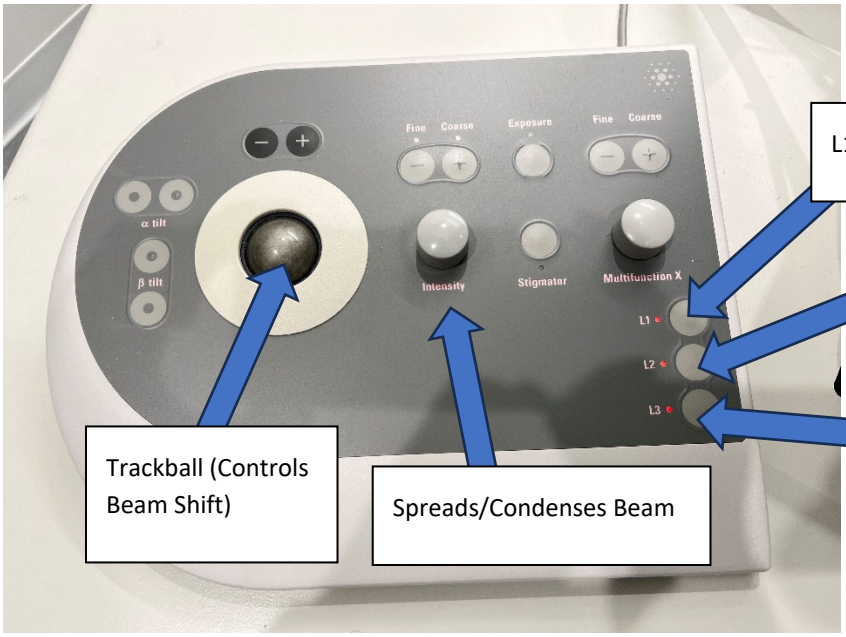
USER GUIDE FOR THE TALOS L120C TRANSMISSION ELECTRON MICROSCOPE

Bio-Imaging Center (Updated 9/29/2023)

Created by Shannon Modla

Overview of the Talos TEM

Left Control Panel



L1 Lifts Viewing Screen

L2 Starts Alpha Wobbler

L3 Decreases Spot Size (Higher Dose)

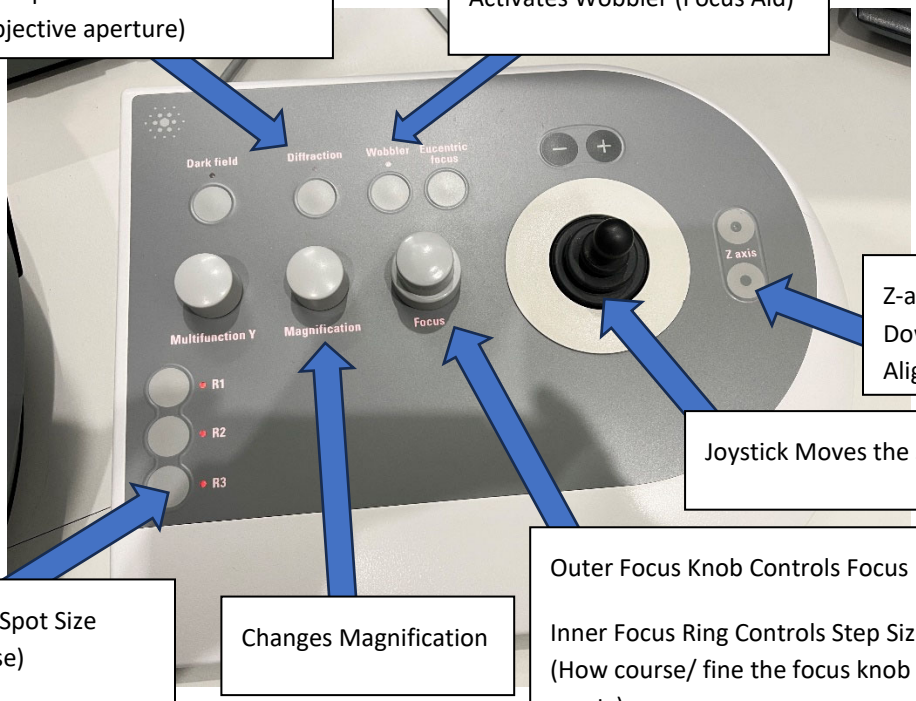
Trackball (Controls Beam Shift)

Spreads/Condenses Beam

Right Control Panel

Puts Microscope in Diffraction Mode (To align objective aperture)

Activates Wobbler (Focus Aid)



Z-axis Moves Stage Up and Down for Eucentric Height Alignment

Joystick Moves the Stage

Outer Focus Knob Controls Focus

Inner Focus Ring Controls Step Size (How course/ fine the focus knob reacts)

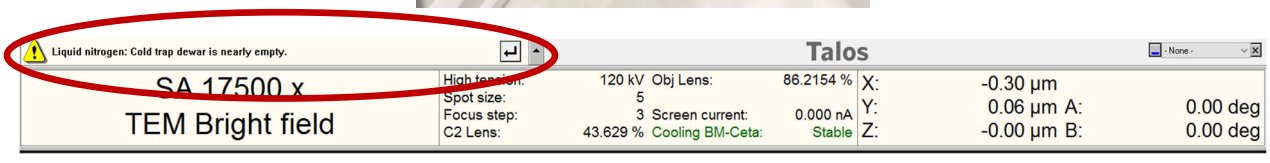
R3 Increases Spot Size (Reduces Dose)

Changes Magnification

User Guide for the Talos L120C TEM

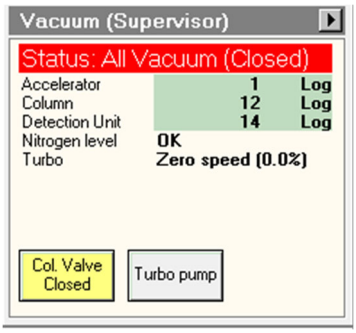
STEP 1: Fill the anti-contaminator with liquid nitrogen

- Why is this important?
    - Cooling the anti-contaminator helps improve the vacuum and keep the column cleaner. The microscope should always be operated cooled.
- Procedure:
    - Open the door on the right side of the instrument and 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 3-4 hours.
    - There is a sensor at the end of the copper wires, and the TEM software will alert you with a message when the liquid nitrogen level is low.

