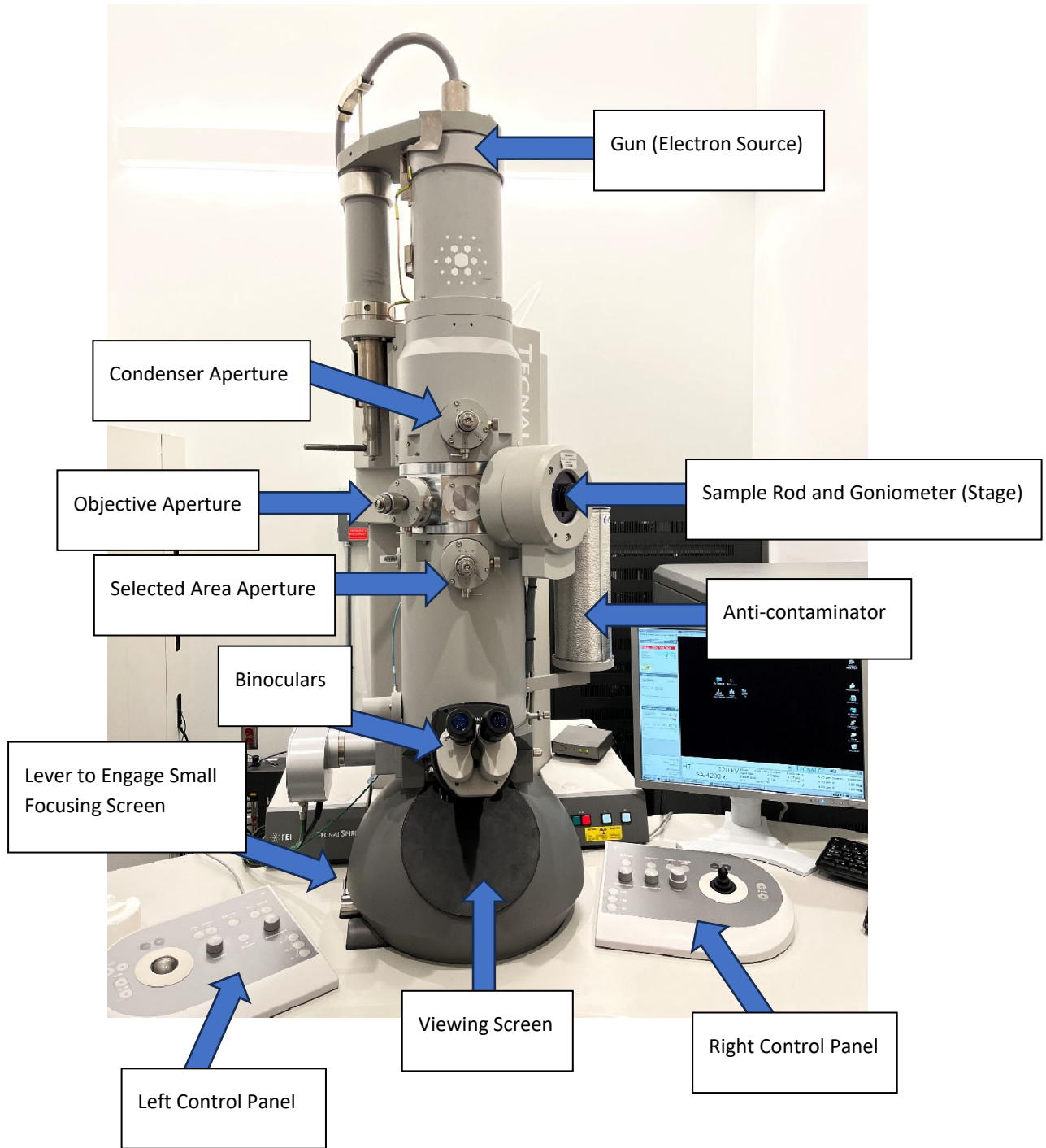


USER GUIDE FOR THE TECNAI G2 SPIRIT BIOTWIN TRANSMISSION ELECTRON MICROSCOPE

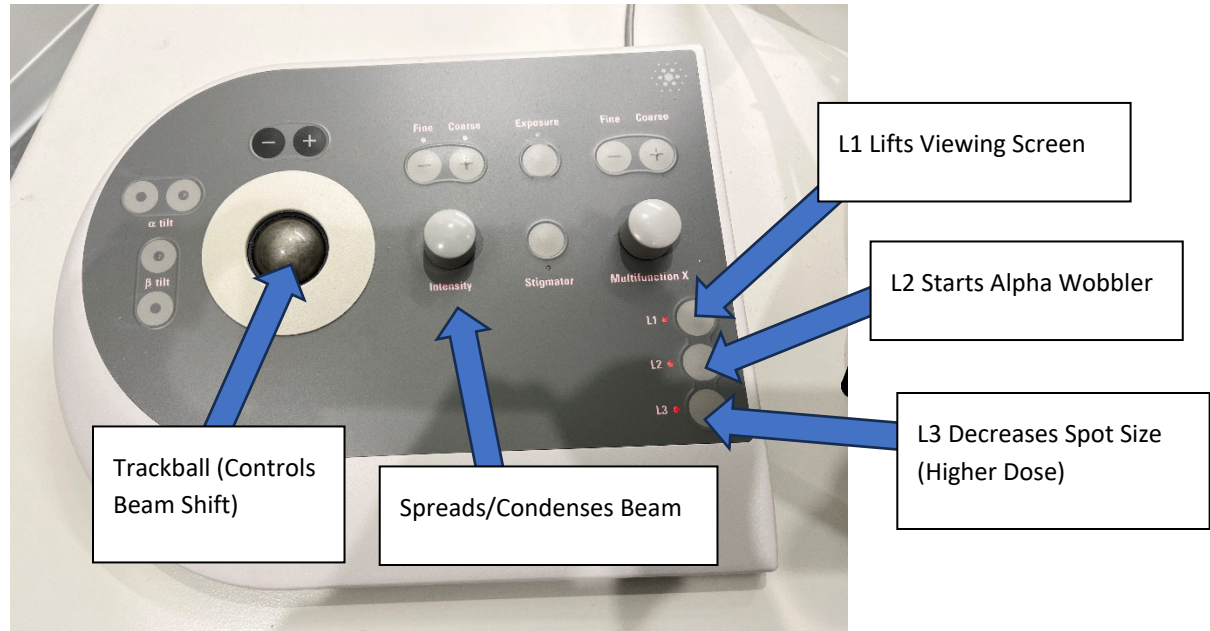
Bio-Imaging Center (Updated 10/12/2023)

