Spread the beam (**Intensity** clockwise from crossover) to 2/3-3/4 the size of the fluoro-screen and center the beam using the X and Y knobs on the aperture (not **MF X/Y** knobs).
  - 4) Repeat steps 2) and 3) until the beam spreads evenly across the screen.



#### 5. Condenser Astigmatism

Note: Condenser lens astigmatism should be corrected when the beam does not expand in a circular fashion when the **Intensity** knob is adjusted. Astigmatism must be corrected separately for each spot size that is used.

- 1) Converge the beam to crossover (LCP **Intensity**) and center it with the beam shift (LCP **Trackball**).
- 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.

#### 6. Specimen Eucentric Height Adjustment

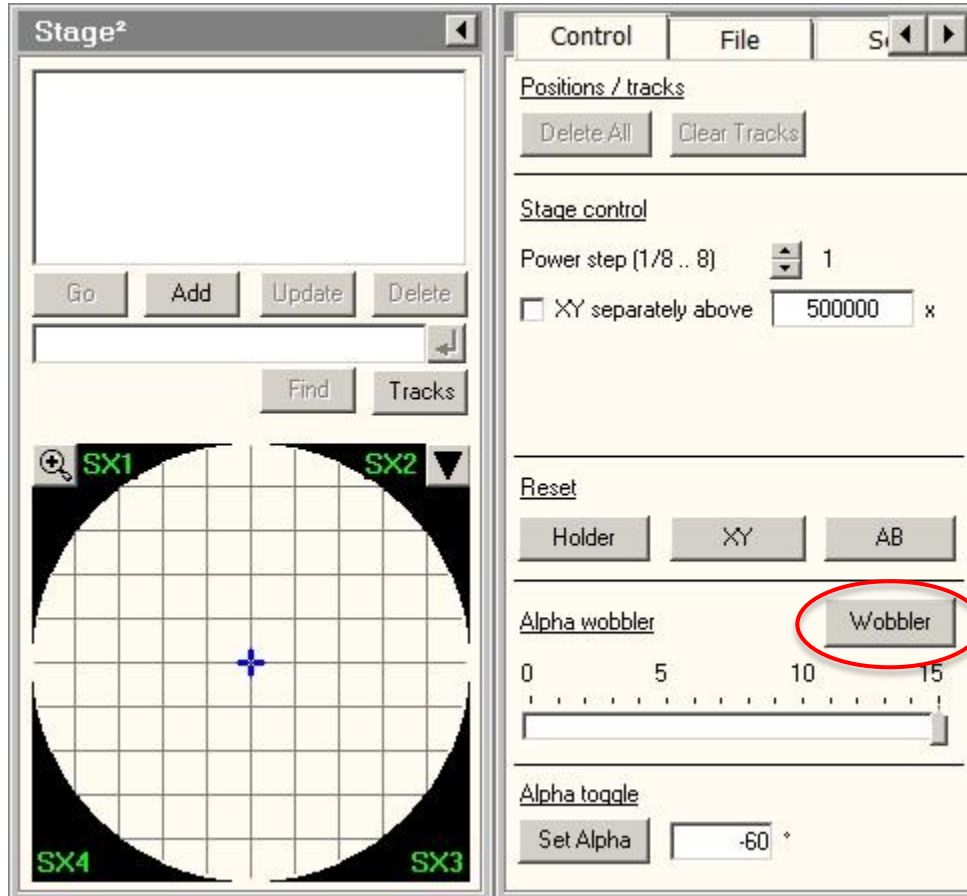
Note: All alignments beyond this point depend on the objective focus. To ensure proper



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alignment, both the specimen and objective lens focus must lie on the eucentric plane of the microscope. This step will position the specimen.

- 1) Find a point of interest on the specimen using the stage **Joystick** (RCP).
- 2) Activate the **Alpha wobbler** in **Stage** control panel (flapout). The stage will begin rocking through a tilt range of  $\pm 15^\circ$ .



- 3) Minimize the specimen movement by adjusting the **Z-axis** control buttons (RCP). These buttons are pressure sensitive; pressing harder = faster change.
- 4) Deactivate the **Alpha wobbler**.
- 5) Click **Eucentric focus** button (RCP) to reset objective lens on eucentric plane.

