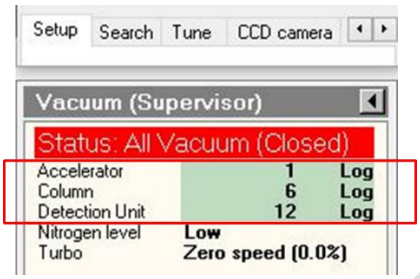
Talos TEM (120kV)
Standard Operation Protocol

Contents

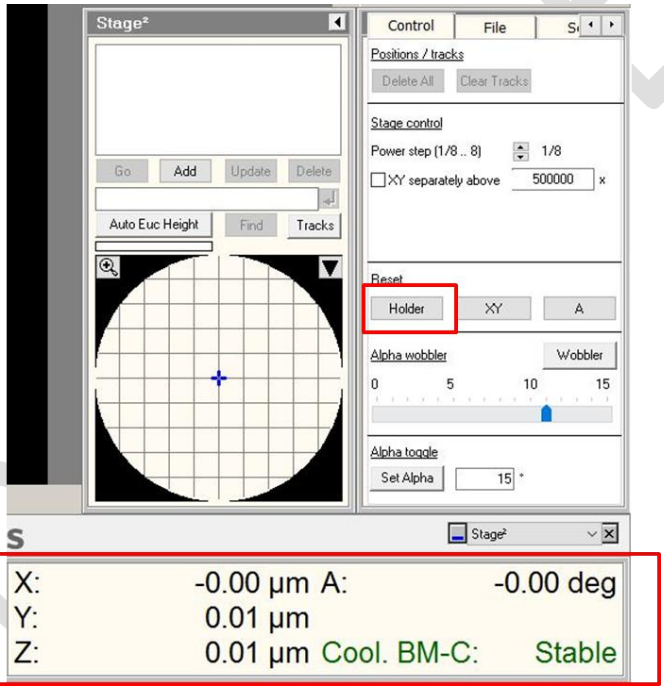
Preparation

Condition Checking

1. Check the condition of the vacuum (<20 logs) in the ***UI***. The statues are green.

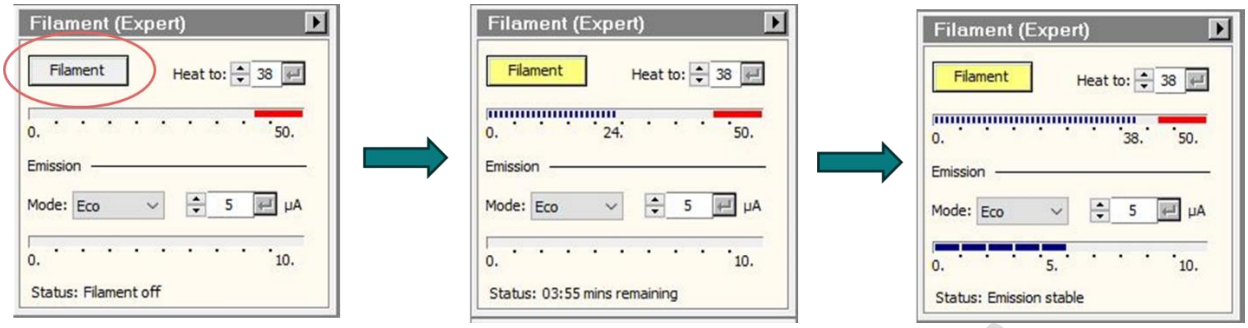


2. Close the column valve.
3. Reset the stage to the home position (X/Y/Z: 0µm) by clicking on ***Holder***.



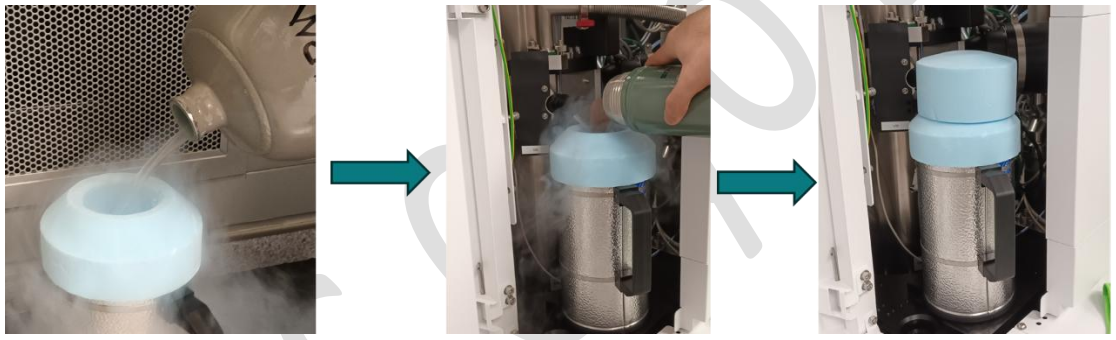
Filament Activation

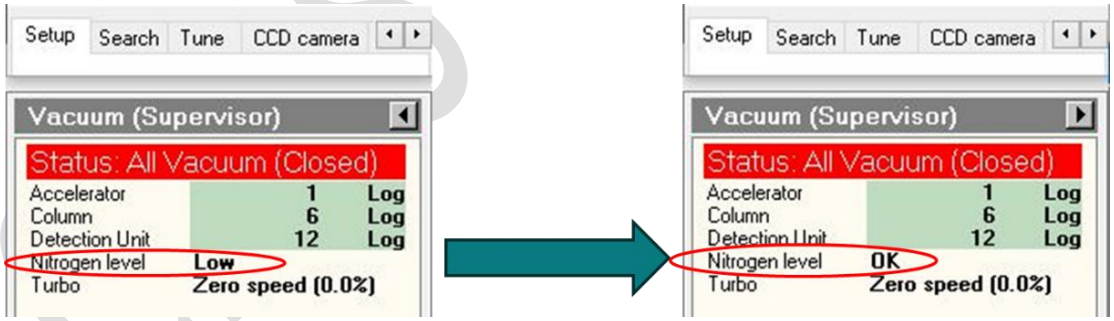
1. Click on ***Filament*** to activate the filament. Do not modify the settings. The settings should be:
    a. Heat to: 38
    b. Emission mode: Eco; 5µA
2. It will take approximately 10 minutes for the status to change to ***Emission stable***. The filament will then be ready.



System Cooling

1. Half-fill the cold trap dewar with LN_2 on the ground.
2. Put the copper wires into the dewar.
3. Fill the dewar with LN_2 using a bottle.
4. Cover the dewar with foam. The LN_2 should be enough for 3-4 hours of operation. Re-fill LN_2 when the ***Nitrogen level*** is ***low***.