Created by Shannon Modla

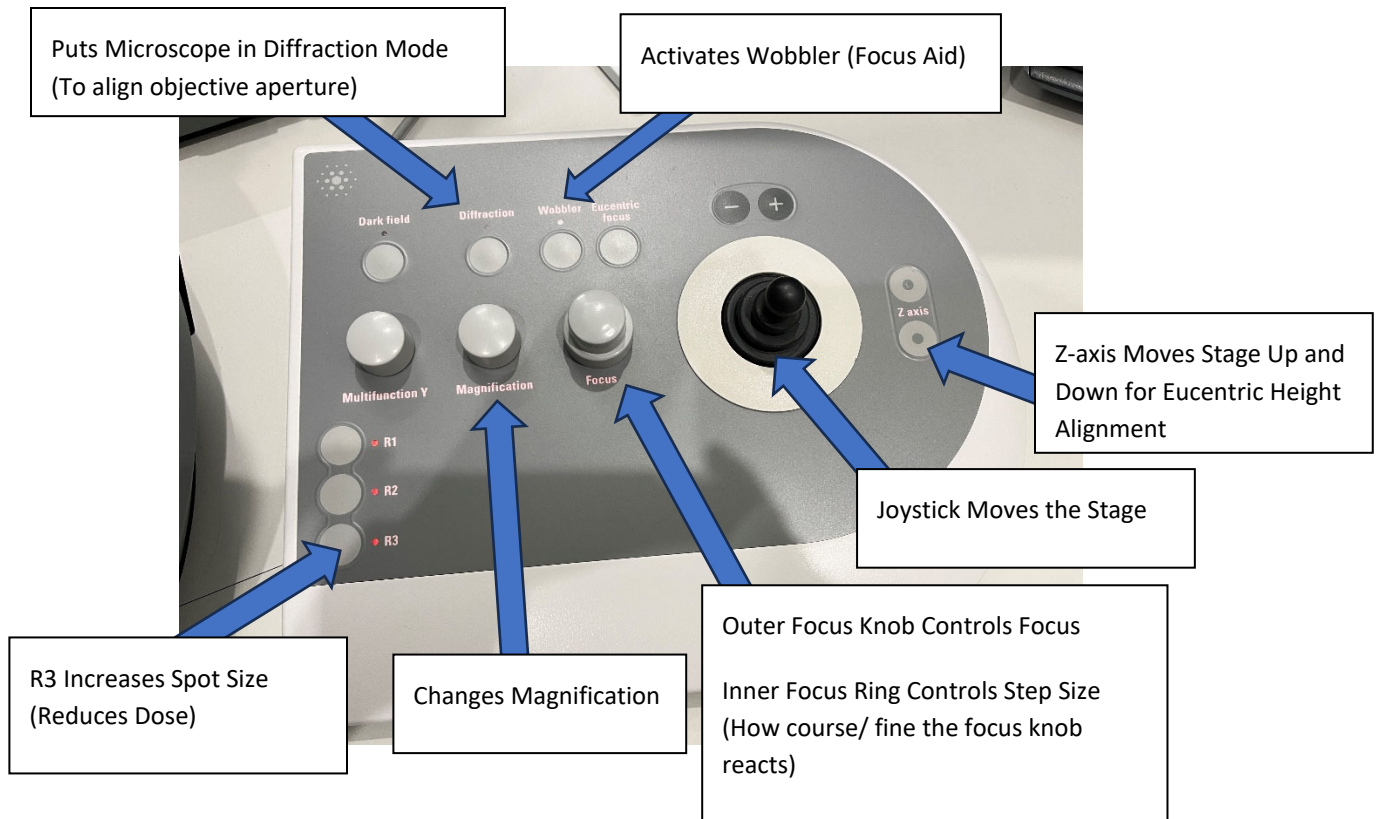
Overview of the Tecnai G2 Spirit BioTWIN TEM



Left Control Panel



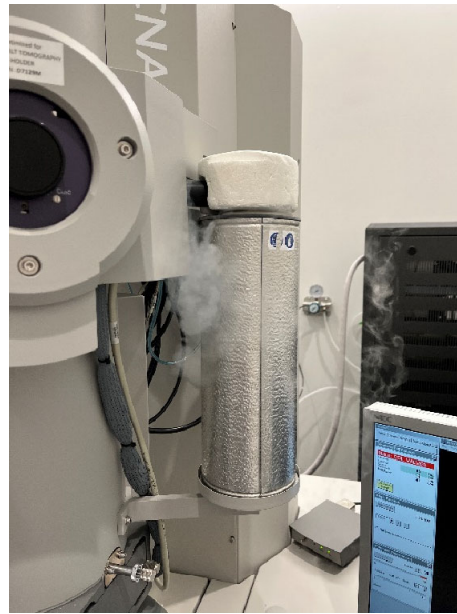
Right Control Panel



User Guide for the Tecnai G2 Spirit BioTWIN TEM

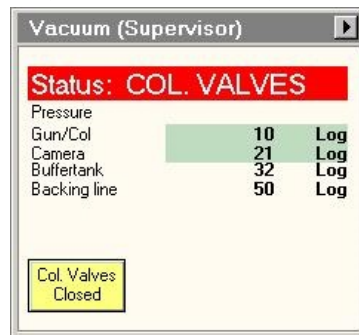
STEP 1: Fill the anticontaminator with liquid nitrogen

- Why is this important?
 - Cooling the anticontaminator helps improve the vacuum and keep the column cleaner. The microscope should always be operated cooled.
- Procedure:
 - Place the anti-contaminator dewar on the platform next to the column with the copper wires extending into the dewar. Fill the dewar halfway with liquid nitrogen, wait until the boiling stops, and fill to the top. Place the Styrofoam cap on top of the dewar when finished.
 - The dewar needs to be refilled every two hours.

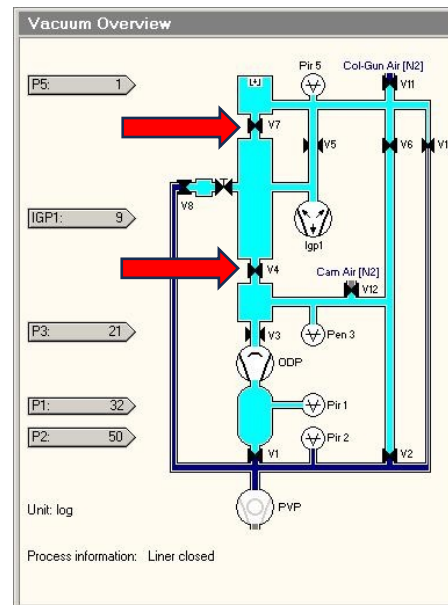
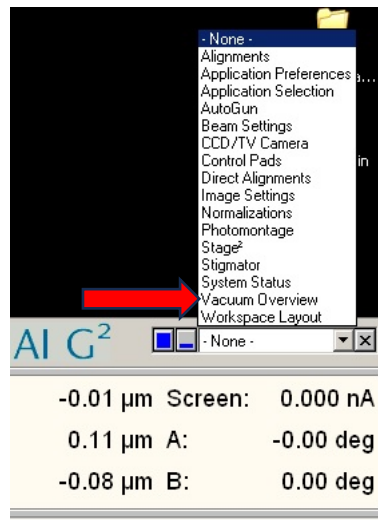


STEP 2: Check the vacuum status

- Why is this important?
 - The TEM should only be used if the vacuum level is within operational range. Electrons require a high vacuum to form an image.
- Procedure:
 - The header to the vacuum box should say "STATUS: COL. VALVES" with a red background and the Gun/Col and Camera values should have a green background. The column valves are closed, and the vacuum is in an operational range. Do not use the system if the cryo-cycle is running.