Alternatively: the eucentric height can also be set by using the **Eucentric focus** button (RCP) to focus the objective lens on the eucentric plane. The specimen can then be brought into focus using the **Z-axis** buttons (RCP). When it is in focus, specimen is located at the eucentric plane of the microscope.

Reference: Single tilt eucentric height:  $\sim 36 \mu\text{m}$

Reference: Cryo holder eucentric height:  $\sim -72 \mu\text{m}$

## 7. Beam Tilt Pivot Points (experienced user)

- 1) Focus (RCP **Focus**) the specimen to minimum contrast.

Note: the **Focus** knob has two parts. The smaller, inner knob changes the focus. The larger, outer knob adjusts the **Focus step** i.e., how much the focus changes with each movement of the inner knob.



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- 2) Move to a non-beam-sensitive area of the sample. Converge (LCP **Intensity**) the beam to crossover and center it (LCP **Trackball**).
- 3) Select **Beam tilt pp X** from **Direct Alignments** control panel.
- 4) Use the **MF X/Y** knobs to superimpose the two beam spots and minimize the beam movement.
- 5) Press **Done** in **Direct Alignments**.
- 6) Repeat steps 2) - 5) for **Beam tilt pp Y**.

8. Beam Shift (experienced user)

- 1) Converge the beam to crossover (LCP **Intensity**) and center it using the LCP **Trackball**.
- 2) Select **Beam shift** from **Direct Alignments** and use the **MF X/Y** knobs to re-center the beam on the screen.
- 3) Press **Done** in **Direct Alignments**.

9. Rotation Centering (experienced user)

Note: Rotation centering is the most important alignment for high-resolution work on this microscope. It should be done carefully and at or above the magnification that will be used for imaging.

- 1) Find a suitable area of the specimen and focus (RCP **Focus**) to minimum contrast at a magnification (RCP **Magnification**) above 100kx.
- 2) Select **Rotation center** from **Direct Alignments**. Use the **MF X/Y** knobs to minimize image movement. The center of the image should pulse in and out of focus, but there should be little or no in-plane movement. The amplitude of the image wobbling can be controlled by the **Focus step** knob (outer ring of the RCP **Focus**).
- 3) Press **Done** in **Direct Alignments**.

Note: If the beam moves appreciably while the rotation center alignment is active, readjust the X and Y pivot points and beam shift (steps 7 and 8) and repeat.

Note: The binoculars can be used to more accurately focus and perform alignments, particularly the rotation center. To use the binoculars, gently raise the small viewing screen (lever, left side of column base).

Note: The eyepieces of the binoculars can be focused by rotating. Inserting the beam stop (knob, just above viewing chamber on right side of column) can aid in focusing the binoculars: adjust each eyepiece until the shadow of the beam stop appears sharp.

10. Objective Aperture Centering (See condenser aperture figure) – Skip if no objective aperture is desired.

- 1) Select an appropriate magnification (50-100kx is a good starting point), select and insert the desired objective aperture (see aperture figure in step 4).
- 2) Enter diffraction mode (RCP **Diffraction**) and center the direct-beam spot (central diffraction spot) using the **MF X/Y** knobs. The aperture should be visible in the diffraction plane with camera length ~ 500mm.
- 3) Center the aperture on the central spot (aperture X and Y knobs, not **MF X/Y**).
- 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, as this can further lose it. Remove the aperture and return to imaging mode.



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Decrease magnification to low mag. mode (“LM” visible by magnification value) and reinsert the aperture. It should be visible in the image plane and can now be roughly centered. Return to the desired magnification and repeat steps 1) - 4).

#### 11. Objective Astigmatism and Focus

- 1) Select an appropriate magnification (50-100kx is a good starting point) and an amorphous region of the sample (support film).
- 2) Objective astigmatism is most easily corrected using the CCD camera and live FFT functionality (see Section VI for more details on the CCD camera). Begin collecting “search” images on the camera. In TIA, click **FFT** to show a real-time fast Fourier Transform of the image.
- 3) You should be able to 1 or 2 Thon rings in **FFT**. Use **Focus** knob on RCP to focus beam on sample/supporting film (outer ring of Focus knob is to set focus step size). The size of Thon ring will increase when beam is moving closer to focus on sample and the size should be infinite large when focusing on sample. Then set defocus to -1um to -1.5um.
- 4) Click **Objective** in the **Stigmator** control panel or press LCP **Stigmator** button.