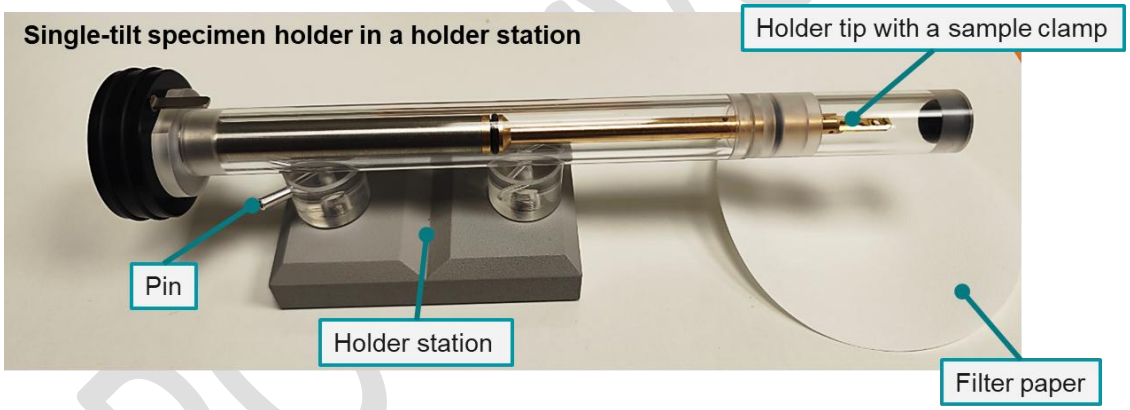




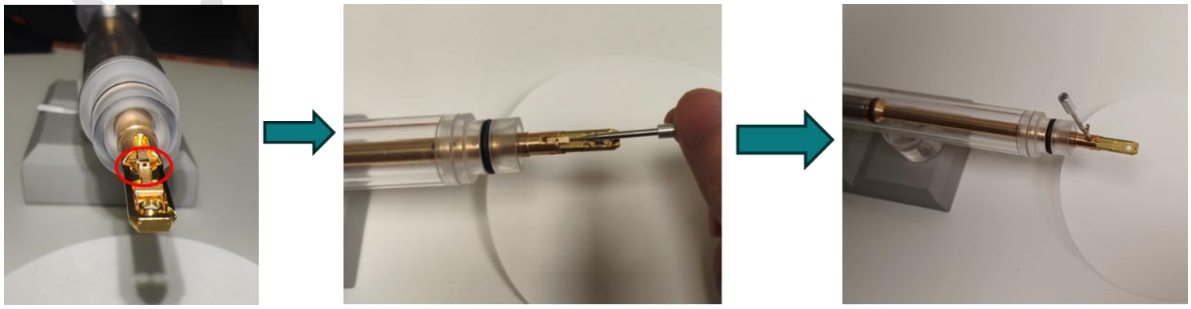
Sample Loading

** Open the ***Velox*** software before loading the sample if you plan to use it for image capture.

1. Take out the specimen holder from the tray. To prevent the holder from sliding out of the station, grip the middle of the station and the holder with both hands.

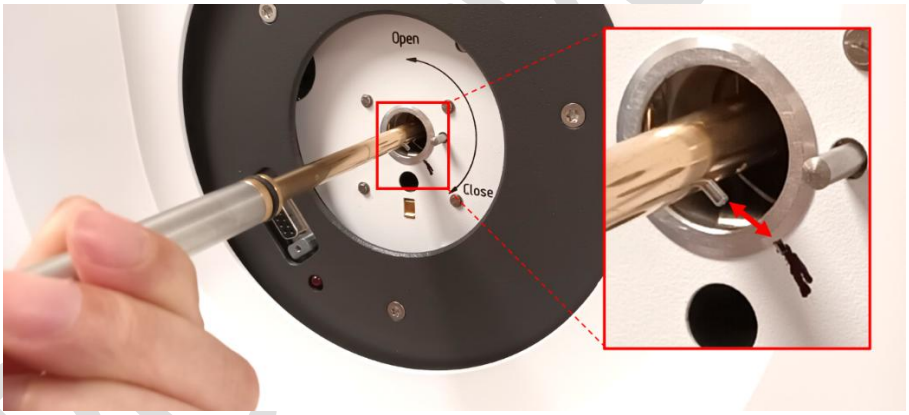


2. Place a filter paper under the holder tip.
3. Carefully remove the plastic cap.
4. Use the pin to slowly lift the sample clamp.

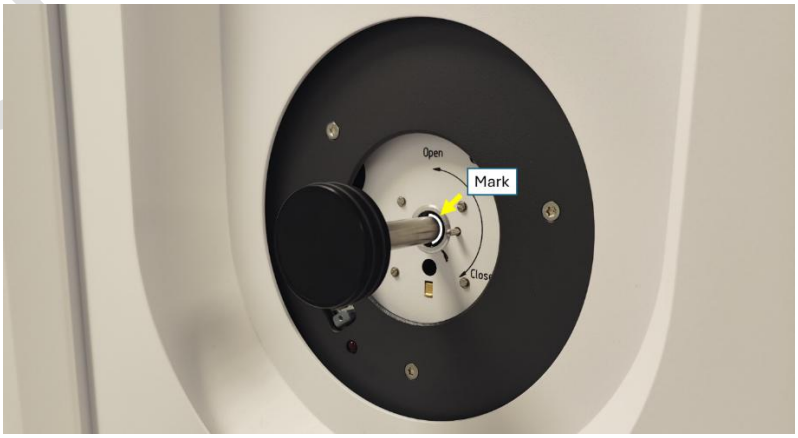


5. Using tweezers, place the grid on the holder tip.

6. Gently tap the holder to position the grid.
7. Secure the grid with the sample clamp using the pin, then remove the pin.
8. Rotate the holder to ensure the grid is fixed at the tip and not falling onto the filter paper.
9. Remove the specimen holder from the station, being careful not to touch the brass holder assembly.
10. Use a dust blower to remove any dust from the rod.
11. Double-check that the stage is set at the home position.
12. Insert the rod into the TEM, aligning the pin of the holder with the mark on the TEM.



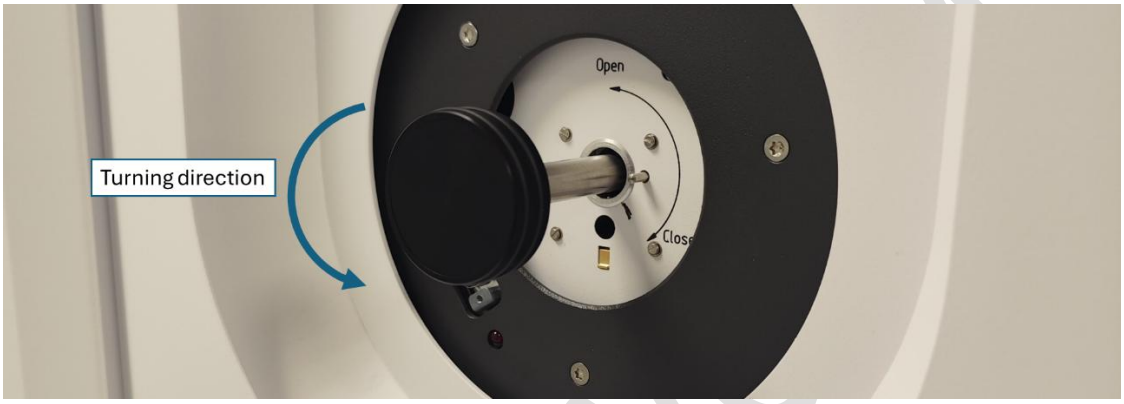
13. Push the rod to the end until the mark on the holder aligns with the edge of the hole. The red light will illuminate.



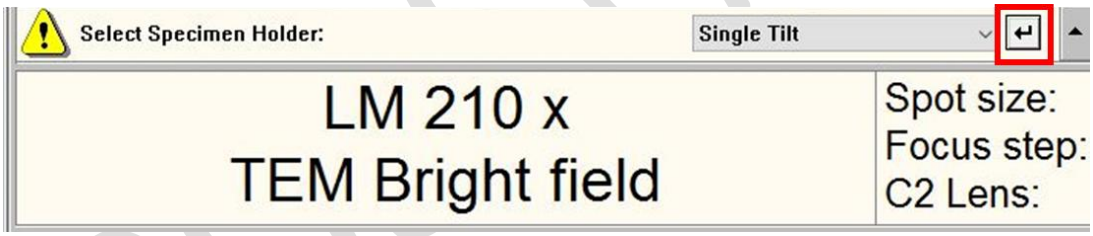
14. The vacuum pump will start automatically. Wait for the vacuum to stabilize; when the **red light turns off** after the countdown (~ 90 to 180 seconds), proceed.