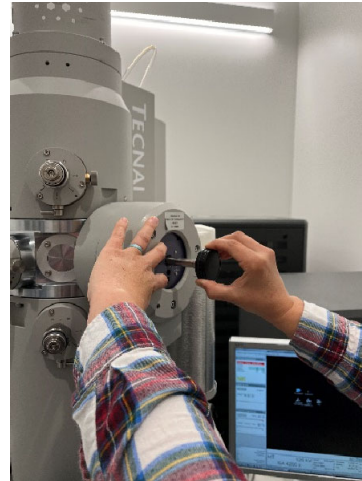
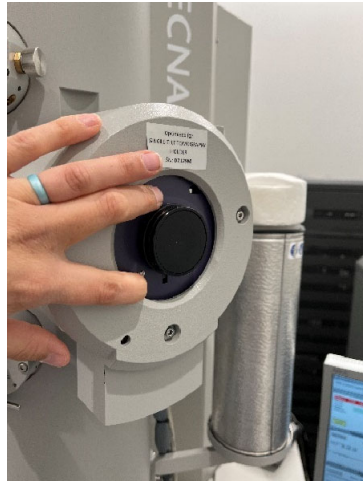
- If the Col. Valves button is yellow, this means the column valves are closed. If the Col. Valves Button is gray, this means the column valves are open. If you want visual confirmation to check on the status of the column valves, access the Vacuum Overview page:



Notice the valves (V7 and V4) are closed

STEP 3: Remove Sample Rod

- Procedure:
 - The column valves must be closed before you remove the sample rod.
 - Press against the plate surrounding the sample rod with two fingers as you retract the sample rod. Pull out the rod until you feel it stop. By pressing against the plate with your fingers, this minimizes movement and reduces the odds of breaking the vacuum.



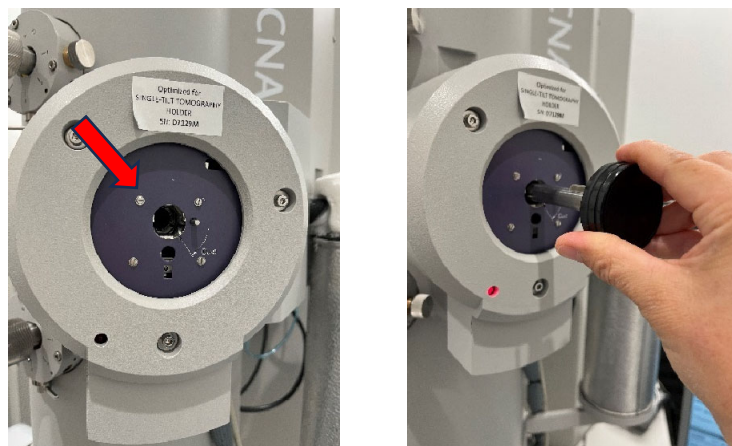
- Rotate the rod clockwise until it stops. This closes the ball valve that separates the airlock from the column.
- Pull the sample rod out completely. Do not touch the sample rod anywhere below the O-ring with your bare fingers. This area needs to be kept clean as it goes into the high vacuum of the microscope.
- Insert the sample rod into the clear plastic holder. Use the pin to lift the grid clamp. Insert your grid, ensuring it is flat within the recess. Lower the clamp using the pin. It is always a good idea to check that the grid is secure within the sample rod before re-inserting it into the column.



Use the pin to lift the grid clamp

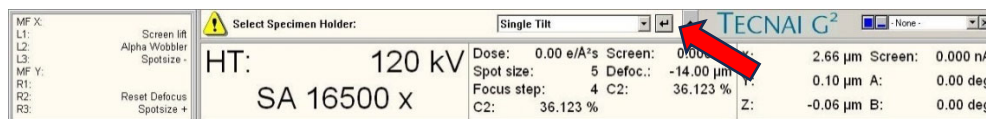
STEP 4: Insert the Sample Rod

- Procedure:
 - Align the rod so the large silver pin is lined up with the upper left screw of the outside of the goniometer.
 - Insert the sample rod. The red light on the goniometer will turn on and you will hear the pump start to evacuate the air lock.

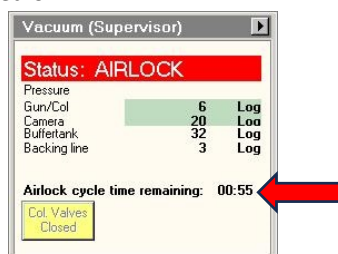


Align silver pin to the screw (arrow) located to the upper left of the hole

- Select single tilt when prompted for the specimen holder.



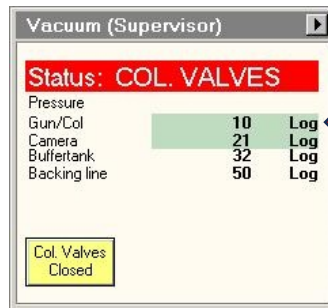
- Wait until the airlock cycle counts down to zero. The airlock will be evacuated for 2 minutes before you can insert the sample rod. Do not insert the sample rod until the airlock cycle is complete and the red light has turned off.



- Once the red light is off, rotate the sample rod counterclockwise, which will open the ball valve and allow the rod to go into the high vacuum of the column. The vacuum of the column will pull in the rod, so you do not have to push it to make it go into the column.

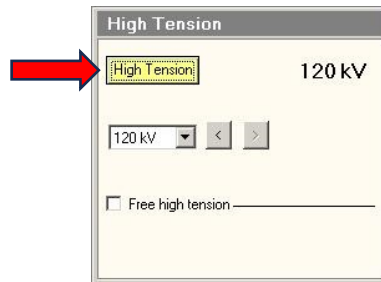
STEP 5: Check the vacuum status again

- Procedure:
 - Wait until the Gun/Col vacuum is <20 Log before proceeding.



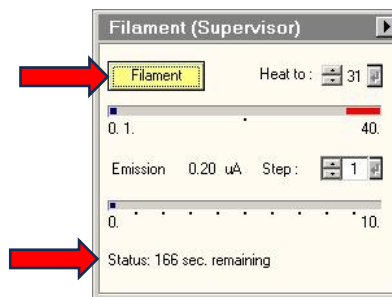
STEP 6: Turn on high tension (HT).

- Click the High Tension button to turn on the high tension. When the HT is on, the HT button turns yellow.



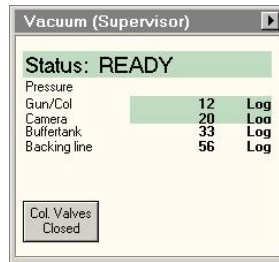
STEP 7: Turn on the filament.

- Click the Filament button to turn on the filament. When the filament is turned on, the Filament button turns yellow. Wait until the filament ramps up to the saturation point. It takes about 165s for the filament to reach saturation.