- 5) 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.
- 6) 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.

## VI. Camera Control and Imaging





## 1. Camera Operation

- 1) The CCD camera is operated by the **CCD/TV Camera** control panel in the **Camera** tab.
- 2) Lift the fluore-screen (RCP button **R1**) so the beam can reach the camera.
- 3) There are three camera modes:

**Search** is a high refresh rate, lower resolution mode used for viewing and focusing on the specimen in real time. Shorter integration time (0.25 sec) and larger bin number (4) are recommended for Search.

**Preview** is a slower, higher resolution mode that uses half the CCD area to preview the final acquired image quality.

**Acquire** is used to capture a single image from the whole CCD detector. Long integration time (1-2 sec) and small bin (1-2) are recommended for Acquire.

Note: The parameters for any of these settings can be configured using the **Settings** flap-out panel on the **CCD/TV Camera** control panel.

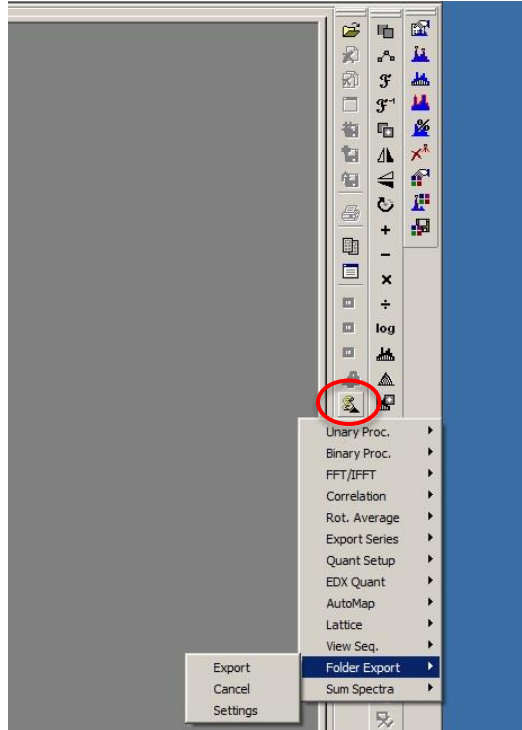
## 2. Saving Images

Note: Images should be saved to the support PC, never to the microscope PC. Any files on the microscope PC are subject to deletion without warning.

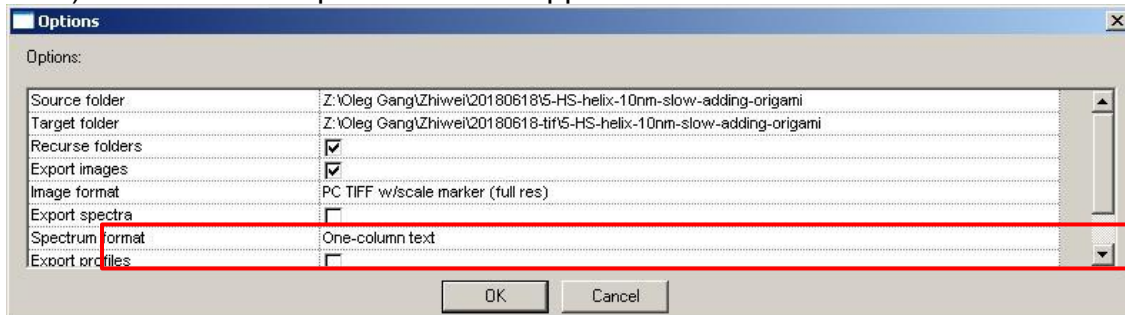
- 1) Save files to the location Z:\<advisor directory>\<user directory>

Note: I recommend that each user create a directory with advisor's name in Z:\. All users from the same lab must put data within the advisor's directory, each user can create his/her own subdirectory.

- 2) Click **Save as** or **Auto Save** to save the images in \*.emi format. Alternatively, right click on the image and select **Export** to save the image as tif file with scale marker and full resolution.
- 3) Export all \*.emi files: after capturing all the images of 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>\.....) and where to save exported tif files. Then click **Export** in **Component > Folder Export** to export all the emi files to tif files.