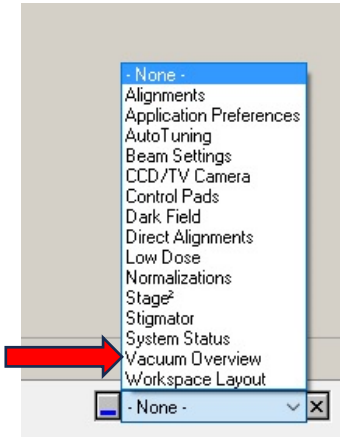


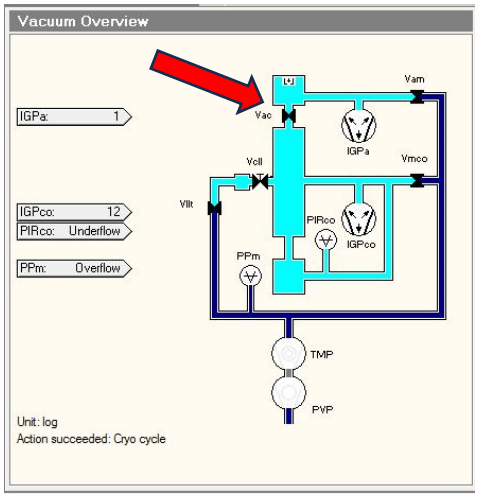
STEP 2: Check the vacuum status.

- 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: All Vacuum (Closed)” with a red background and the Accelerator, Column, and Detection Unit 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:





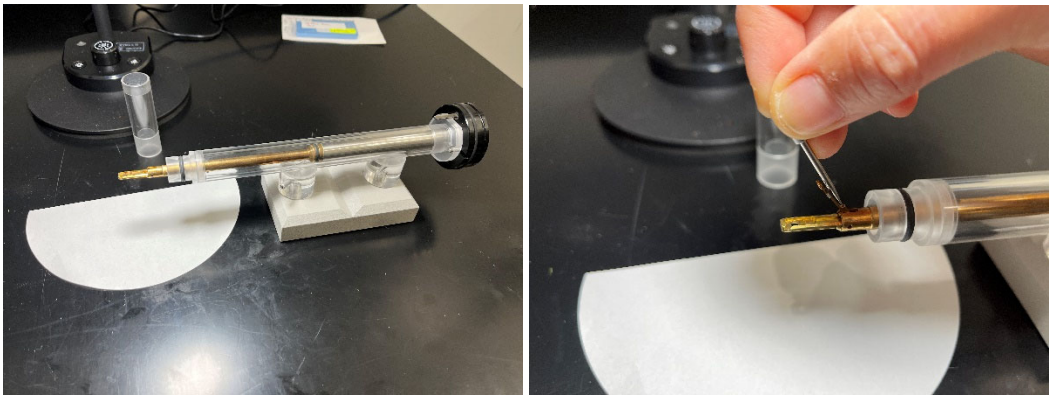
Notice the valve between the gun and the column is closed

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.

Press against the plate as you retract the rod from the column.

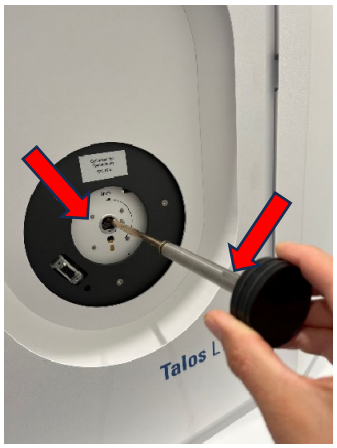
    - Rotate the rod clockwise until it stops. This closes the ball valve that separates the airlock from the column.
    - Pull the sample rod out completely. Do not touch the sample rod anywhere below the O-ring with your bare fingers. This area needs to be kept clean as it goes into the high vacuum of 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

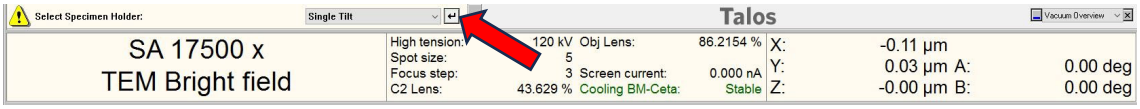
STEP 4: Insert the sample rod

- 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 turbo pump start spin up to 100% speed. It takes a minute or so for the turbo pump to accelerate to 100% speed.

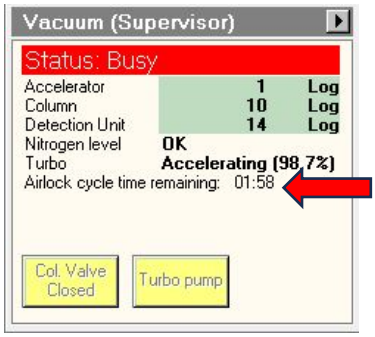


Align large silver pin on the sample rod with the screw above and to the left of the hole

    - Select single tilt when prompted for the specimen holder.



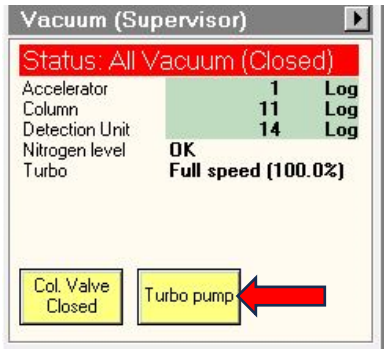
    - Wait until the airlock cycle counts down to zero. The airlock will be evacuated for 2 minutes before you can insert the sample rod. Do not insert the sample rod until the airlock cycle is complete and the red light has turned off.



    - Once the red light is off, **quickly 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 want to pull in the rod. **Slowly** guide the rod into the column.

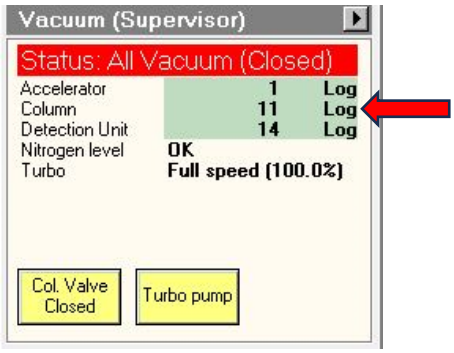
STEP 4: Insert the sample rod (cont.).

- You can now turn off the Turbo Pump when the software permits (the system will automatically turn off the Turbo Pump when the column valves are opened). When the turbo pump is turned off, the Turbo pump button turns from yellow to gray.



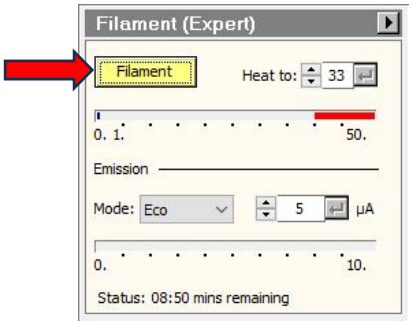
STEP 5: Check the vacuum status again

- Procedure:
  - Make sure the Accelerator, Column, and Detector Unit are green, indicating the vacuum is within operational limits.