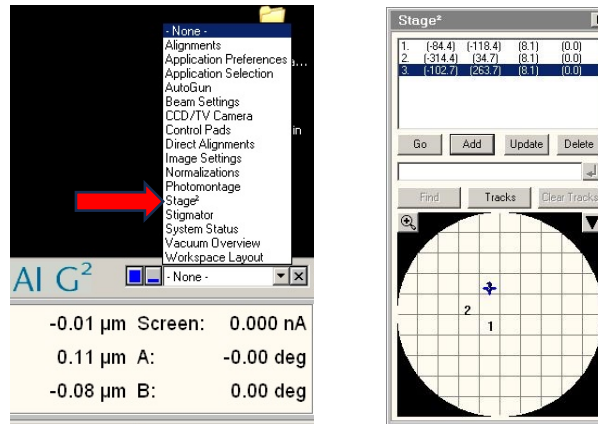
STEP 8: Open the column valves by pressing the Col. Valves

- When the column valves are opened the Col. Valves Closed button turns gray and the header to the Vacuum box says Status: Ready with a green background.

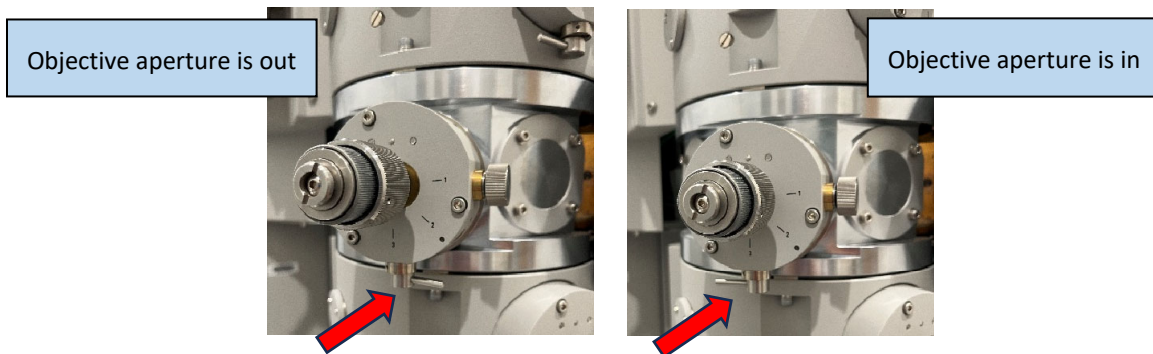


STEP 9: Find a region of interest on your grid.

- It is best to start at a lower magnification to find a suitable area and then increase the magnification. If you want to save locations on your grid, this can be done under Stage², which can either be accessed in the Search Tab or the dropdown menu in the lower right corner.



- When you drop the magnification within the LM mode, you will have to retract the objective aperture to see the entire field of view. The levers under the apertures are used to insert and retract them from the optical path. When the lever is pointed to the right, the aperture is out. When the lever is pointed to the left, the aperture is in.

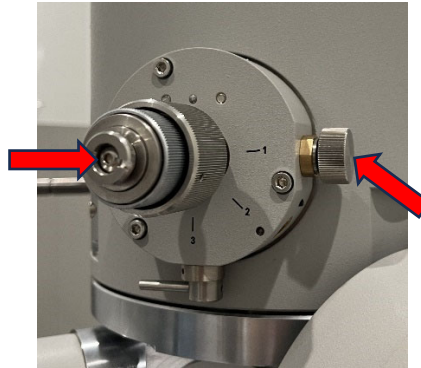


- When returning to a high magnification, remember to re-insert the objective aperture. At higher magnifications, the objective aperture enhances the contrast of the sample.

STEP 10: Basic Alignments

1. Align the C2 aperture.

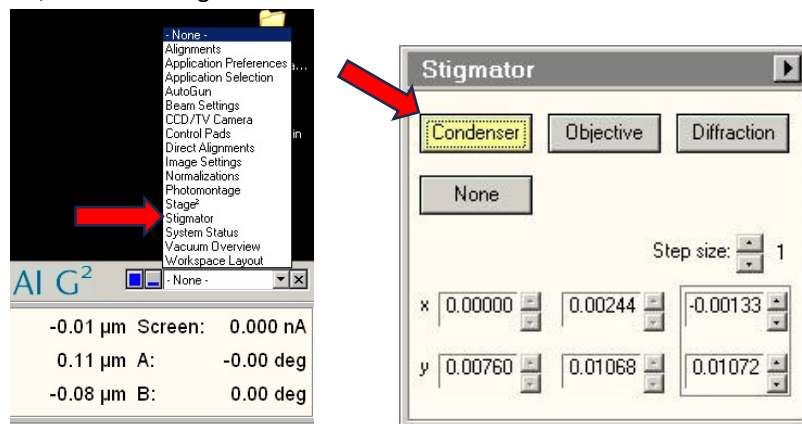
- Use the Intensity knob to bring the beam to crossover.
- Center the beam to the middle of the viewing screen using the track ball, which controls beam shift.
- Expand the beam to the 40 mm circle on the viewing screen.
- Always expand the beam by rotating the intensity knob clockwise from crossover.
- If the beam is no longer centered on the 40 mm circle on the viewing screen, use the two condenser aperture knobs (arrows below) to center it.



- When the condenser aperture is properly aligned, the beam should open and close concentrically within the middle of the viewing screen.
- If the condenser aperture is not aligned, the beam will appear to swing as you go through crossover.

2. Correct C2 astigmatism if needed.

- If the beam does not appear circular, there is an astigmatism in the C2 lens. To correct for an astigmatism, access the Stigmator control.



- Click on Condenser to activate the stigmator control for the condenser lens. When active, the condenser button turns yellow and the multifunction X, Y knobs now say Cond. Stig X and Cond. Stig. Y.
- Use the MFX and MFY knobs to make the beam appear circular.
- When you are finished adjusting the stigmator, click None to deactivate the MFX and MFY knobs.
- Note: Condenser Stigmator adjustments only apply for the current spot size. This may need to be readjusted if you change spot size.

STEP 10: Basic Alignments Continued.

3. Center the objective aperture

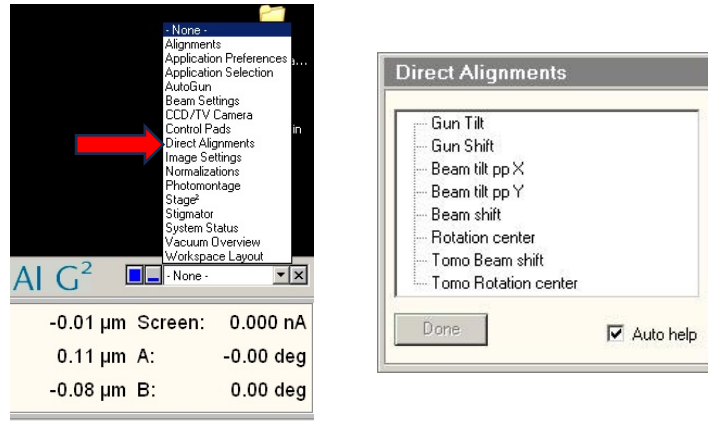
- The objective aperture improves contrast in your image.
- For the best image quality, the objective aperture should be centered. The objective aperture cannot be seen under normal imaging conditions. It can only be seen when you go into diffraction mode, which makes the back focal plane of the objective lens the imaging plane.
- Procedure:
 - Go into diffraction mode. You will see a bright central diffraction spot and a diffuse ring that delimits the edge of the objective aperture.
 - If the ring is not centered around the central diffraction spot, use the two objective aperture knobs (as you did earlier for aligning the C2 aperture) to center it.
 - Press diffraction mode again to return to normal imaging mode.