** **IMPORTANT NOTE**: Step 15 must be performed (1) immediately (2) after the countdown. Otherwise, the vacuum will crash, and Cryo-cycle (~ hours) will be required.

15. Turn the knob anticlockwise.



16. Slowly insert the rod further into the TEM.
17. Wait until the vacuum reaches below 20 logs before starting the experiment.
18. Select ***Single Tile*** and click the arrow button before proceeding.



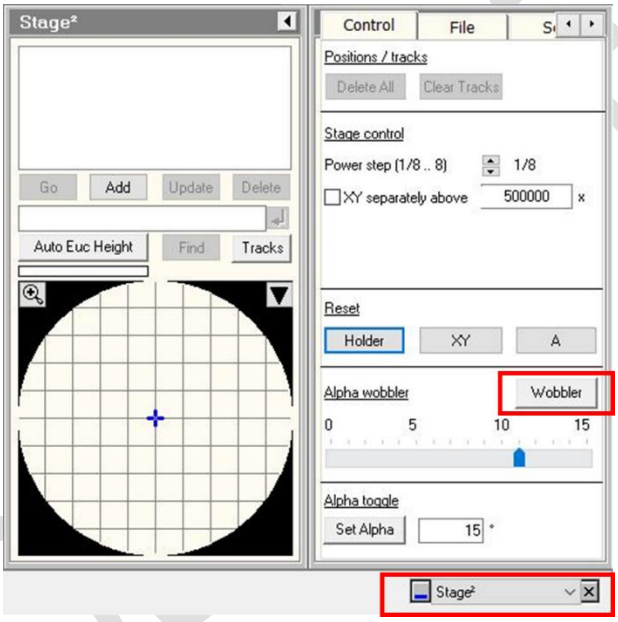
Microscope Operation

Find the target area

1. Open the column valve.
2. Utilize the control panel to adjust the magnification and intensity.
3. Move the stage to position the target area at the center.

Adjust eucentric height

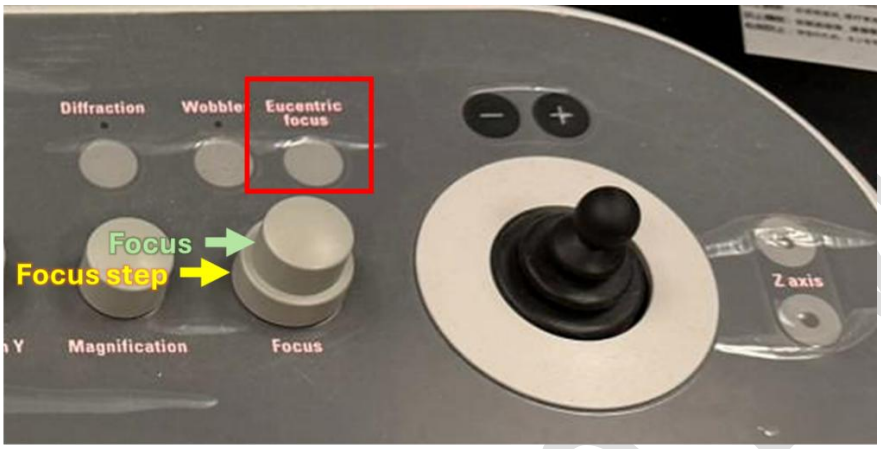
1. Low magnification is preferred. Set the magnification to LM mode
2. Identify a feature and position it at the center of the screen.
3. At ***Stage2***, click ***Wobbler***.

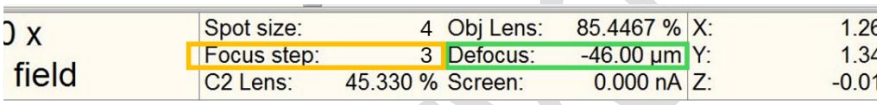


4. The image will appear to "move" on the screen.
5. Use the ***Z axis buttons*** on the control panel to adjust the stage height and minimize the movement of the object in the image.

Focus the image

1. Click on ***Eucentric focus*** on the control panel to set the defocus to zero.
2. Set the ***Focus step*** to 3 by adjusting the bottom knob (yellow) at Focus.
3. Use the top knob (green) at ***Focus*** to focus the image.

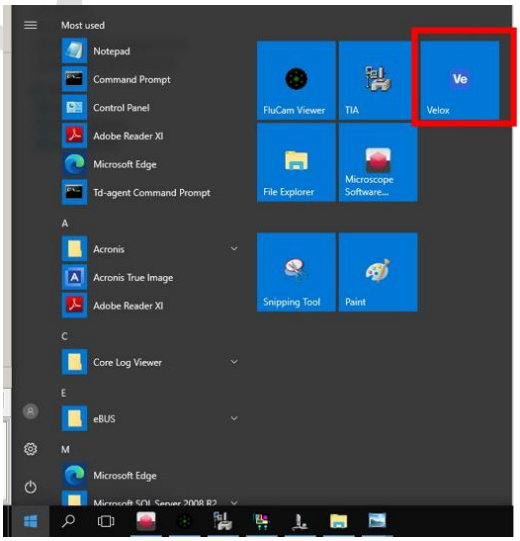




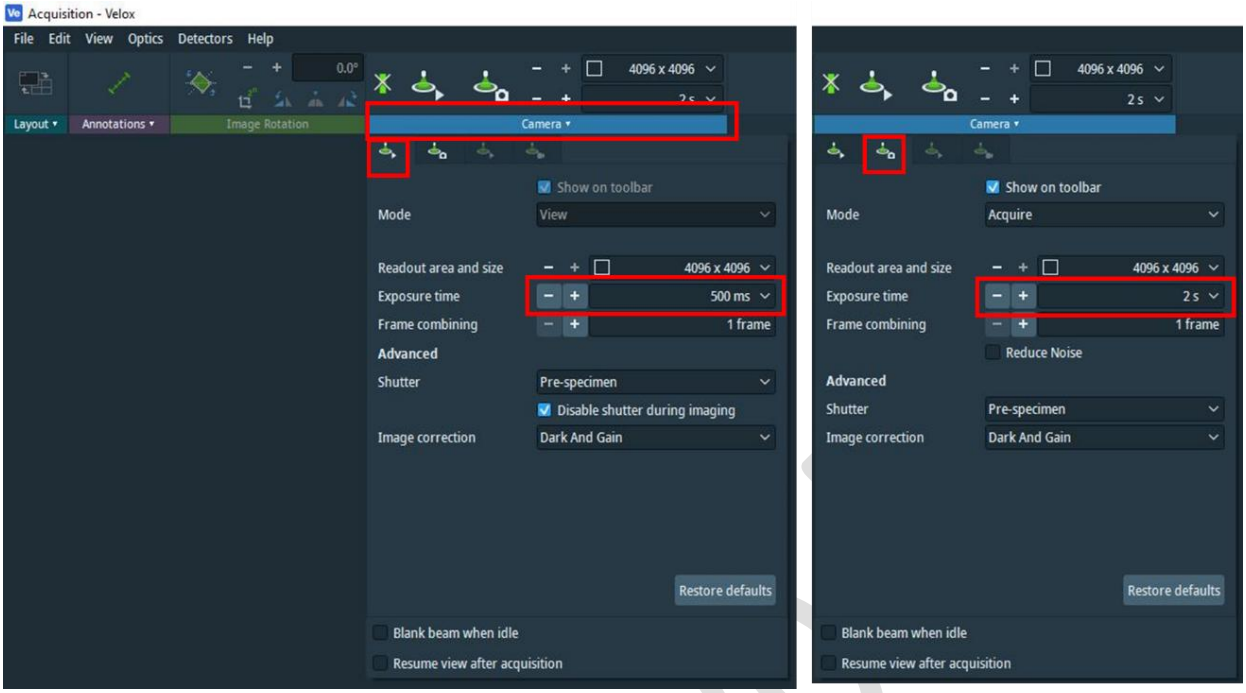
4. When the stage is at eucentric height and the image is in focus, the contrast will be minimal.
5. To increase the contract, set defocus by adjusting the focus knob as the step 3.