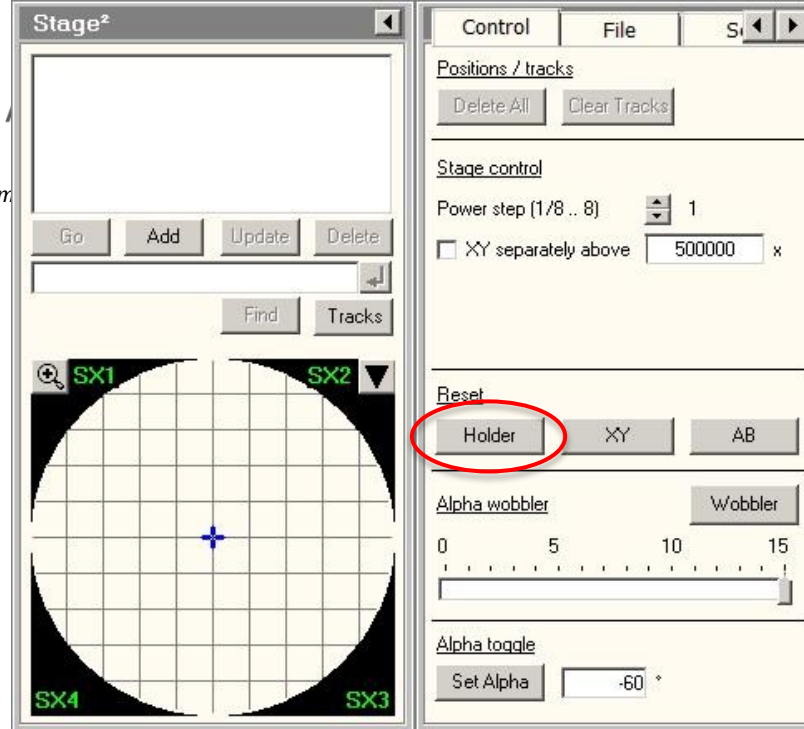


4) Files can be copied from the support PC.



## VII. Switch Samples

- 1) Remove any objective aperture (out); leave condenser aperture inserted (In).
- 2) Set the column valves closed (**Col. Valves Closed** is yellow).
- 3) Place the viewing screen down; cover the window with the rubber mat.
- 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.  
Note: experienced user can skip this step.
- 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 A are zero or close to zero.



- 6) Wait until the **Filament** reading below 10, remove the sample holder, remove your sample, load another sample, and insert the holder back to the stage. See section III for details.
- 7) Switch the filament on by clicking **Filament** button (its color changes to yellow) in the **Filament** control panel. Required time (~7 mins) to reach target temperature is visible at the bottom of **Filament** control panel.
- 8) Find beam, move sample to eucentric height, insert objective aperture if needed and use Camera for imaging.