4. Find the eucentric height.

- The sample should be moved in Z to the eucentric height. The eucentric height is the vertical position within the goniometer that exhibits the best performance of the optics. All samples should be placed in the eucentric position for imaging.
- Procedure:
 - Activate the alpha wobbler (L2). This will cause the specimen to tilt back and forth from -15° to $+15^\circ$.
 - Note alpha wobbler and wobbler have two different functions. Do not confuse them. Alpha wobbler (tilts stage) is used to find the eucentric height. Wobbler (tilts beam) is used as a focus aid.
 - Minimize the specimen movement using the Z-axis up and down buttons on the right control panel. The buttons are touch sensitive, so the harder you press, the greater the change. When the specimen is at eucentric height, a feature of interest on your viewing screen will remain stationary throughout the tilt range.
- When the eucentric height is found, deactivate the alpha tilt (L2).

STEP 11: Direct Alignments

- Why are direct alignments important?
 - Direct alignments should be done once daily so the microscope operates at peak performance. They do not need to be done for every sample.
- Procedure:
 - The sample should be at eucentric height and in focus before you begin the direct alignments.
 - Access the direct alignments box and proceed from the top of the list to the bottom. If the auto help box is ticked, it will provide a help page that provides guidance for each step.



- Direct Alignments Step 1: Gun Tilt
 - Purpose:
 - Aligning the gun tilt ensures as many electrons as possible are getting from the gun to the sample.
 - Procedure:
 - Condense the beam so that it is centered around the 40mm circle on the viewing screen.
 - Click Gun Tilt from the direct alignments.
 - Observe the screen current. First adjust MFX (Gun Tilt X) until you achieve the highest possible screen current reading. Next, adjust MFY (Gun Tilt Y) until you achieve the highest possible screen current reading. Do MFX and MFY one at a time.

Dose:	21.6 e/A ²	Screen:	18.1 nA
Spot size:	4	Defoc..	-10.34 μm
Focus step:	2	C2:	35.892 %
C2:	35.892 %		

- Click done under Direct Alignments

STEP 11: Direct Alignments Continued

- Direct Alignments Step 2: Gun Shift
 - Purpose:
 - Gun shift alignment ensures the beam is going down the optical axis of the microscope.
 - Procedure:
 - Click Gun Shift, go to Spot Size 9. Condense the beam to crossover and center it using the trackball (beam shift).
 - Go to Spot Size 3. Condense the beam to crossover. Use MFX and MFY knobs (which now are assigned to Gun Shift X and Gun Shift Y) to center the beam to the viewing screen.
 - Click done under Direct Alignments
 - This is an iterative process. Repeat the steps above until the spot remains centered between Spot Size 9 and Spot Size 3.

- Direct Alignments Step 3: Beam Pivot Points
 - Procedure:
 - The sample must be in focus for these alignments to be accurate. To check the sample is in focus, activate the wobbler and minimize movement with the focus knob. It is helpful to use the small focusing screen and binoculars to focus more accurately while using the wobbler.
 - The beam should be slightly condensed for beam pivot point alignments.
 - Activate Beam tilt pp X.
 - Look at the edge of the beam, not the sample itself.
 - Use MFX and MFY to minimize the movement at the edge of the beam. Do MFX and MFY one at a time.
 - Click done under Direct Alignments.
 - Repeat the above procedure for Beam tilt ppY.

- Direct Alignments Step 4: Beam Shift
 - Procedure:
 - Activate Beam shift
 - Use MFX and MFY (which now are assigned to Beam Shift X and Beam Shift Y) to center the beam to the viewing screen.
 - Click done under Direct Alignments.

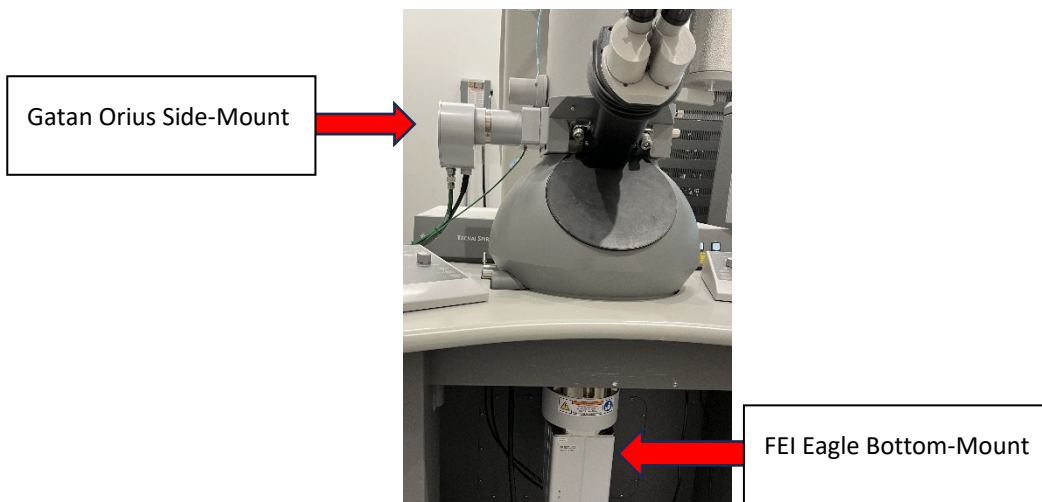
STEP 11: Direct Alignments Continued

- Direct Alignments Step 5: Rotation Center
 - Why is it important?
 - When rotation center is activated, it wobbles a current through the objective lens. Having an aligned rotation center improves resolution by eliminating an optical aberration known as coma.
 - Procedure:
 - Go to 60,000-80,000X magnification and spread the beam out. You will be looking at the sample for this alignment, not the edge of the beam.
 - Activate rotation center.
 - When the rotation center is aligned, the center of the specimen should appear to pulse. If the rotation center is not aligned, you will see a preferential, lateral movement of the specimen.
 - Use MFX and MFY to adjust the rotation center so the center of the specimen appears to pulse in and out. It is helpful to use the small focusing screen and binoculars to align the rotation center more accurately.
 - The amplitude of the pulsing can be increased or decreased using the bottom ring of the focus knobs.
- Direct Alignments: Tomo Beam Shift and Tomo Rotation Center
 - Do not do these alignments. They are not necessary as this system is not used for tomography.