Acquire image

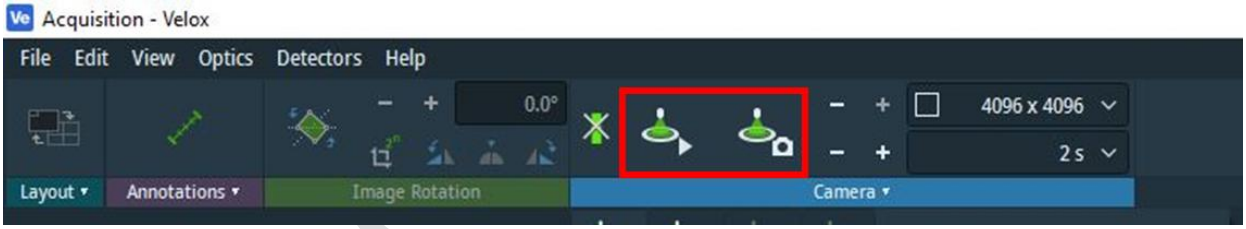
1. Perform alignment if necessary, following the instructions in the "Alignment" section.
2. Open software ***Velox***.



3. In ***Acquisition – Velox***, configure the exposure time for viewing and capturing.



4. Use the icons to switch between live view and acquisition modes.

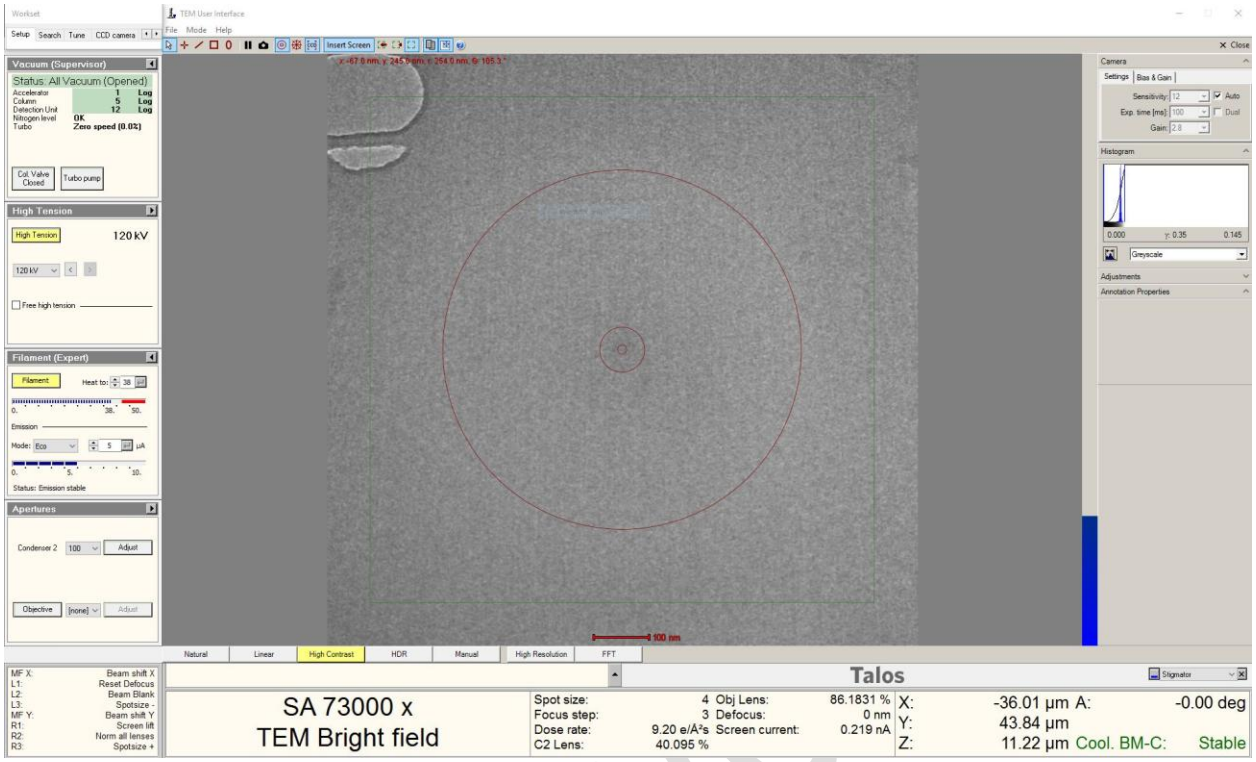


5. Preview the captured images ***Process – Velox***.

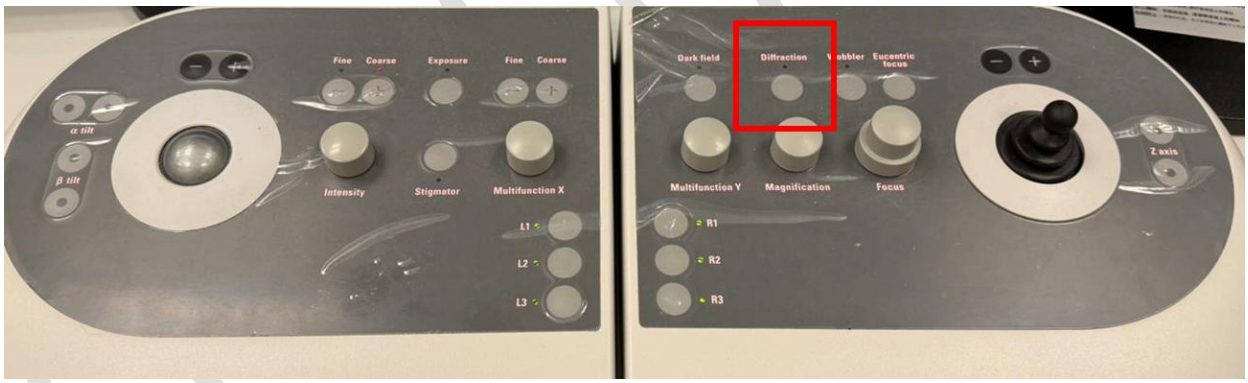
Acquire diffraction pattern for crystal structure analysis

Diffraction mode is used to obtain information about the crystal structure and orientation of materials. However, when switching the microscope to diffraction mode, the intensity can be very strong, potentially damaging the camera. Therefore, caution is necessary when using this mode. Here, we use a Graphene Oxide (GO) TEM grid as an example.

