

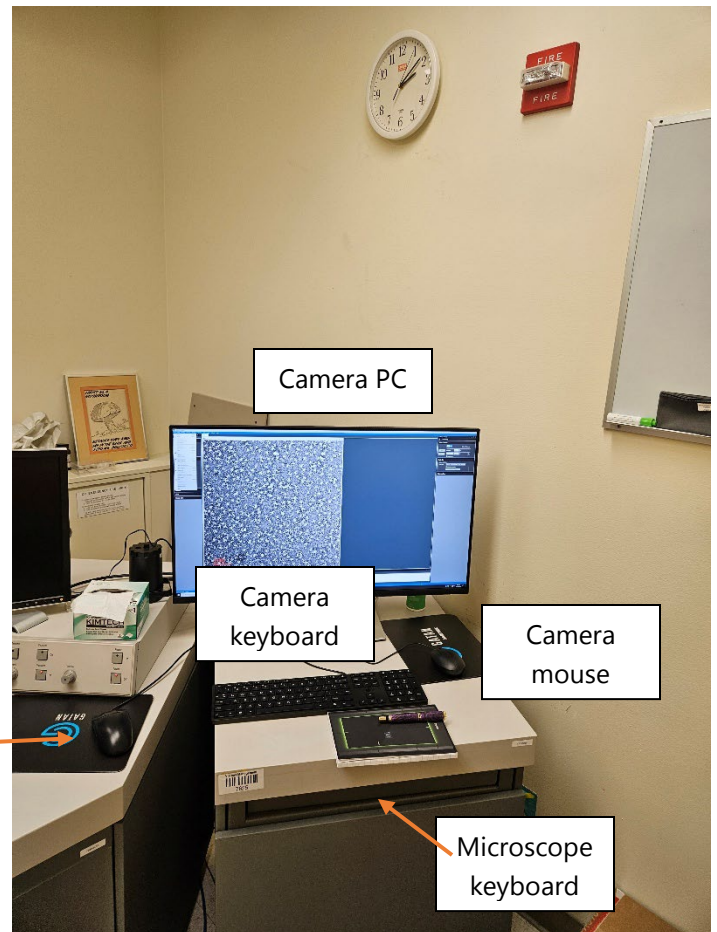
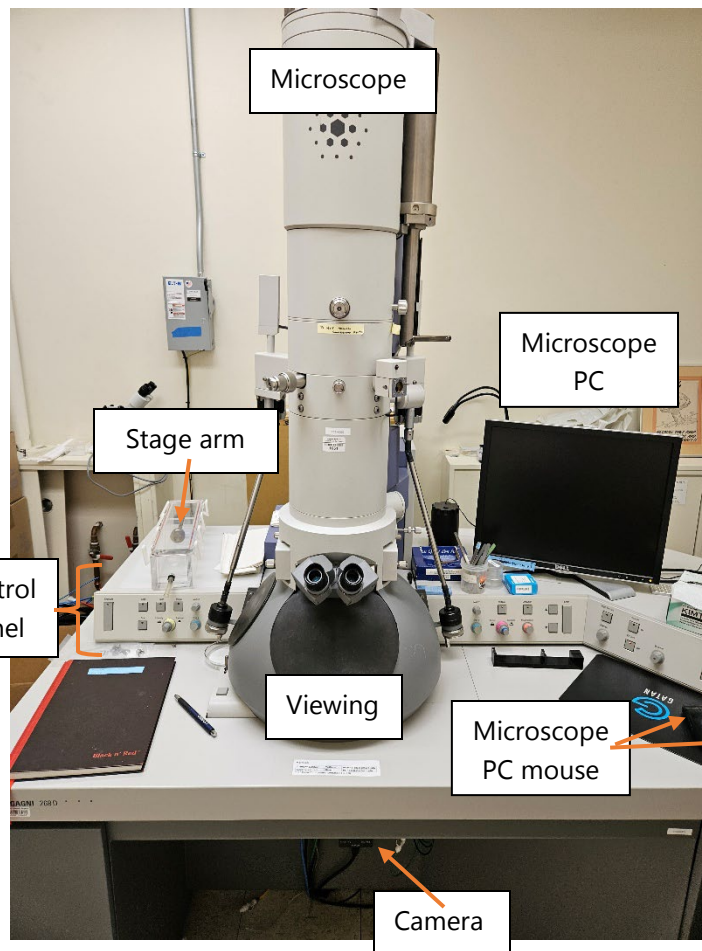
Morgagni Protocol