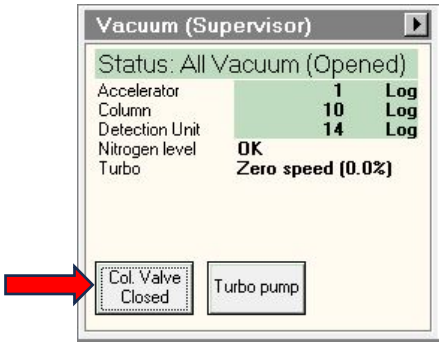
STEP 6: Turn on the filament.

- On this system, the HT remains on at all times. 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 9 min for the filament to reach saturation.



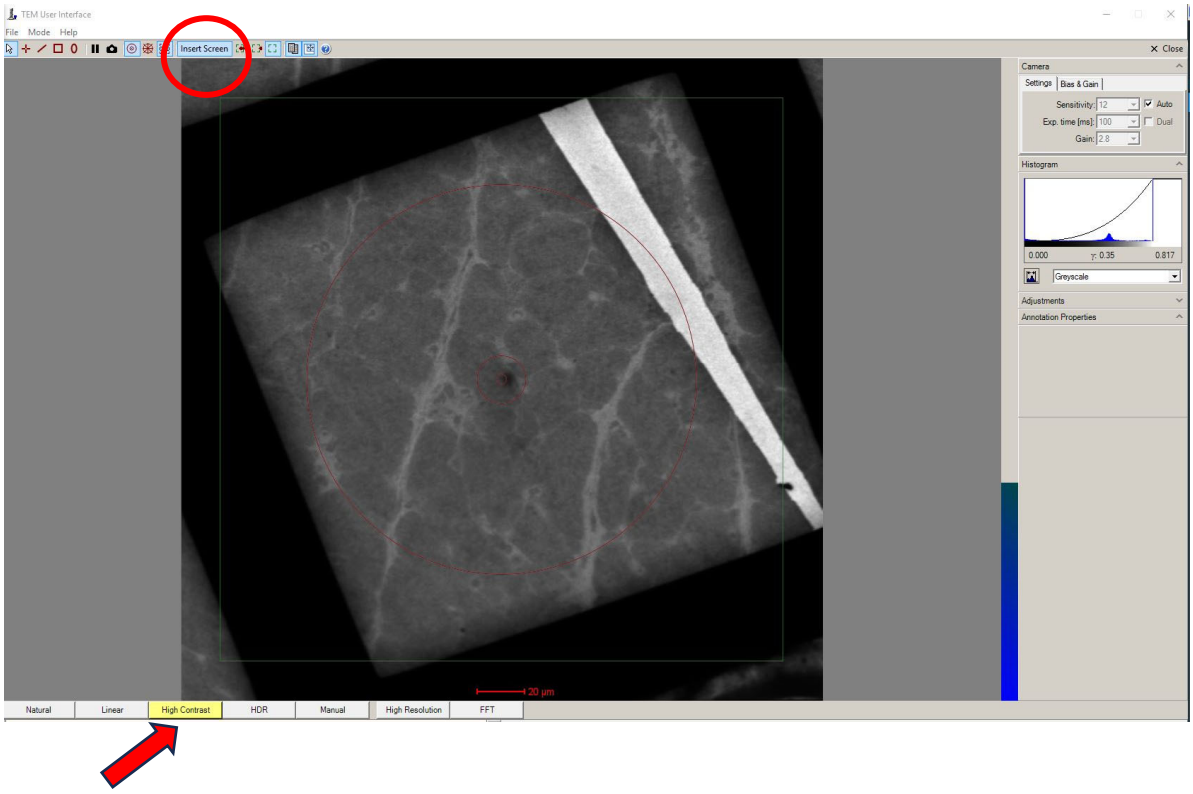
STEP 7: Open the column valves.

- Click the Col. Valve Closed button to open the column valves. When the column valves are opened the Col. Valves Closed button turns gray and the header to the Vacuum box says “Status: All Vacuum (Opened)”



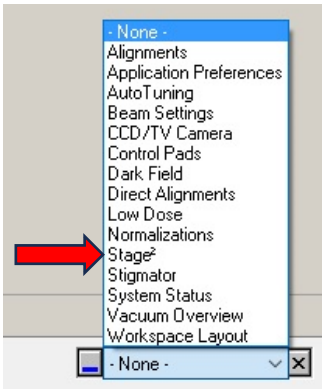
STEP 8: Find a region of interest on your grid.

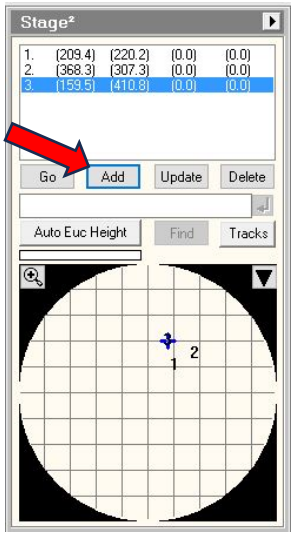
- The Talos uses a FluCam to help navigate your samples. The FluCam is a camera directed at a phosphorescent screen in the viewing chamber.
    - Click Insert Screen and the Play Button if needed to see a live view of the grid on the FluCam
    - The most used mode of the FluCam is High Contrast Mode. Manual mode is useful when needing to view the diffraction spot as turning the mouse wheel allows you to manually adjust the exposure to see very bright features.



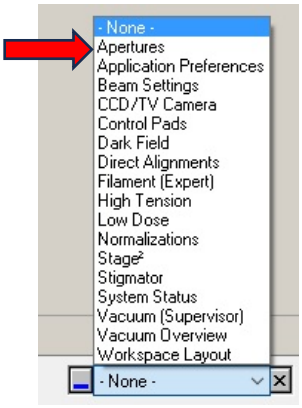
STEP 8: Find a region of interest on your grid (cont.)

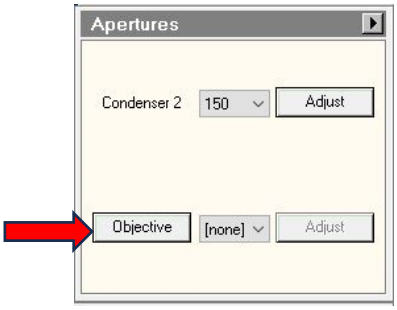
- It is best to start at a lower magnification to find a suitable area and then increase the magnification. If you want to save locations on your grid, this can be done under Stage^2.





- 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 Talos should automatically remove and insert the objective aperture when you go from M to LM or from LM to M magnification ranges. However, sometimes it forgets to re-insert the objective when going from LM to M. To insert the objective aperture through the software, go to Apertures and click Objective. When the Objective button is yellow, it is inserted into the column.