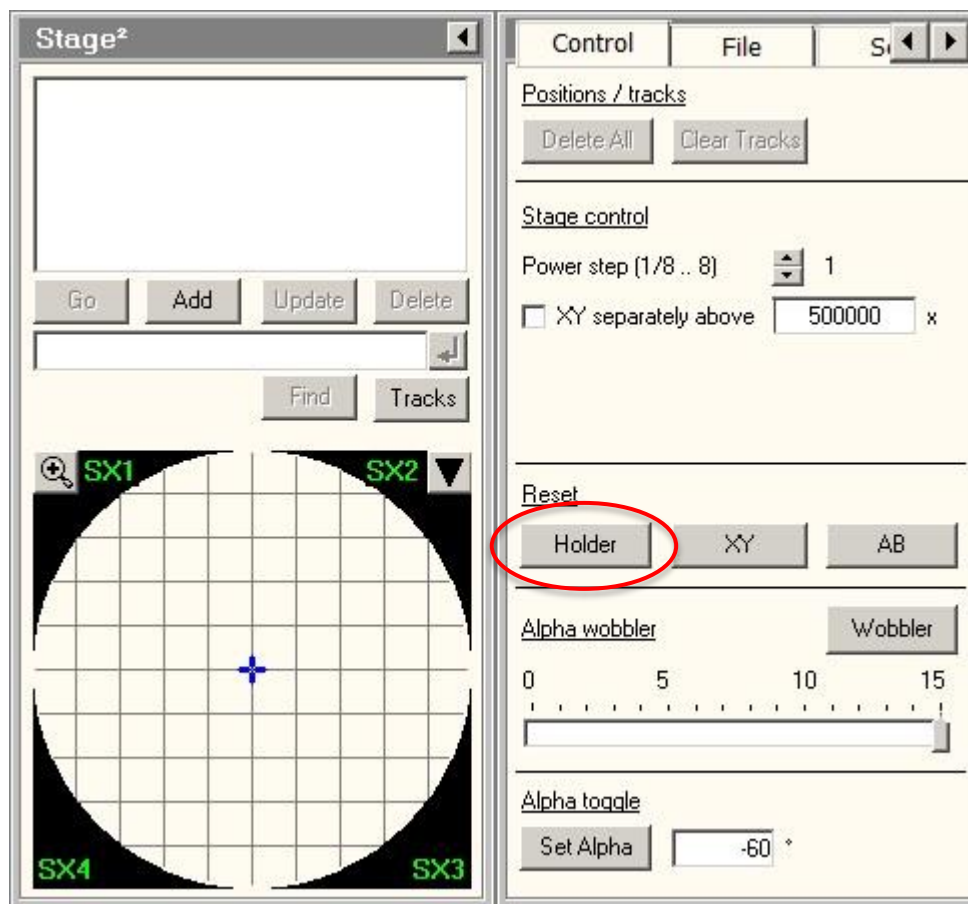
## VIII. End of Session

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



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- 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, remove your sample, See section III for details.
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) You are done from your session, clean up TEM table and sample prep area.
3. If you are the last user of the day:
  - 1) Switch HT off by clicking **High Tension** button (its color changes from yellow to grey) in the **High Tension** control panel. Choose 20 kV in the drop-down HT list.
  - 2) Disable **FEI Spirit 120kV TEM** in Badger.
  - 3) Remove the LN2 dewar, empty the LN2 in the dewar, and place the dewar upside-down on the table. Place a paper towel below the cold finger on the microscope to collect condensing moisture.
  - 4) Watch Gun/Col vacuum reading on **Vacuum** tab of **Setup** panel, **run the Cryo Cycle immediately after Gun/Col vacuum rises above 20 Log. Cryo Cycle** is located in the flap-out panel from the **Vacuum** tab; Standard settings are **Start after** = 0 min, **Duration** = 240 min.





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

5) You are done fro your session, clean up TEM table and sample prep area.

Pressure			
Gun/Col	6	Log	
Camera	18	Log	
Buffertank	35	Log	
Backing line	59	Log	

Col. Valves Closed

Cryo Settings Control

Start after: 0 min

Duration: 240 min

Remaining time: min

- Airlock

Prepump Airlock Empty Buffer Cryo Cycle Step Back

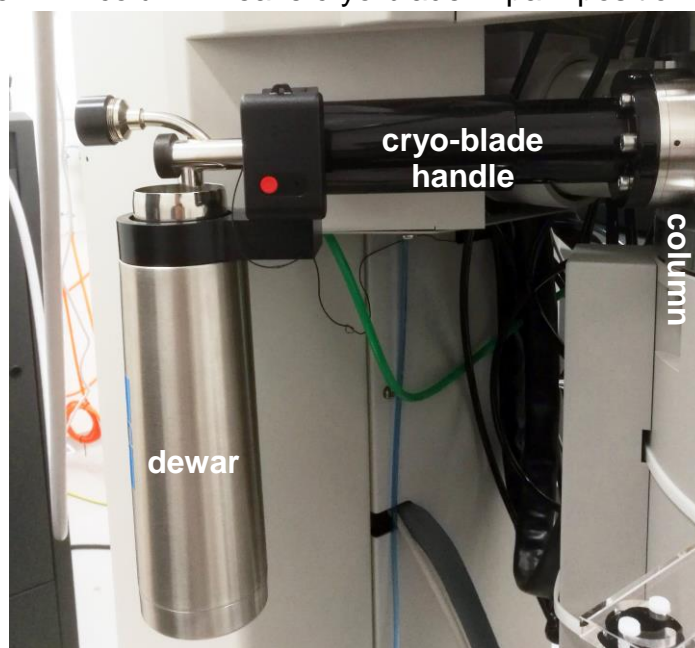


## Appendix

- **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. Leave cryo-blade in park position 0 or P1.