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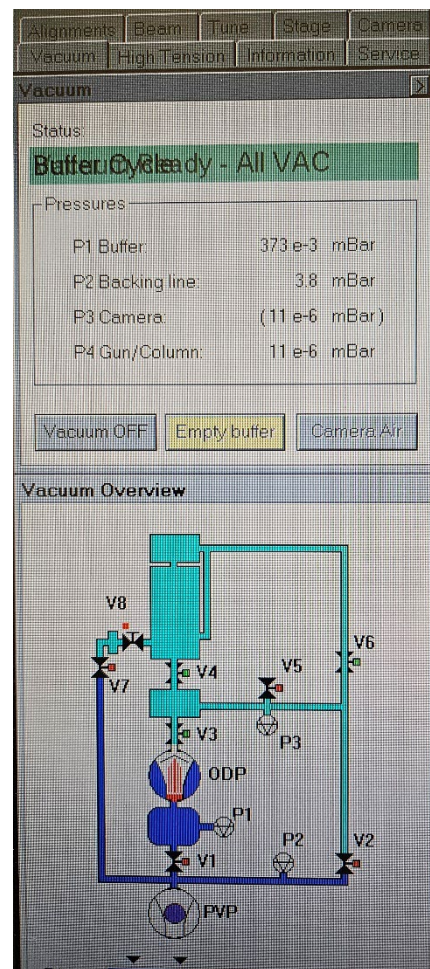
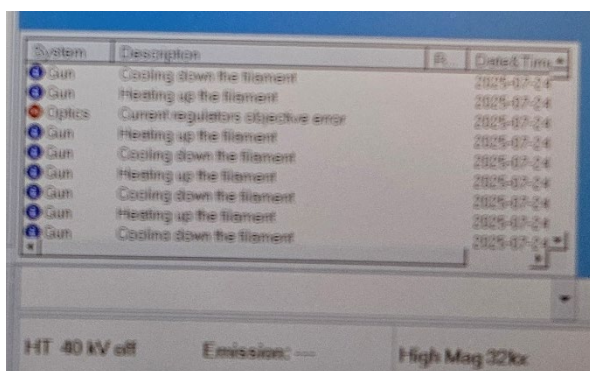
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Getting to know the Morgagni

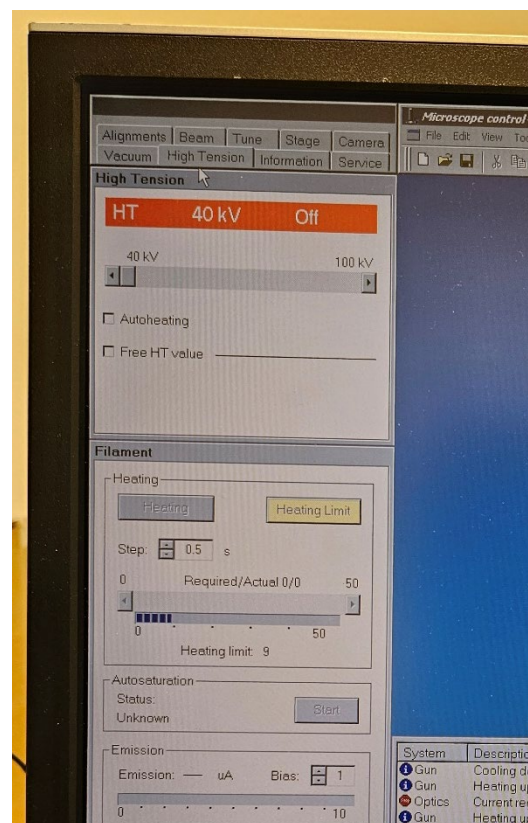
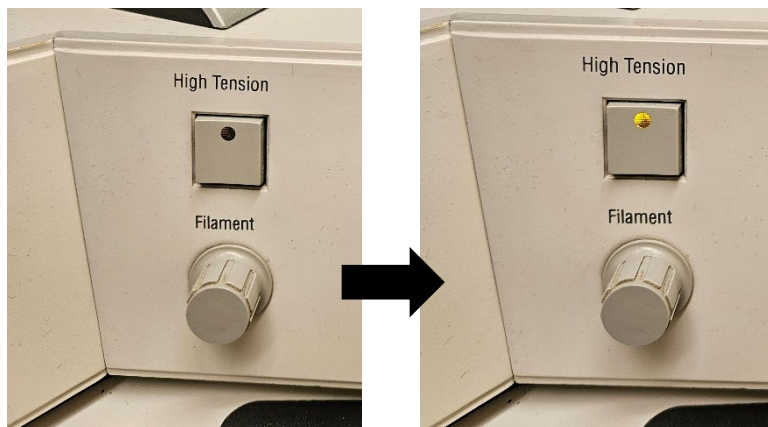