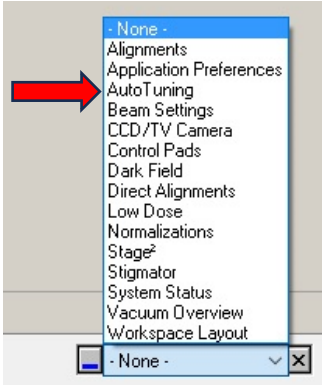
STEP 9: Find the eucentric height and focus

1. 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:
        - Go to a magnification range (usually SA) you will be using for image acquisition.
        - Activate the alpha wobbler (L2). This will cause the specimen to tilt back and forth from -15˚ to +15˚.
        - 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).
2. Find focus
    - The sample must be in focus for subsequent alignments to be accurate.
    - Procedure:
        - Turn on the wobbler
        - Adjust focus using the focus knob until the image stops moving.

STEP 10: Direct Alignments: Auto Tuning

The Talos TEM has an Auto Tuning feature that permits automatic execution of the direct alignments.

- Why are direct alignments important?
    - Direct alignments should be done <u>once daily</u> 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 Auto Tuning

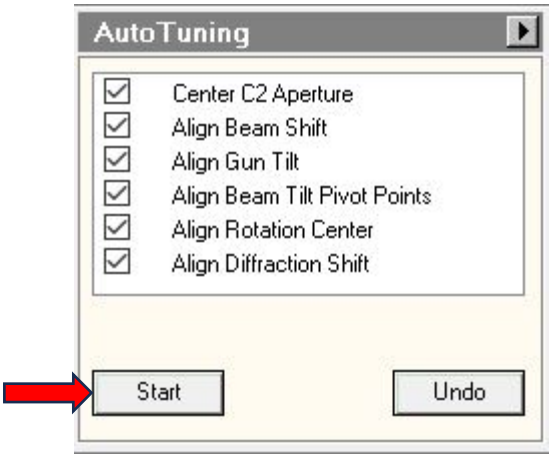


STEP 10: Direct Alignments: Auto Tuning (cont.)

- Condense the beam to crossover and center it to the screen using the trackball.
- Expand the beam clockwise from crossover so it is within the 40mm circle on the FluCam

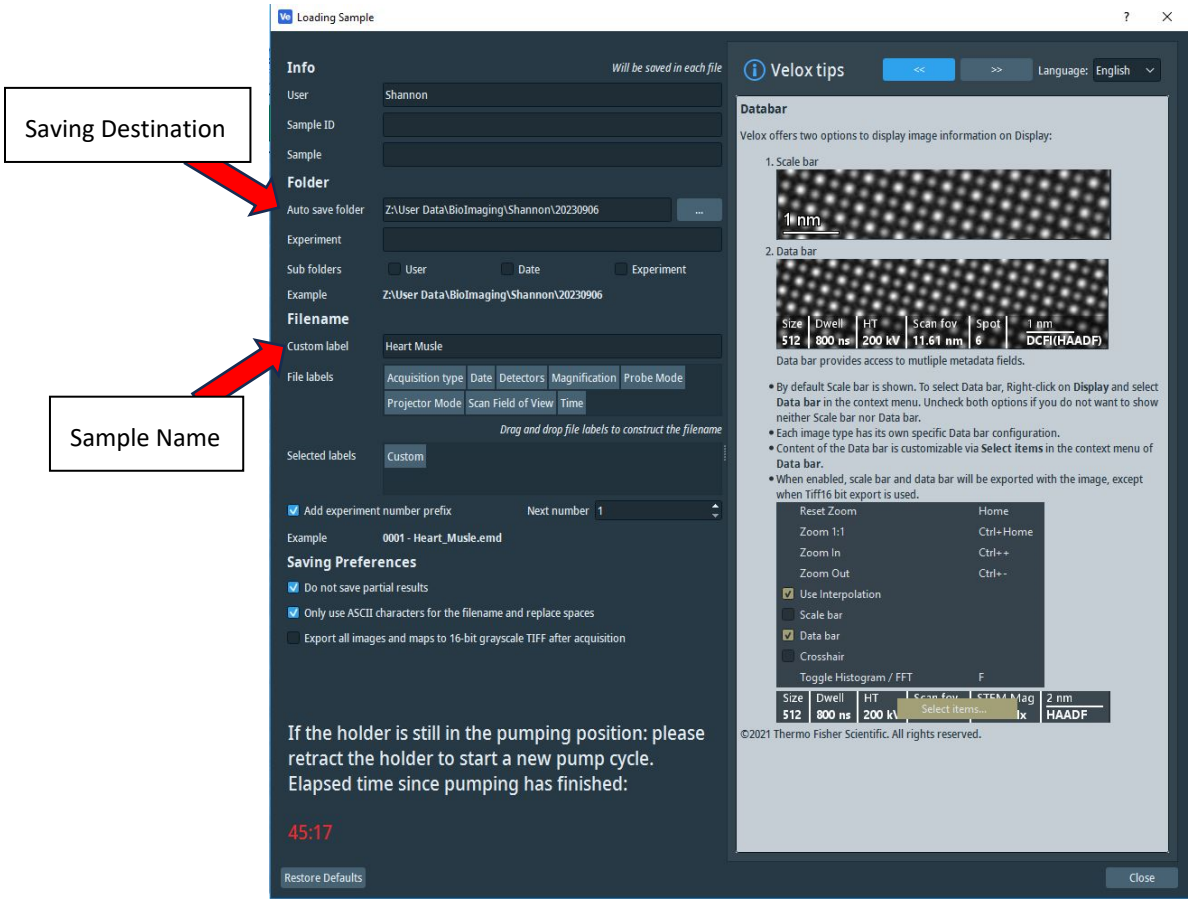
> Always expand the beam by rotating the intensity knob clockwise from crossover.

- Go to spot size 5, set the magnification to be between 13,000X-20,000X, and ensure you are in microprobe mode
- Click Start under AutoTuning. A green checkmark will be shown after each alignment as it passes. If an alignment fails, you will see a red X. Wait until all alignments are completed.
- If alignments fail, you will have to do them manually. Refer to the Appendix.