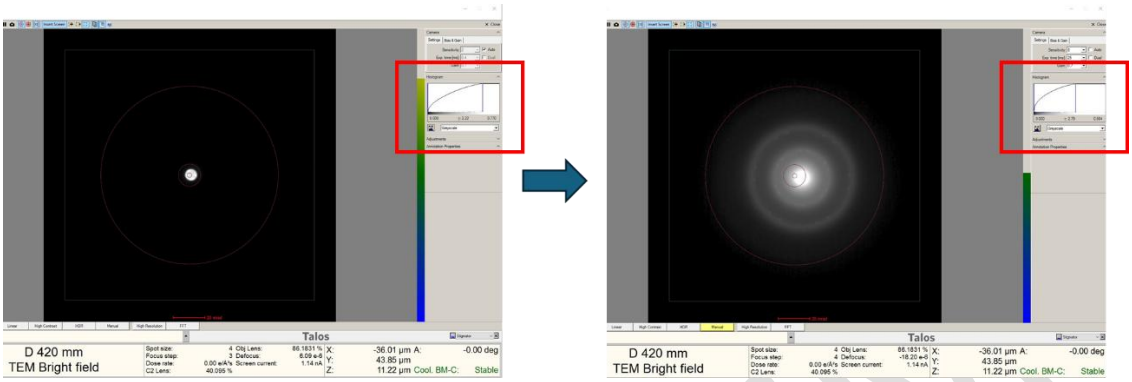
1. Align the microscope with the appropriate beam and position the sample at the eucentric height. This ensures that the sample is centered and at the optimal position for imaging.
2. ***Insert screen*** in ***UI***.
3. Change the magnification to SA mode, set the spot size to 4, and locate the position of the GO on the grid.



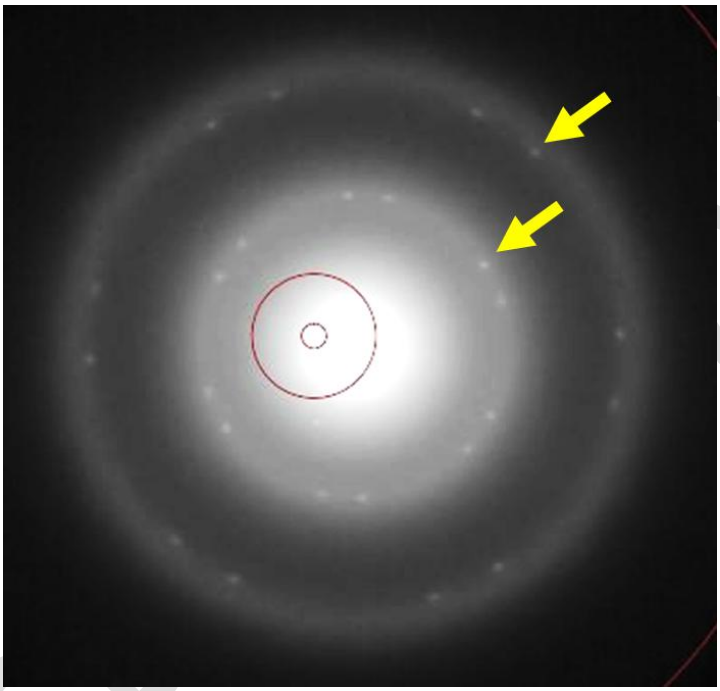
4. Click the ***Diffraction*** button on the control panel to switch from imaging mode to diffraction mode.



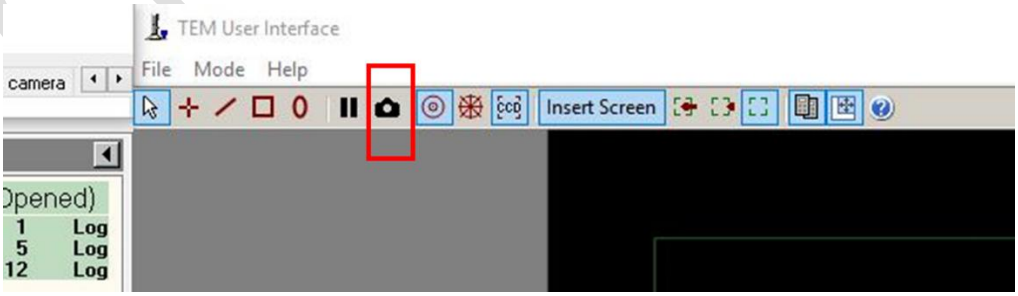
5. Use the mouse wheel or adjust the histogram to change the contrast. If necessary, adjust the focus for optimal clarity of the diffraction pattern.