STEP 12: Acquiring Images

The Tecnai has two CCD cameras available for acquiring images:

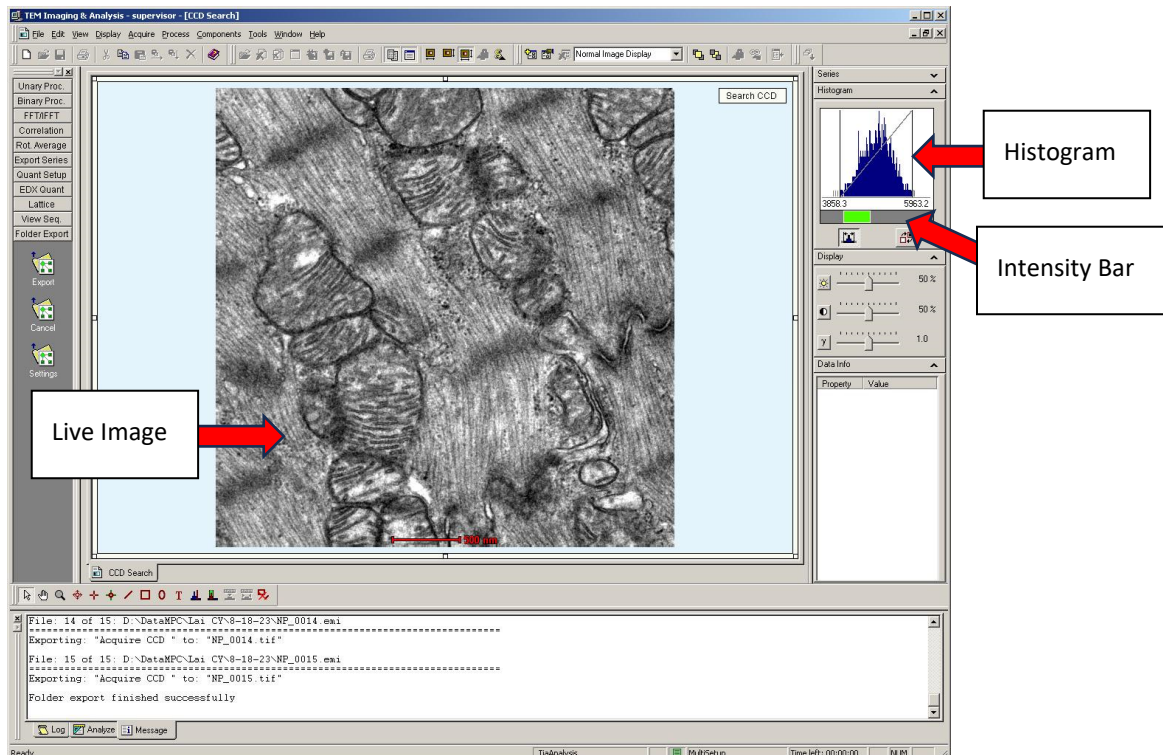
- Gatan Orius is a side-mount 2k x 2k CCD.
 - It is a lower resolution camera and collects lower quality images than the Eagle camera, but it has a very fast readout speed and provides a larger field of view than the Eagle camera. I recommend using it for navigational purposes to locate regions of interest.
- FEI Eagle is a bottom-mount 4k x 4k CCD.
 - It is a high-resolution camera, but it is slow. Use it when you want the highest image quality.



STEP 12: Acquiring Images Continued

Using the Gatan Orius Camera

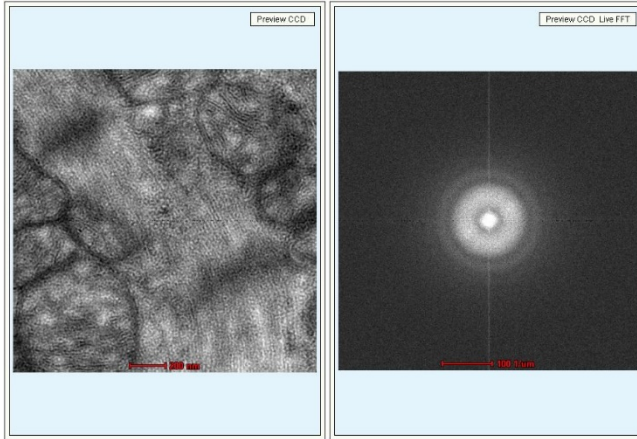
- Ensure Gatan Digital Micrograph software is running. Digital Micrograph must be open in the background for the camera to function.
- Select WA-Orius in the dropdown under CCD/TV Camera box.
- Spread out the beam so you do not expose the camera to a very high electron dose.
- Make sure the binoculars will not interfere with the insertion/retraction of the side-mount camera.
- Click Insert. The camera will be inserted above the viewing screen, and you will see its shadow appear on the viewing screen.
- Recommended settings for the Orius:
 - Search Mode (Used for searching):
 - Integration Time: 0.25s
 - Binning: 2
 - Readout Area: Full
 - Preview Mode (Used for focusing):
 - Integration Time: 0.25s
 - Binning: 1
 - Readout Area: Half
 - Acquire Mode (Used for taking an image):
 - Integration Time: 1
 - Binning: 1
 - Readout Area: Full
- Click Search. You will now see a live image appear in TIA (TEM Imaging and Analysis software).



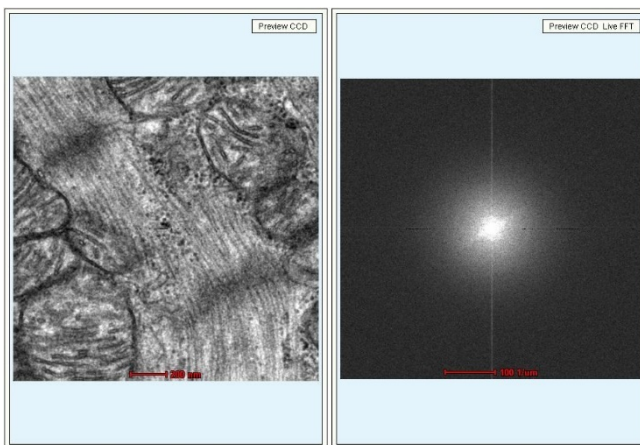
STEP 12: Acquiring Images Continued

- Adjust illumination.
 - If the camera is receiving too high of an electron dose, you will see a pattern of polygons on the screen, which are the fiber optics of the camera. Reduce your brightness until the intensity bar is within the green of the histogram! Brightness can either be reduced by:
 - Spreading out your beam. Turn the intensity knob clockwise to spread out the beam.
 - OR
 - Increase spot size. Increasing the spot size results in more of the beam being cut off by the C2 aperture, causing the dose hitting the specimen and camera to be reduced. On average, one change in spot size results in a twofold change in dose.
- Focus the image. There are multiple strategies to focus the image. Here are two:
 - Focus by looking at the image:
 - Click Preview in the CCD/TV Camera box
 - Preview mode is set to a fraction of the readout which means only a portion of the CCD will be displayed. This gives a digitally zoomed image, which can help with focusing.
 - Focus by using the FFT:
 - FFT=Fast Fourier Transform; it is a mathematical representation of your image whereby high frequency information is represented along the periphery and low frequency information in the center. The FFT appears as a glowing orb.
 - When using the FFT to aid with focus, it is recommended you use it on a live image with the same binning as the image to be acquired. The FFT loses information when binning is applied to the image, resulting it in having a different appearance.
 - While in preview (Bin=1), click Live FFT in the CCD/TV Camera box. A live FFT will be displayed alongside the image in TIA.
 - When the FFT is a spread-out circle, you are in focus. At focus, there is minimum contrast in the image.
 - If you turn the focus knob counterclockwise from focus, you are in a defocus. At a strong defocus, the central spot of the FFT will condense and you will see rings, known as Thon rings, in your FFT.
 - If you turn the focus knob clockwise from focus, you are in an overfocus. At a strong overfocus, the central spot of the FFT will condense and Thon rings will appear. In overfocus, electron dense objects have a hollow appearance.
- Acquire the image. Click the acquire button in the CCD/TV Camera box.