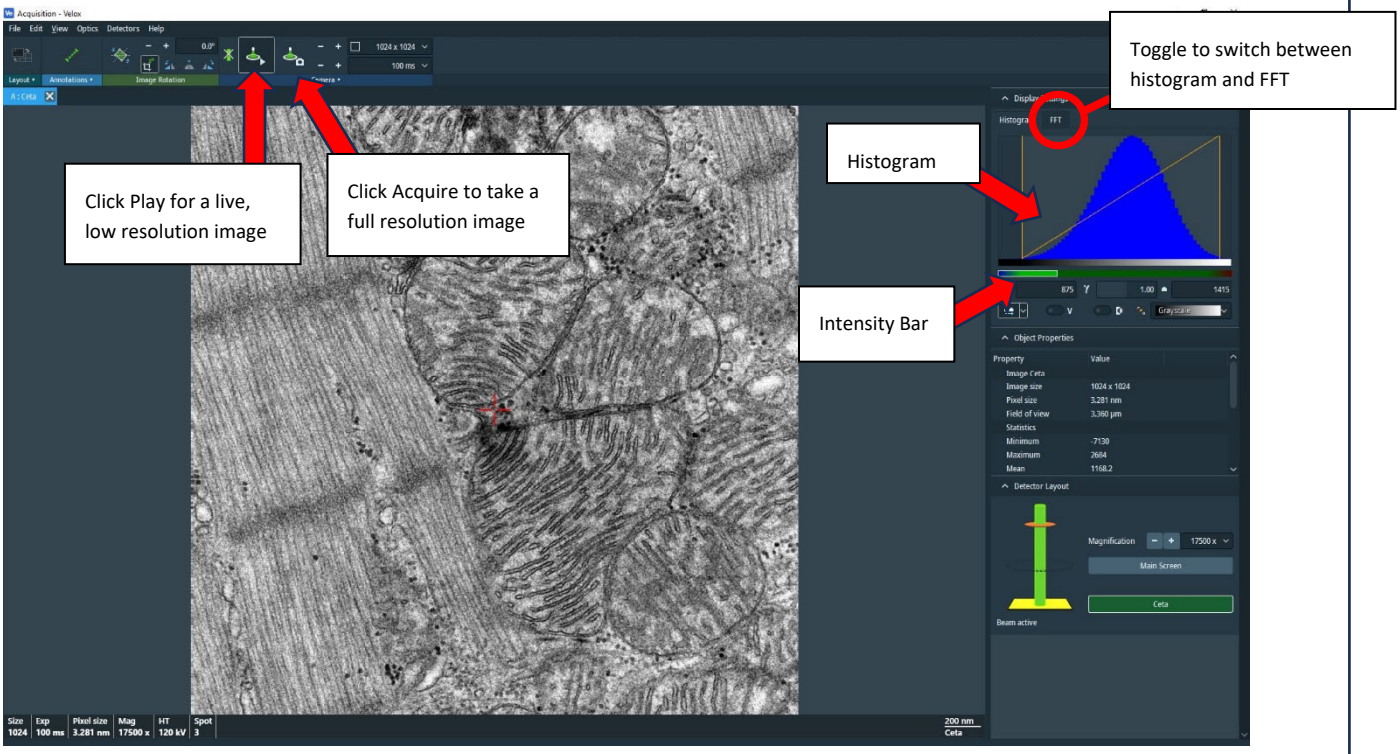
STEP 11: Acquiring Images

- Images are acquired in Velox Acquisition software using a bottom-mount 4k x 4k Ceta camera
- When you insert a new sample into the column, you will be prompted by Velox to enter information about your sample (if not prompted, this window can be accessed under the Edit>Preferences menu of Velox – Acquisition):
    - Save images to the Transfer Server going to the Support PC (\\NPC9958295) using the following folder hierarchy: Lab>User>Date



STEP 11: Acquiring Images (cont.)

- Acquiring Images in Velox:



- Click the Play Button to see a live image
  - Clicking Play automatically raises the phosphorescent viewing screen so the camera can receive the signal.
  - For basic imaging of room temp samples that can receive a high dose, an exposure time of 100ms, a bin of 4 (1024 x 1024), and 1 frame is sufficient for a live image.

- Adjust the illumination.
  - If the camera is receiving too high of an electron dose, it will be over-exposed, and you will see the fiber optics of the camera. Reduce your brightness until the intensity bar is within the lower green of the histogram. Brightness can either be reduced by:
    - Spreading out your beam. Turn the intensity knob clockwise to spread out the beam.

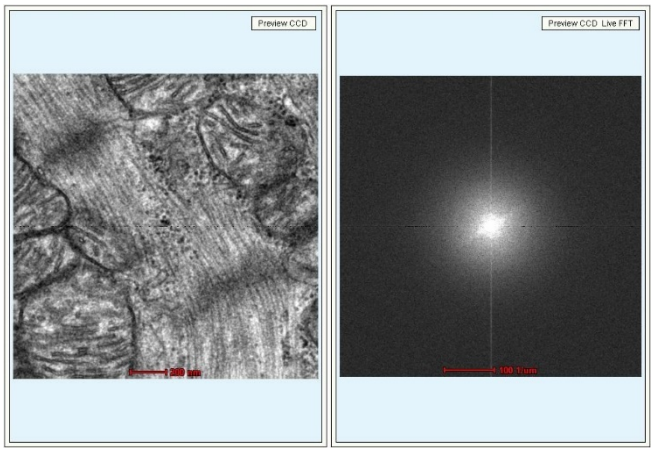
OR

    - 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.

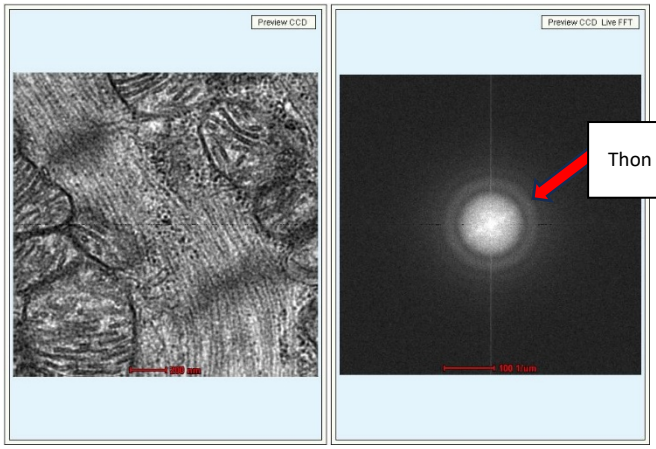
STEP 11: Acquiring Images (cont.)

- Focusing the image
  - There are multiple strategies to focus the image. Here are a few:
    - Focus by looking at the image:
      - While within the live image, use the middle mouse wheel to scroll up. This will digitally zoom in on the image, which may help to see details better while adjusting focus.
    - Focus by using the wobbler
      - Turn on the wobbler. This will cause the beam to tilt back and forth very quickly. When you are out of focus, the image will appear to shake. When you are in focus, the image remains stationary.
      - Adjust focus until the image stops shaking.
    - 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.
        - 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.
  - Other comments about focusing.
    - You will need to adjust the focus step (inner focus knob) depending on magnification.
      - The focus step dictates how course or fine the focus knob behaves.
      - Low magnifications require a larger focus step and higher magnifications require a smaller focus step.
      - If you ever use too large of a focus step and get lost, press the eucentric focus button and that will return you close to focus.
    - When you move from one part of your grid to another, you may need to redo the eucentric height alignment as the grid may not be perfectly flat.

STEP 11: Acquiring Images (cont.)