6. After making these adjustments, you should be able to see the diffraction pattern clearly.

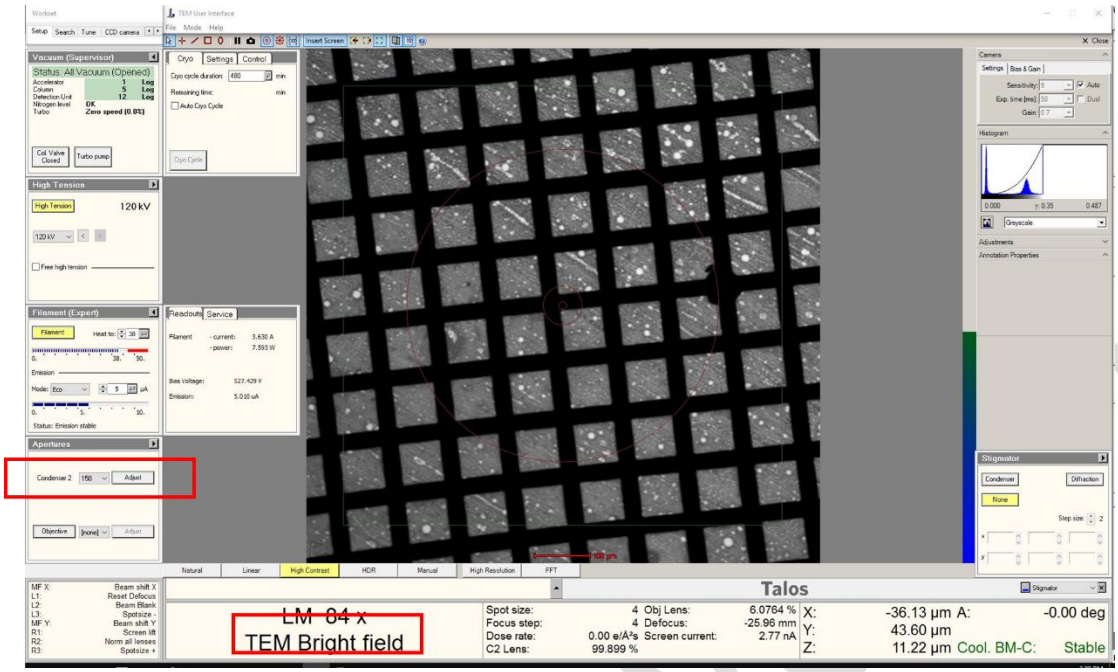


7. Capture the images in ***UI***. (NEVER use Ceta Camera for diffraction image capturing)

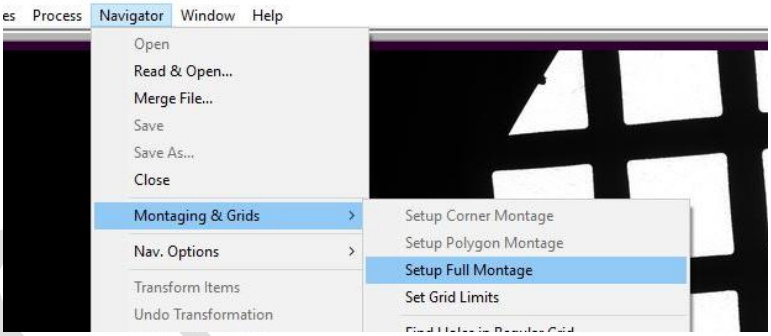


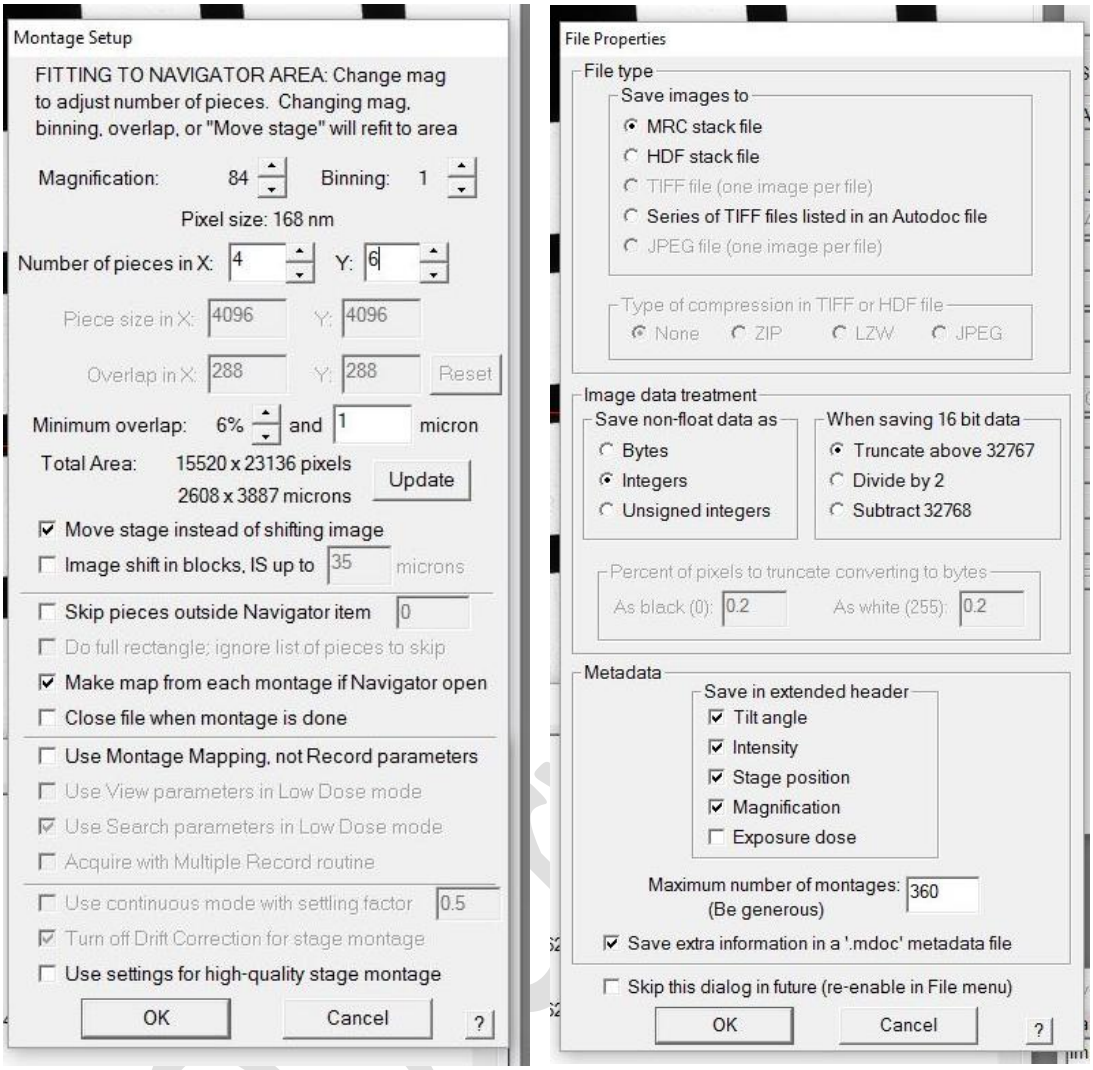
Acquire atlas / grid map

1. Set magnification as ***84x*** and use ***150***um at C2 aperture.

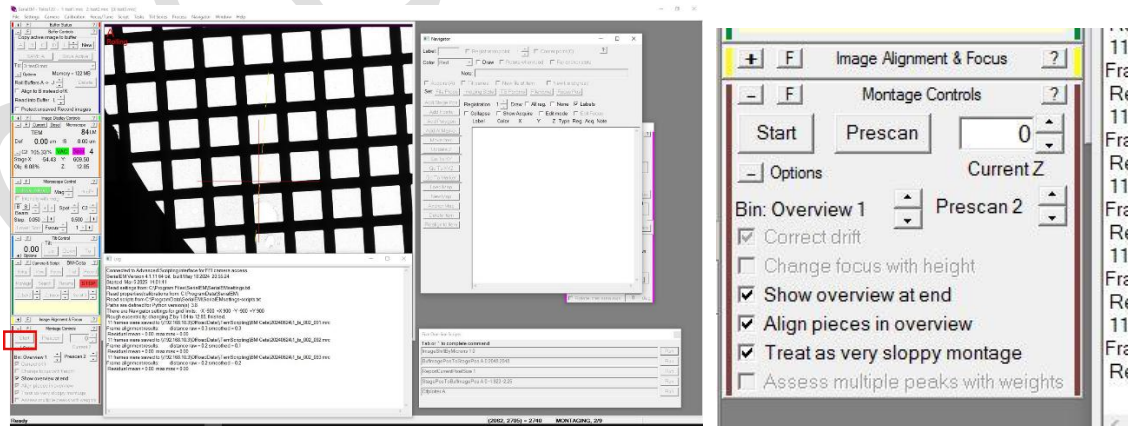


2. Open software ***SerialEM***.
3. Go to eucentric height: ***Tasks → Eucentric Rough***.
4. Open Navigator: ***Navigator→ Open***.
5. Set up Montage: ***Navigator → Montaging & Grid → Setup Full Montage***.





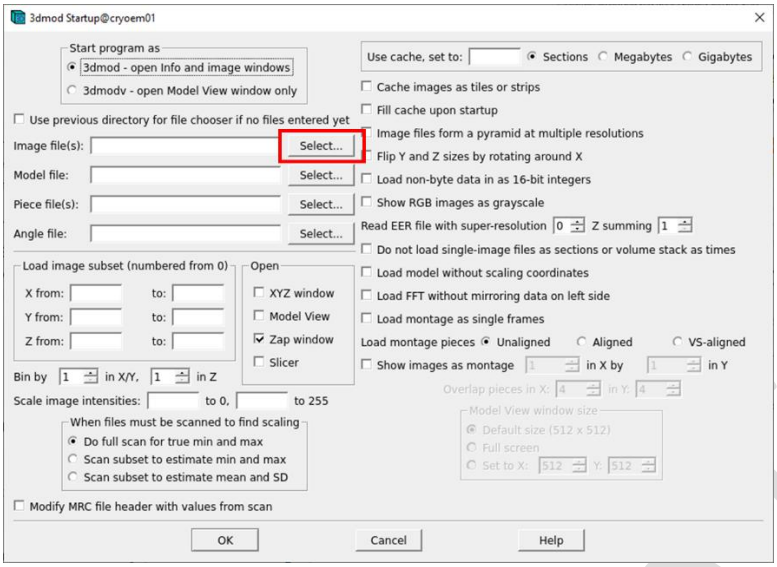
6. Press ***Start*** in ***Montage Controls*** window.