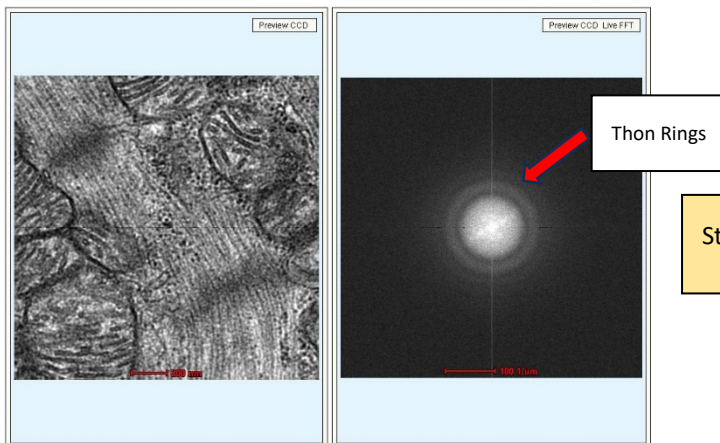
STEP 12: Acquiring Images Continued



Strongly overfocused image and its FFT



Focused image and its FFT

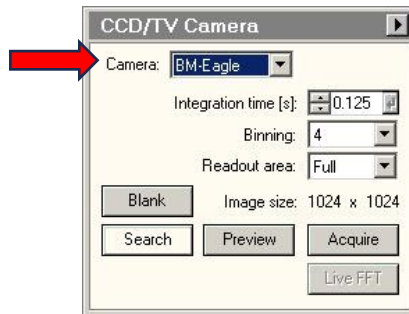


Strongly defocused image and its FFT

STEP 12: Acquiring Images Continued

Using the FEI Eagle Camera

- Select BM-Eagle in the dropdown under CCD/TV Camera box. This will cause the Orius side-mount camera to retract if it had not been retracted previously.



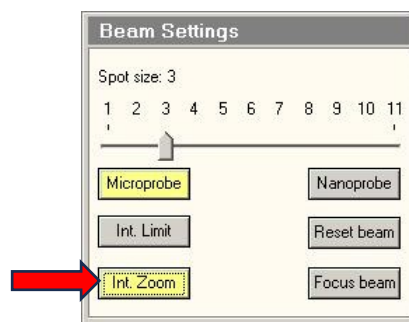
- Spread out the beam so you do not expose the camera to a very high electron dose.
- Press L1 to lift the viewing screen. Since the camera is located under the viewing screen, the screen must be lifted for the camera to receive the image.
- Recommended settings for the Eagle camera:
 - Search Mode (Used for searching):
 - Integration Time: 0.125s
 - Binning: 4
 - Readout Area: Full
 - Preview Mode (Used for focusing):
 - Integration Time: 0.25s
 - Binning: 1
 - Readout Area: Quarter
 - Acquire Mode (Used for taking an image):
 - Integration Time: 1
 - Binning: 1
 - Readout Area: Full
- Click Search. You will now see a live image appear in TIA (TEM Imaging and Analysis software).
- Adjust the illumination. Use the same strategies to adjust the illumination as discussed above under “Using the Gatan Orius Camera” heading.
- Focus the image. Use the same strategies to focus as discussed above under “Using the Gatan Orius Camera” heading.
- Acquire the image. Click acquire the CCD/TV Camera box.

Please note that with the above settings for the Eagle camera, the acquired image will have approximately 3.3x the signal as the search mode, so adjust your illumination accordingly to avoid acquiring an overexposed image. Check the histogram of your acquired image to make sure you are not clipping information.

STEP 12: Acquiring Images Continued

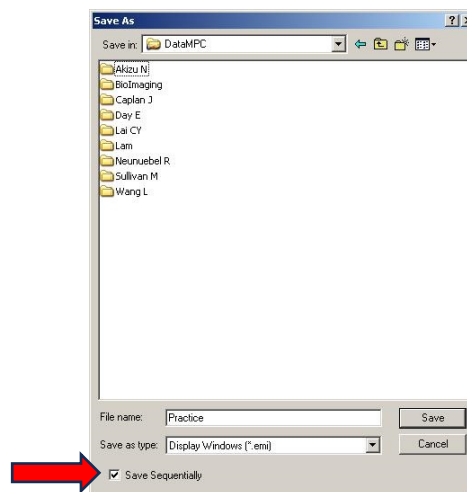
Intensity Zoom

- Under normal circumstances, the intensity of the beam hitting the screen changes with magnification. As you go up in magnification, the intensity of the beam on the screen decreases, and as you go down in magnification, the intensity of the beam on the screen increases. The user must continuously adjust the beam intensity when magnification is changed.
- Intensity Zoom is a useful feature that keeps the intensity of beam on the screen constant.
- To use Intensity Zoom:
 - Define a good spread of the beam using the intensity knob.
 - Click Int. Zoom button. When it is active, it appears yellow.
 - The system will now automatically change the spread of the beam with magnification.



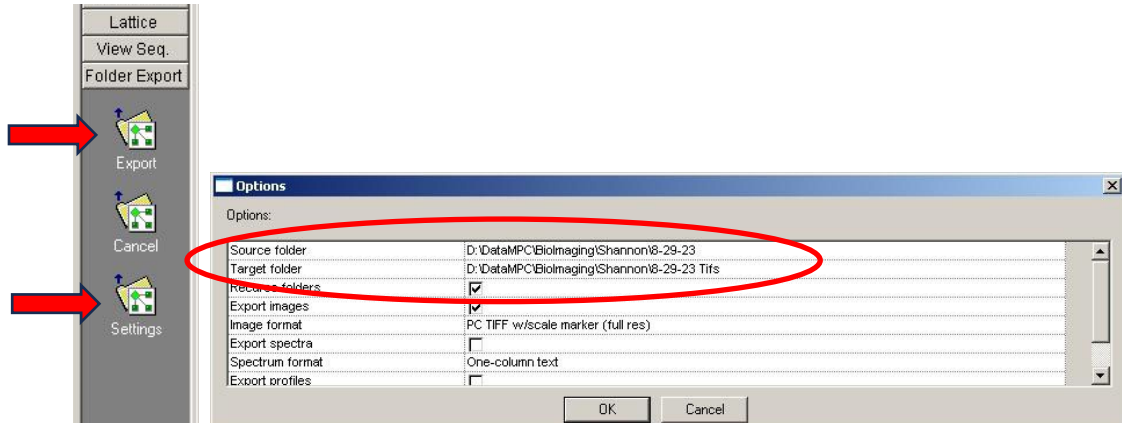
Using TIA (camera acquisition software)

- Saving images
 - Go to File> Save As
 - Type in the sample name and any other information you wish to include in the file name.
 - Files are saved to the Data (D:) Drive. Please save in the following folder hierarchy:
Lab>Student>User> Date
 - By ticking the box Save Sequentially, other images with the same file name can simply be saved by clicking on the save icon (floppy disk) on the tool bar.