2. Specimen Loading:
  - 1) Remove sample holder from pumping station (see pumping station SOP for details).
  - 2) Gently and fully insert the sample holder horizontally into the cryo-workstation.

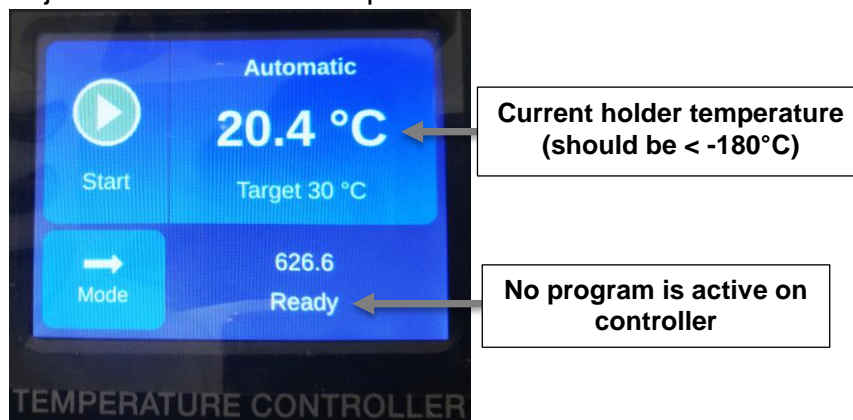




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- 3) Keep cryo-holder connected to its controller, fill LN2 to the holder dewar and cryo-workstation, wait for the holder temperature to drop below  $-180^{\circ}\text{C}$  (minimum) before loading grid to the holder.

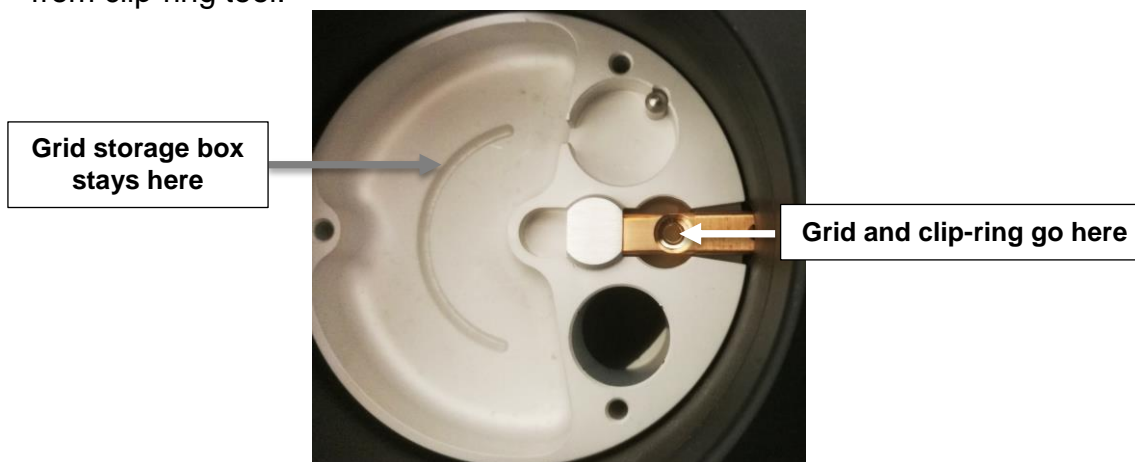
Note: keep surface of LN2 just above the holder tip.



- 4) 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 touch the storage box, sample grids, and holder tip must be precooled to LN2 temperature.

- 5) 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.
- 6) Secure the clip-ring on the clip-ring tool, load clip-ring straight down to holder tip and push down straightly to secure clip-ring on the holder tip, release clip-ring from clip-ring tool.



- 7) **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.**

- 8) Close specimen shutter on holder; fill LN2 in transfer station to just over holder tip.

Note: specimen shutter must be close all the time during holder insertion into and removal from microscope.