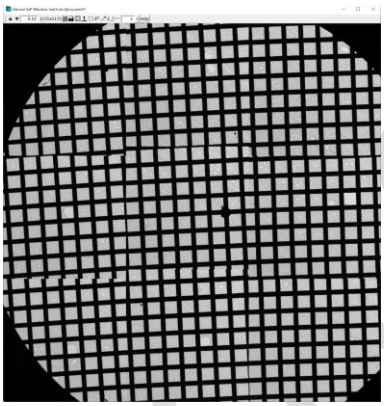
7. To view the montage, you can access HPC and load module imod.

```
$ module load imod
$ 3dmod
```

8. Open the mrc file in 3dmod.



9. The whole image can be viewed.



10. To get the individual images, you can use mrc2tif in imod.

```
$ module load imod
$ mrc2tif
$ mrc2tif <filepath.mrc> <tiff name>
```

11. The files will be generated automatically in the root folder.

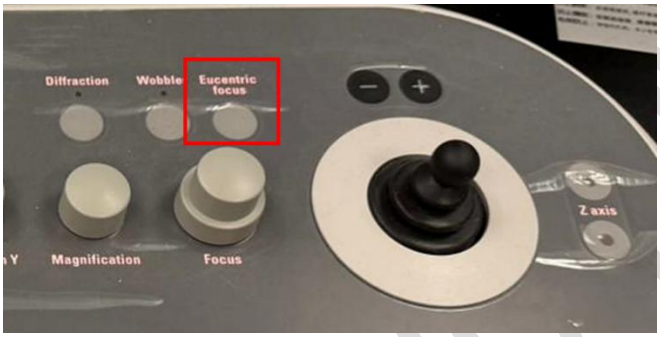
Alignment

Microscope alignment is crucial for image quality. Follow these steps to adjust alignment if necessary:

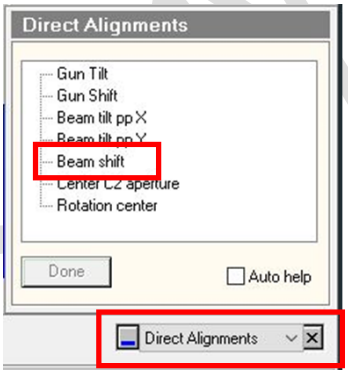
The beam is not at the center / The light at the screen is not even.

Beam Shift

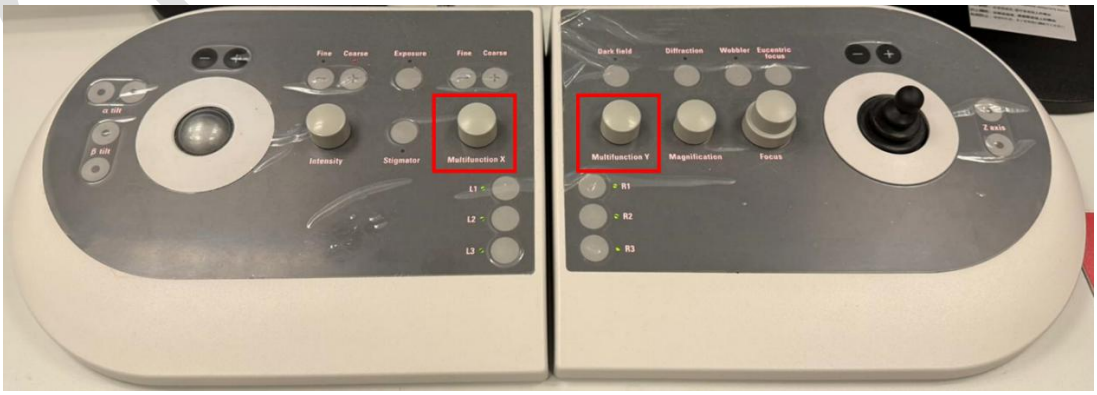
1. Press ***Eucentric Focus*** on the control panel.



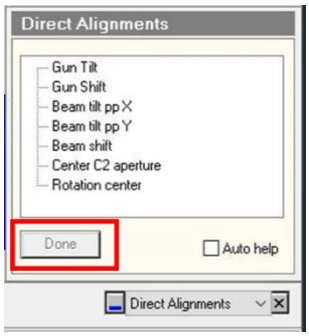
2. In ***UI***, select ***Beam shift*** in ***Direct Alignments***.



3. Reduce the beam size by adjusting the ***intensity*** on the control panel.
4. Move the beam spot to the center by adjusting ***MF X / Y*** on the control panel.

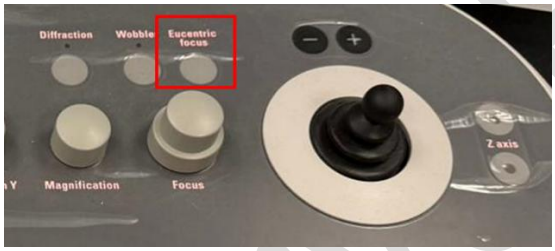


5. Click ***Done*** after adjustment.

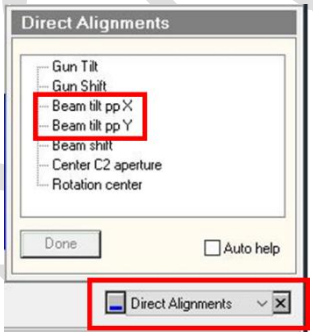


Beam tilt ppX / ppY

1. Press ***Eucentric Focus*** on the control panel.



2. In ***UI***, select ***Beam tilt ppX or Beam tilt ppY*** in ***Direct Alignments***.



3. Reduce the beam size by adjusting the ***intensity*** on the control panel.
4. You will see a shaking spot on the screen. Adjust ***MF X / Y*** to reduce the shaking.
5. Click ***Done*** after adjustment.

The beam is not circular.

Adjust condenser stimatism

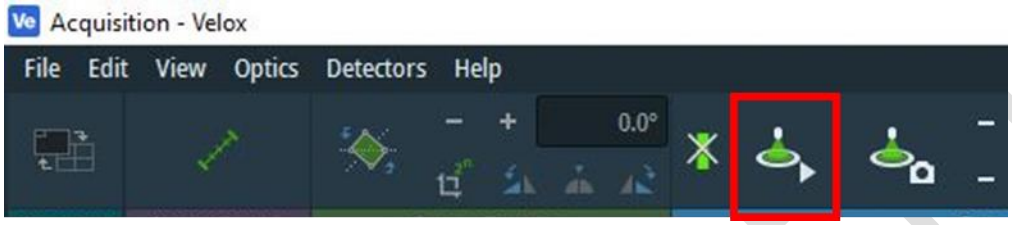
If you find that the beam is not circular on the screen:

1. Reduce the beam size by adjusting the ***Intensity***.
2. Click ***Condenser*** at ***Stigmator***.
3. Adjust ***MF X / Y*** to change the shape of the beam.
4. Click ***None*** to exit the mode.

The shape of the thon ring is not circular.

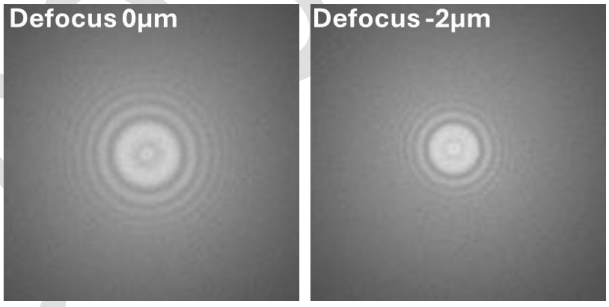
Adjust objective stigmatism

1. Switch to high magnification (e.g.73kx).
2. Move the stage to carbon film.
3. To ensure the intensity will not damage the detector, spread the beam size that is larger than the screen.
4. In ***Velox***, activate the live mode.