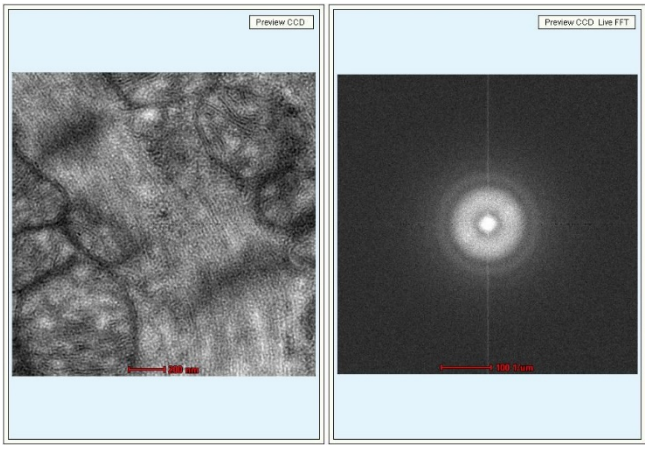


Focused image and its FFT



Thon Rings

Strongly defocused image and its FFT

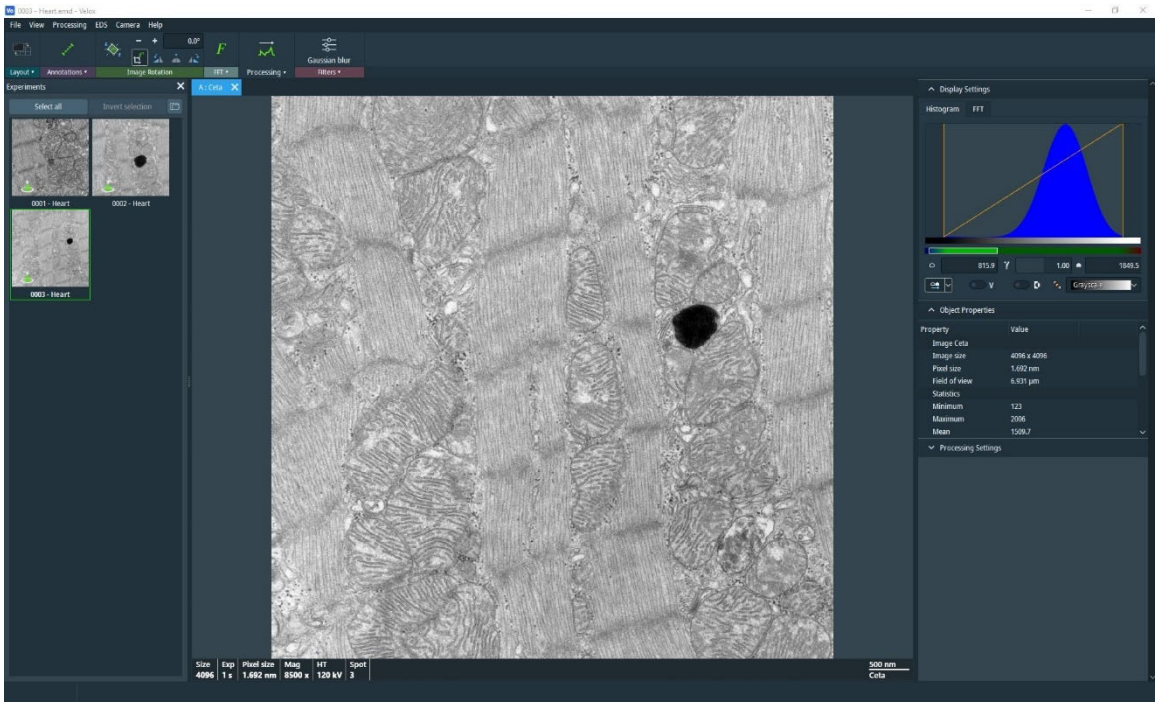


Strongly overfocused image and its FFT

STEP 11: Acquiring Images (cont.)

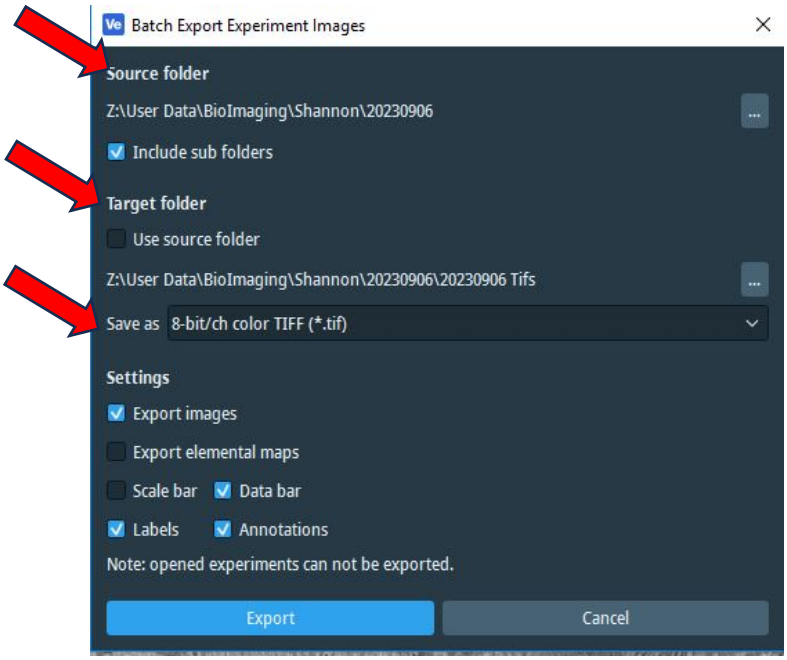
- Acquire the image.
  - Click the acquire button to acquire a full resolution image. View the acquired image in Velox- Processing to ensure it is not overexposed (the live and acquire settings result in slightly different intensities in the final image).

  - For basic imaging of room temp samples that can receive a high dose, an exposure time of 1s, a bin of 1 (4096 x 4096), and combining 4 frames is sufficient.
  - The Ceta camera is equipped with a CMOS chip that allows for frames to be summed.
    - Summing frames permits multiple frames to be acquired without oversaturating the sensor so you can expand the dynamic range of the image
    - For example, a 1s exposure with 4 frames summed means 4 images are acquired at 0.25s exposures and the signal from each frame is summed to create the final image.
- Saving the images
  - Images are automatically saved based on the criteria initially set up in Velox (see above)
  - To view images, open Velox – Processing
    - Images are displayed in a tile layout on the left.

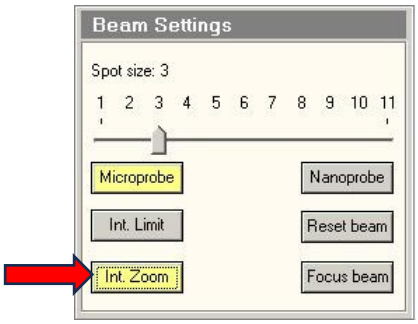


STEP 11: Acquiring Images (cont.)

- Exporting images to tifs
  - In Velox – Processing, go to File> Batch Export.
    - Define source folder, target folder, and file type.
    - Click Export.