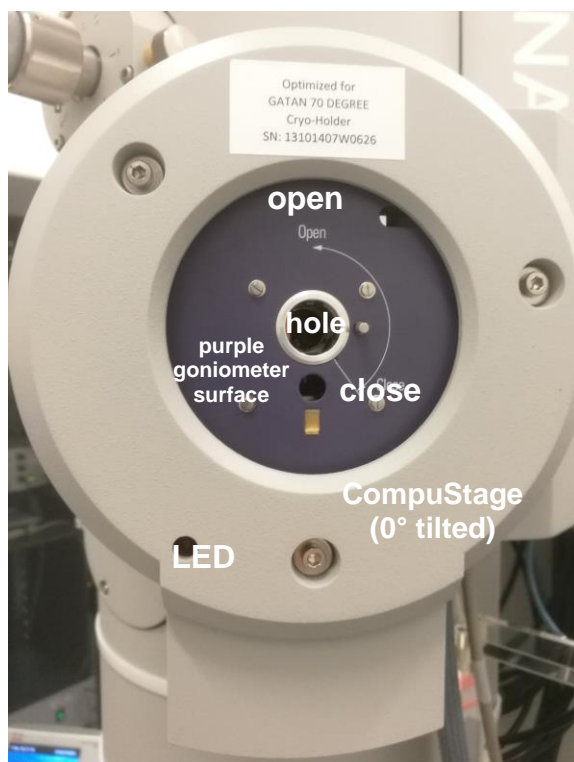
3. Sample Holder Insertion:



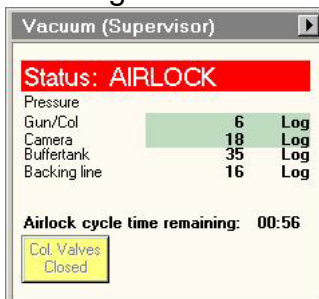
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Note: Before inserting or removing the sample holder, make sure that the column valves are closed (**Col. Valves Closed** button is yellow), cryo-blade is in park position 0 or P1, the objective aperture is not inserted (aperture lever is switched 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) Click **Prepump Airlock** and wait for ~10 sec to finish.
- 3) Gently and quickly retract holder from cryo-workstation, align holder rod well with stage tube 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. Continue without stop to push the holder further in for 8 mm with slightly increased force.



- 4) 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.
- 5) The airlock pumping time counting-down is visible in the **Vacuum** control panel.

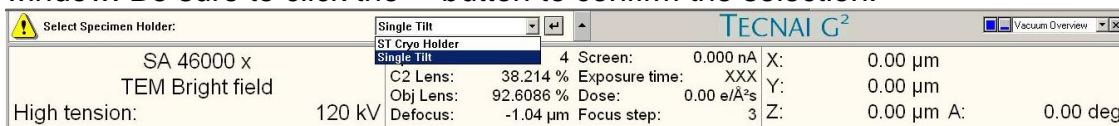


- 6) Select the specimen holder type (ST Cryo Holder) from the box in TUI message

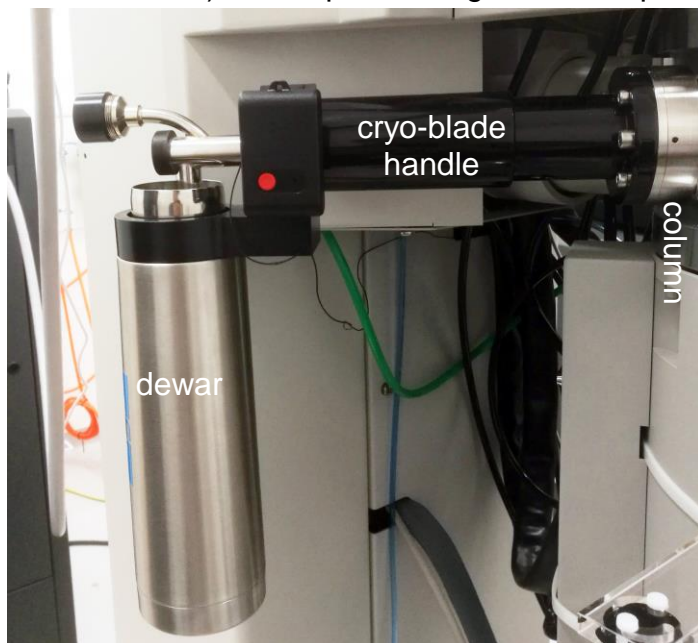




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- 7) When the pumping time counting-down finishes (**Status: COL. VALVES**) and the red stage LED is off, Turn the holder counterclockwise 150°. This rotation moves the guide pin to 10 o'clock (Open). Remember the stage is still tilted at -60°.
- 8) 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) Tilt CompuStage back to 0° by clicking **Search** control tab>**Stage** (flapout)> **Set Alpha: -60°**.
- 10) Insert Gatan cryo-blade from park position 0 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.