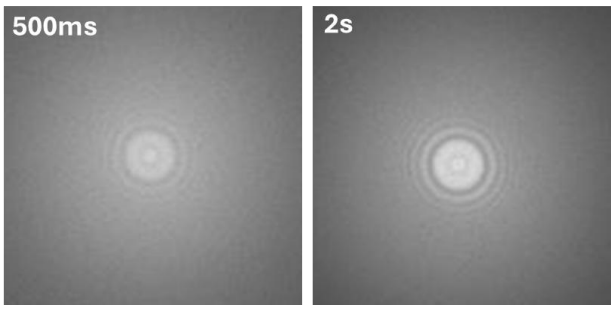


5. Observe the thon ring in FTT mode.

   a. Check the size of the thon ring. Adjust defocus (e.g. -2µm) to enhance visibility of the thon ring.



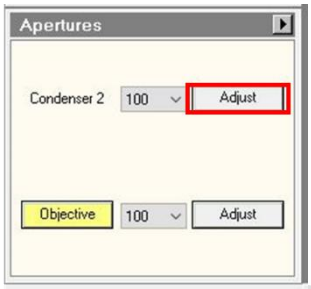
   b. If the contrast of the thon ring is low, increase exposure time in velox.



6. Click ***Objective*** at ***Stigmator***.
7. Adjust ***MF X / Y*** to change the shape of the beam.
8. Click ***None*** to exit the mode.

The beam is not concentric when the intensity is changing.

1. Reduce the beam size by adjusting the ***Intensity***.
2. Move the beam to the center by adjusting the ball onto the panel.
3. Click ***Adjust*** at the ***Apertures – Condenser 2***.



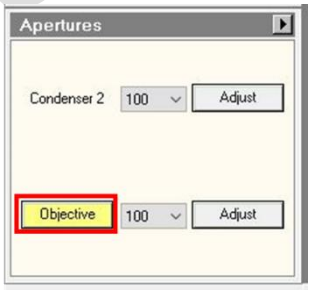
4. Enlarge the beam size by adjusting the ***Intensity***.
5. Use ***MF X / Y*** to move the beam to center.
6. Uncheck ***Adjust*** at the ***Apertures – Condenser 2***.
7. Repeat the steps 2- 3 times.

The contrast of the image is low.

Insert objective aperture

If you would like to increase the contrast at high magnification, you can insert objective aperture.

1. Click ***Object*** at ***Apertures***.

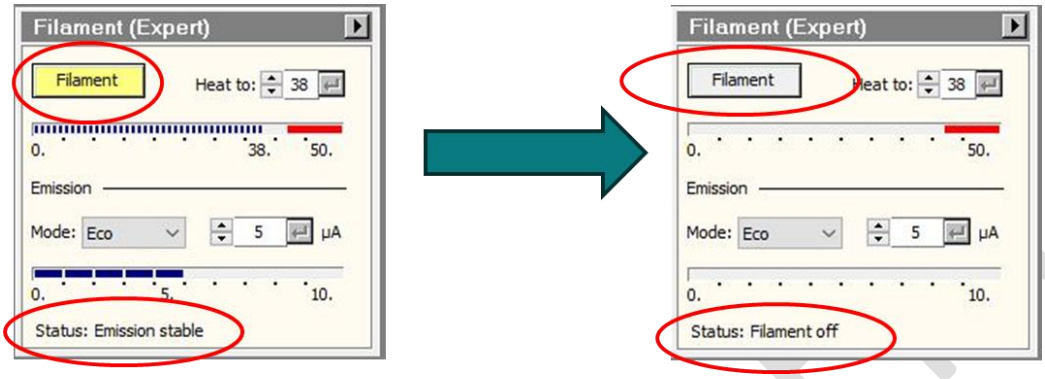


2. After inserting the aperture, check Stigmatism and adjust if necessary.

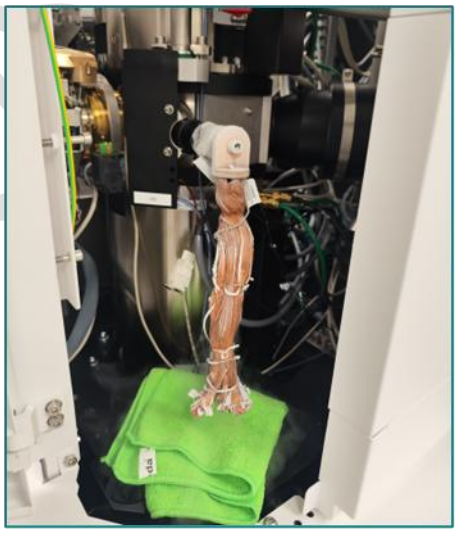
Note: Inserting an aperture may enhance contrast but could lower image resolution. Consider this trade-off when adjusting.

After Experiment

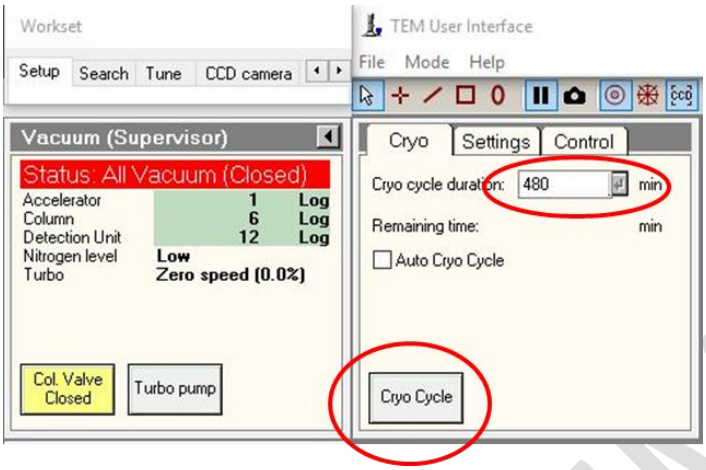
1. Close the column valve.
2. Turn off the filament.



3. Unload the sample. Pull the rod from the TEM until it stops. Rotate the rod clockwise. And pull again slowly.
4. Carefully place the holder back to the holder station. Unclamp the grid with the pin. Rotate the holder to drop the grid onto the filter paper.
5. Put the plug at the TEM.
6. Remove the LN2 cooling bottle in TEM and place a towel at the bottom of the copper wires.



7. Check with staff if there is another scheduled for the next session. If not, perform ***Cryo Cycle***.



8. Sign the logbook and take away your own belongings.

Data Transfer

Note: The data stored on the local computer will be removed on the 1st working day of each calendar month.

1. Refer ***CryoEM Microscope Data Management User Guide*** to transfer your data using FileZilla.
2. In your own computer, use FileZilla or other similar SFTP client software to download the data from the SFTP server.

SFTP Host: cmsftp01.cpos.hku.hk Username: contact staff for CMSFTP account Password: contact staff for CMSFTP account Port: 22 *Important Note:* • *Files uploaded to FileZilla are just temporary. All data residing in the SFTP server will be removed after 14 days.* • *There will be no backup of the data.* • *Please make sure you have successfully downloaded the data to your local computer within 14 days.* • *Do not disclose your Username and Password to others.*
HPC Host: cmsftp01.cpos.hku.hk Username: your HPC account Password: your HPC account Port: 22