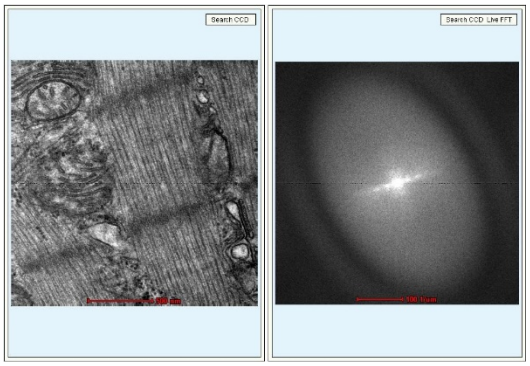


- **Intensity Zoom**
  - Under normal circumstances, the intensity of the beam hitting the screen changes with magnification. As you go up in magnification, the intensity of the beam on the screen decreases, and as you go down in magnification, the intensity of the beam on the screen increases. The user 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.

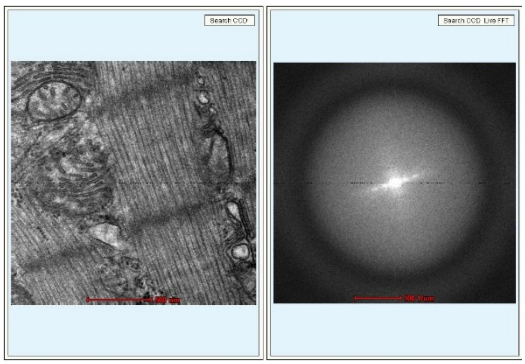


STEP 12: Correcting Objective Astigmatism

- 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.

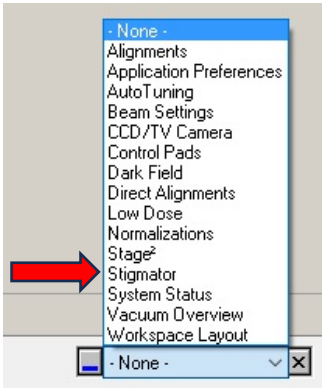


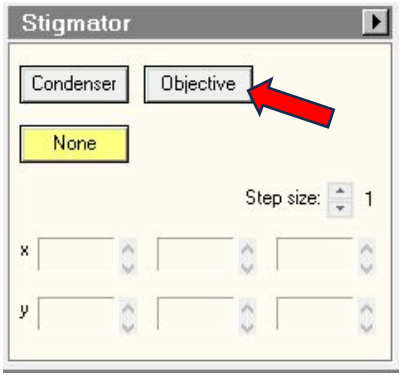
Objective Astigmatism Present



Objective Astigmatism Absent

- Correcting Objective Astigmatism:
    - Access the Stigmator control (or press Stigmator on the left hand panel).
    - 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 (MFX and MFY) now say Obj. Stig X and Obj. Stig. Y.



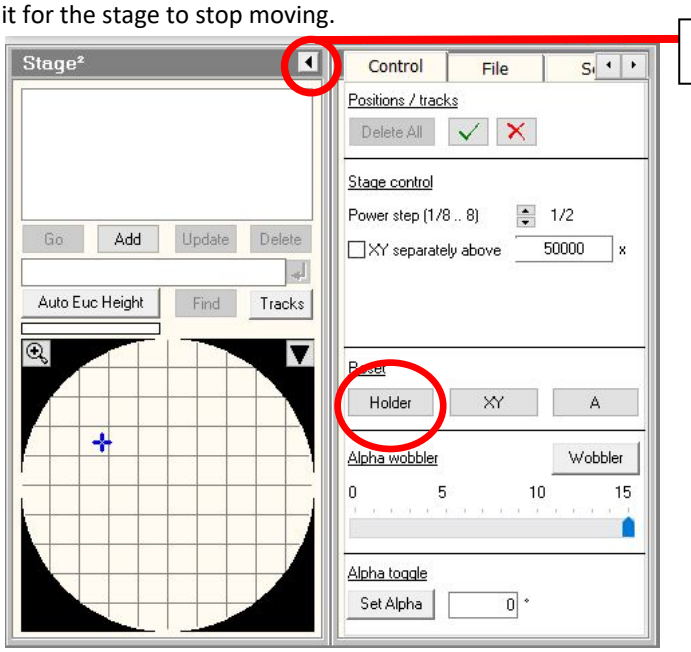


    - While looking at the live FFT, use the MFX and MFY knobs to make the FFT appear round.
    - When you are finished adjusting the stigmator, click None to deactivate the MFX and MFY knobs.

STEP 13: Sample Exchange

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

1. Reset the holder.
    a. Go to Stage^2 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.



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. The HT and Filament can remain on.
4. Remove the sample rod and insert a new sample as described earlier.
5. With every new sample, you will have to redo the eucentric height alignment.

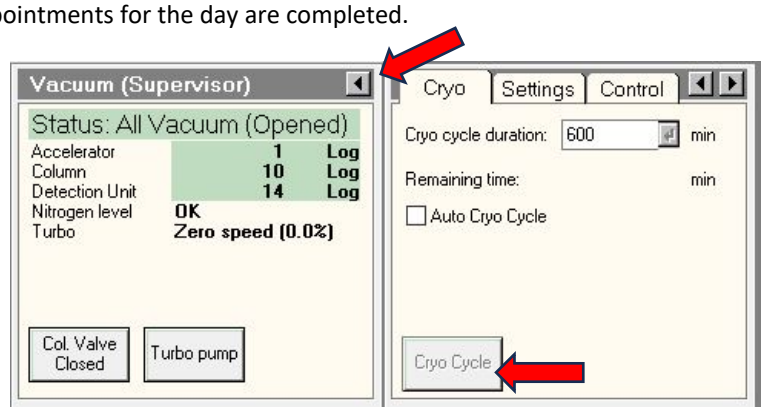
STEP 14: Data Transfer

- Option 1: Drag files from the Support PC Transfer Server to the Bio-Imaging transfer server (//force.dbi.udel.edu/transfer).
- Option 2: Zip files together and transfer them using UD Dropbox
- Personal flash drives and external hard drives are not permitted

STEP 15: Shut Down

- At the end of your appointment, turn off the filament, reset the holder, close the column valves, remove your sample from the microscope, 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 (look at the iLabs calendar!), 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 vacuum box will say STATUS: CRYO CYCLE
       - The cryo-cycle will turn off the ion-getter pump (IGP) and turn on the turbo 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!