- 11) Open specimen shutter on cryo-holder and wait for 1 min before opening column valve.
- 12) 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), cryo-blade is in park position 0 or P1, 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.

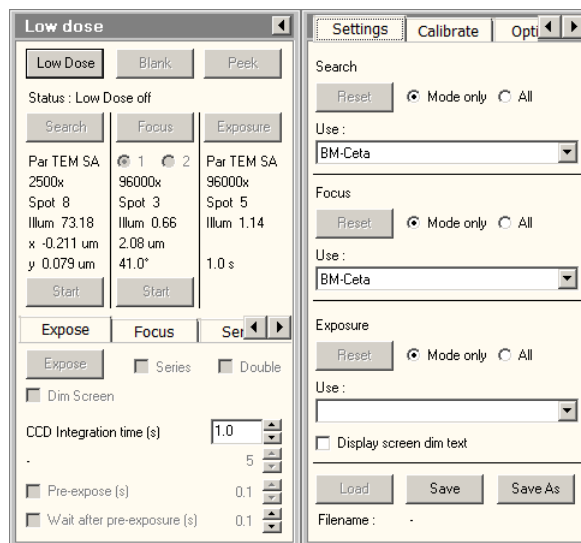


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- 2) Close shutter on the holder and tilt CompuStage  $-60^\circ$  by clicking **Search** control tab>**Stage** (flapout)> **Set Alpha:  $-60^\circ$** .
- 3) Always use left hand to keep light pressure on the purple goniometer surface when removing 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) Gently and quickly insert holder into precooled cryo-workstation if you plan to load another grid.
- 9) If you finished your cryo-imaging session. Empty LN2 from the holder dewar after removing it from microscope and insert sample holder into the pumping station, open valve 1 and 2 on the pumping station, connect the cable, start Warm-up cycle or Automastic with new controller (see pumping station SOP for details).

- **Low Dose Setup for CryoEM (carbon film grid only)**

1. Click **Low Dose** button in **LowDose** panel in workset to enter low dose mode (Figure below).



2. Set up individual LD mode. Use CCD camera for all three modes.
  - a. **SEARCH** mode: choose larger spot size 5-8, low magnification that must be in the same SA range with **FOCUS** and **EXPOSURE** (the lowest magnification in SA range is 2400x), spread beam. Apply large defocus (50-100 um) to achieve good contrast. Note: the principle of setting up **SEARCH** is to keep minimal but enough electron density at sample to observe sample on CCD camera.



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- b. **FOCUS/EXPOSURE** mode: use the same settings for the two modes, choose magnification of 40-100 kx, spot size 3-5, use small fluo-screen to measure dose rate to be around 30-60 e/A<sup>2</sup>/s.
  3. Set up connections between LD modes.
    - a. **EXPOSURE** mode: this is most important of all three modes. Perform **Direct Alignments** and center a feature (a big black dirt, holey carbon holes, or corner of copper bar, it should be something that has good contrast and easy to center) by moving stage (joystick on right control pad).
    - b. Go to **SEARCH**, adjust **MF X/Y** (they should be Image Shift) to center the same feature that is centered in **EXPOSURE** mode.
    - c. Go to **FOCUS**: make sure it has the same microscope setting with **EXPOSURE**, define focus distance and angle. Focus distance should be at least 2 times of beam size; focus angle can be set to 0-360°, for Cflat/Quantifoil grid, use focus distance and angle to set focus position in the center of four grid holes.
  4. Always suggest to cycle LD modes: **SEARCH>FOCUS>EXPOSURE>SEARCH**. Find area of interest for imaging in SEARCH, define defocus in FOCUS using Thon ring method (-2.5um for cryo-imaging), and take image in EXPOSURE.