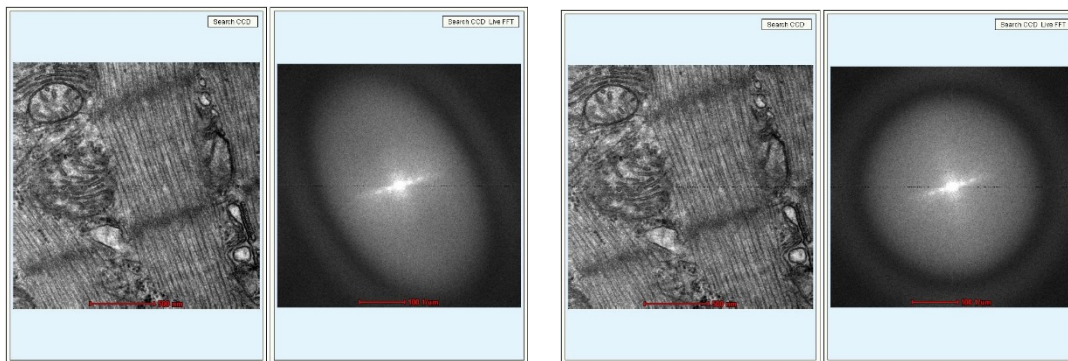
STEP 12: Acquiring Images Continued

- Converting images
 - Raw images are saved as a .emi extension. To save as tif, they must be converted.
 - Under Folder/Export, click Settings
 - Define the path for the Source Folder
 - Define the path for the Target Folder
 - Click OK
 - Under Folder/Export, click Export. The status of the file conversion appears in the message log.



STEP 13: Correcting Objective Astigmatism

- If the objective lens exhibits an astigmatism, it needs to be corrected. Astigmatism means the lens is not equally strong in all directions, and it will cause the image to have a smeared appearance along a preferred direction.
- Observing Objective Astigmatism:
 - It is easiest to see an astigmatism by looking at a live FFT on a camera.
 - Objective astigmatism is more obvious at higher magnifications (>18,500X) than at low magnifications, so increase the magnification.
 - Set the focus to be at a slight defocus (one Thon ring is visible).
 - If there is no astigmatism in the objective lens, the FFT will appear round. If there is an astigmatism present in the objective lens, the FFT will appear stretched in one direction.

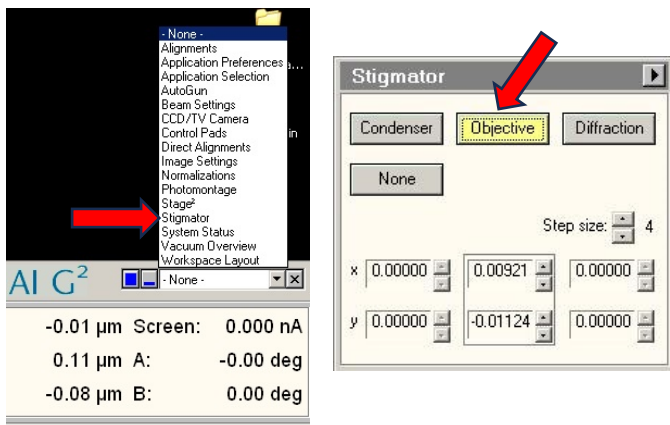


Objective Astigmatism Present

Objective Astigmatism Absent

STEP 13: Correcting Objective Astigmatism Continued

- Correcting Objective Astigmatism:
 - Access the Stigmator control.
 - Click on Objective to activate the stigmator control for the objective lens. When active, the objective button turns yellow and the multifunction X, Y knobs now say Obj. Stig X and Obj. Stig. Y.

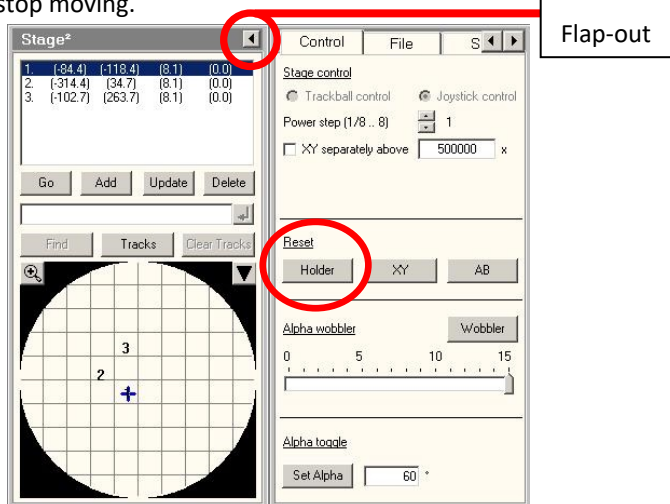


- While looking at the live FFT, use the MFX and MFY knobs to make the FFT appear round.
- When you are finished adjusting the stigmator, click None to deactivate the MFX and MFY knobs.

STEP 14: Sample Exchange

When you are ready to put a different sample into the microscope:

1. Reset the holder.
 - a. Go to Stage² and open the flap-out.
 - b. Click Holder under Reset. This will bring the stage back to its home coordinates in X, Y, and Z. Wait for the stage to stop moving.



STEP 14: Sample Exchange Continued

2. Close the column valves by clicking on Col. Valves Closed in the Vacuum box. Column valves must always be closed prior to removing the sample.
3. Turn off the filament by clicking the Filament button. Wait until the filament is completely off.
4. Remove the sample rod and insert a new sample as described earlier.
5. After a new sample has been inserted, turn the filament back on once the Gun/Column vacuum is <20 Log.
6. With every new sample, you will have to redo the eucentric height alignment.

STEP 15: Data Transfer

- Drag files from the Data (D:) drive to the networked support PC. There is a shortcut on the desktop called "Shortcut to data on FEI Support Computer".
- From the support PC, data can be moved off the computer either by using the Bio-Imaging transfer server ([//force.dbi.udel.edu/transfer](http://force.dbi.udel.edu/transfer)) or UD Dropbox.

STEP 16: Shutdown

- At the end of your appointment, reset the holder, turn off the filament, remove your grid, and place the empty sample rod back into the vacuum of the microscope as described earlier.
- If there is another appointment scheduled after you later in the day, top off the anti-contaminator with liquid nitrogen.
- If you are the **last appointment of the day**:
 1. Remove the anti-contaminator dewar and pour the extra liquid nitrogen into the wide mouth dewar. Do not leave liquid nitrogen in the anti-contaminator dewar as it will cause the dewar to be wet the next morning.
 2. Start the Cryo-Cycle:
 - Click the flap-out to the Vacuum Box.
 - Under the Cryo tab, click Cryo Cycle.
 - The HT will turn off.
 - The vacuum box will say STATUS: CRYO CYCLE
 - The cryo-cycle will turn off the ion-getter pump (IGP) and turn on the oil diffusion pump to remove moisture from the vacuum as the anti-contaminator warms.
 - The cryo-cycle takes **10 hrs** to complete, so it is important not to start it until all appointments for the day are completed.

Only start the Cryo Cycle if you are the LAST appointment of the day!