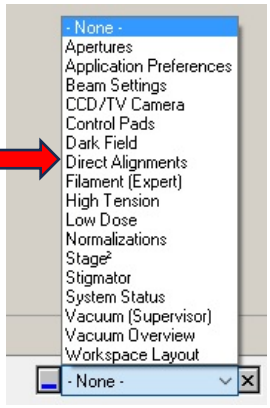


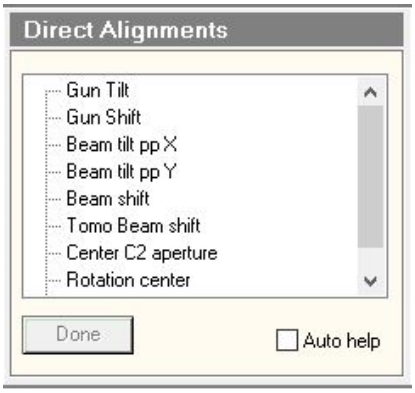
Appendix: Manual Alignments

There may be times when autotuning fails and you will need to do the direct alignments manually:

Direct Alignments (Manual):

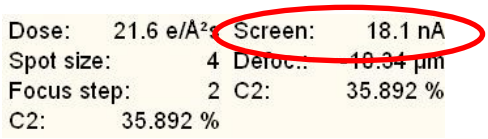
- Procedure:
    - The sample should be at eucentric height and in focus before you begin the direct alignments.
    - Access the direct alignments box and proceed from the top of the list to the bottom. If the auto help box is ticked, it will provide a help page that provides guidance for each step.





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.



    - Click done under Direct Alignments

Direct Alignments Step 2: Gun Shift

- Purpose:
    - Gun shift alignment ensures the beam is going down the optical axis of the microscope.
- Procedure:

- Go to Spot Size 9. Condense the beam to crossover and center it using the trackball (beam shift).
- Click Gun 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.
  - 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.

Direct Alignments Step 5: Tomo Beam Shift

- Procedure:
  - Activate Tomo Beam Shift
  - Use MFX and MFY to center the beam to the viewing screen.
  - Click done under Direct Alignments.

Direct Alignments Step 6: Center C2 Aperture

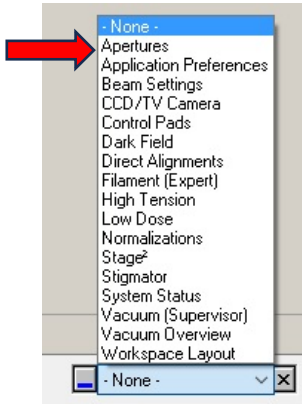
- Procedure:
  - Activate Center C2 Aperture. This will wobble current through the C2 lens.
  - Use MFX and MFY (which are now assigned to Condenser Aperture) to minimize movement of the beam on the viewing screen.
  - Click done under Direct Alignments.

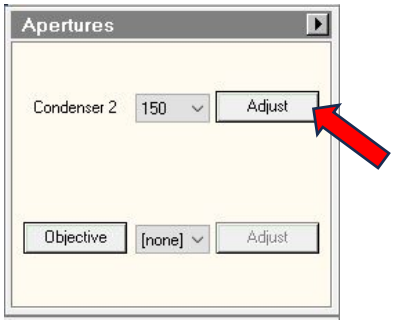
Appendix: Manual Alignments (cont.)

- Alternate Procedure:
  - If you find the above procedure to center the C2 aperture difficult, here is an alternative method:
  - 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 Flu 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, access the Aperture Control, click Condenser, and use MF X and MFY knobs to center the beam to the 40 mm circle on the Flu screen





  - 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.

Direct Alignments Step 7: Rotation Center

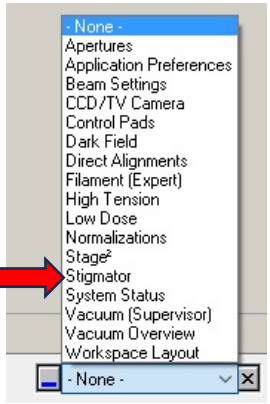
- Why is it important?
  - When rotation center is activated, it wobbles a current through the objective lens. Having an aligned rotation center improves resolution by eliminating an optical aberration known as coma.
- Procedure:
  - Go to 60,000-80,000X magnification and spread the beam out. You will 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.

- The amplitude of the pulsing can be increased or decreased using the bottom ring of the focus knobs.

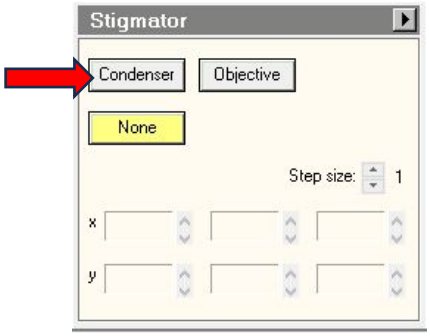
Other Alignments:

Correct C2 astigmatism if needed.

- If the beam does not appear circular on the FluCam, 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.

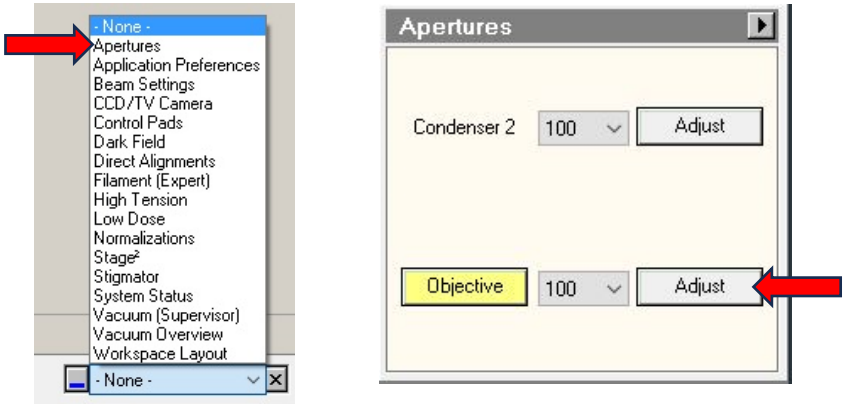


- Use the MFX and MFY knobs to make the beam appear circular.
- When you are finished adjusting the stigmator, click None to deactivate the MFX and MFY knobs.
- Note: Condenser stigmator adjustments only apply for the current spot size. This may need to be readjusted if you change spot size.

Appendix: Manual Alignments (cont.)

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:
  - Use the FluCam to center the objective aperture. Do not do this procedure on the Ceta camera.
  - Go into diffraction mode. Insert the objective aperture by clicking the Objective button from the Apertures control panel. You will see a bright central diffraction spot and a diffuse ring that delimits the edge of the objective aperture. You will need to use Manual Mode on the FluCam (it should automatically switch from High Contrast to Manual when you enter diffraction mode) and use the middle mouse wheel to manually adjust the exposure to clearly see the central diffraction spot.
  - If the ring is not centered around the central diffraction spot, go to Apertures control panel. Click Adjust after Objective and use the multifunction X (MFX) and multifunction Y (MFY) knobs to center the objective aperture around the bright diffraction spot.



- Press diffraction mode again to return to normal imaging mode.