Starting up the microscope

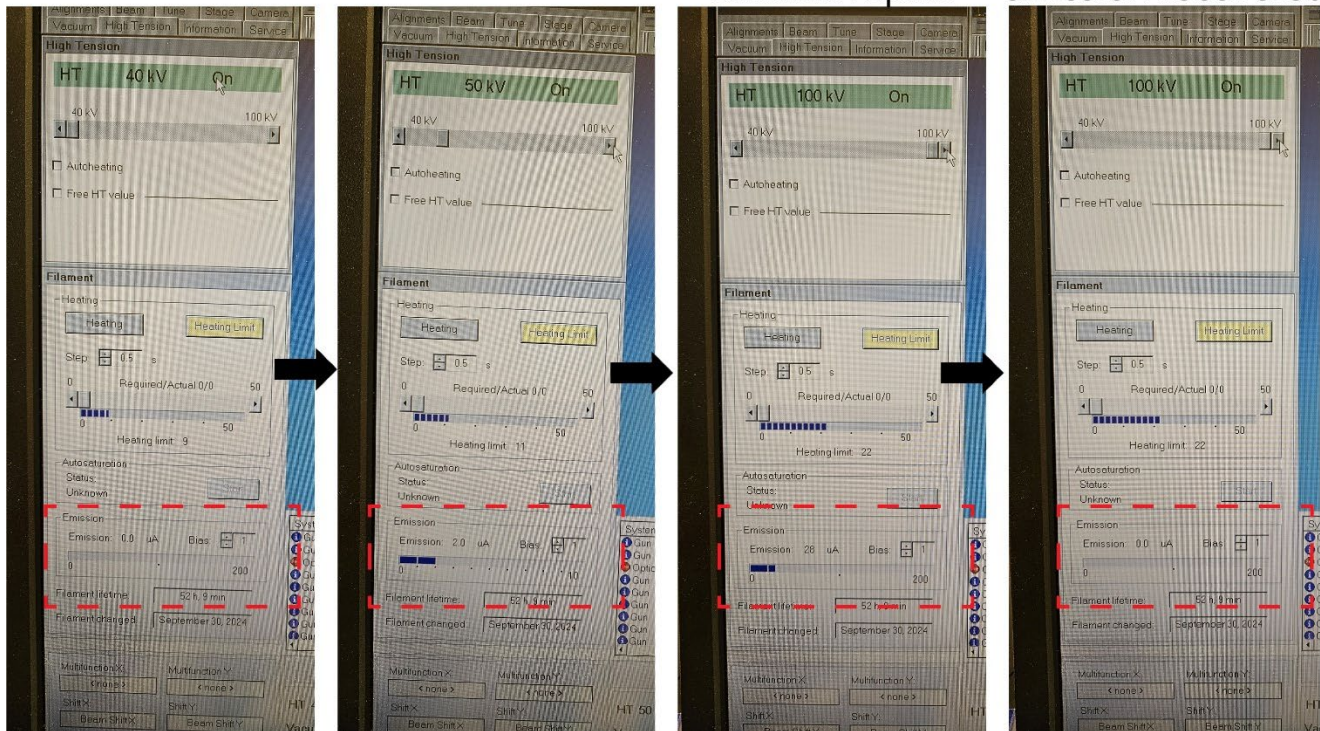
- Before starting, check the following on the vacuum:
 - Status says "Vacuum Ready – All VAC" and has a green background.
 - P1 is in the $100 \text{ e}^{-3} \text{ mBar}$ range
 - P2 is in the mBar range
 - P3 is in the $10\text{-}20 \text{ e}^{-6} \text{ mBar}$ range
 - P4 is present and in the $10\text{-}20 \text{ e}^{-6} \text{ mBar}$ range
 - ODP (bottom left of screen) reads above 60° C
- Check the message box (shown below) for any other errors and note the time of the occurrence. Some errors fix themselves, like the vacuum leak error.



- Click on the High Tension tab (shown right). High tension should be off when not in use. Below the monitor is the control panel. Press the High Tension button to turn it on. The button will light up indicating it is on.



- 100 KV
emission recovered



-

Loading the sample

1. The stage arm is stored in a plexiglass container beside the Morgagni. There is also a black metal stand to hold the arm while the grid is being placed. This stand is usually found by the Morgagni near the computer. Take out the arm and place it onto the metal stand with the pin on the shaft pointed up.

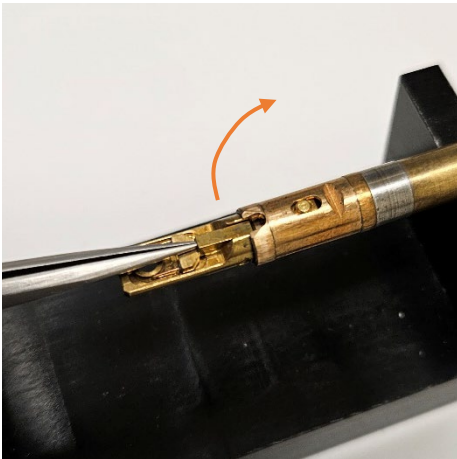


2. At the end of the arm is the stage to hold the grid. The grid is sandwiched between the stage and a metal ring that is attached to a few more metal pieces. What is important to notice right now is that there is a rectangular piece with a hole on the end that faces towards where the ring is.



3. Insert a pair of closed tweezers into the hole. Lift up to swing the holder open. Place the grid onto the stage inside the circular cutout. It does not matter which way you place the grid. Gently tap the arm to let the grid center into the cutout. Carefully use the tweezers to close the holder. The holder is spring-loaded and so it may jostle the stage and knock the grid out of alignment if done too roughly.

ONLY lift the holder by the rectangular box. **NEVER** lift the holder by the ring, as this will cause damage to the system. The ring is delicate and can easily be broken or lose its grip on the grid, allowing the grid to fall into the column and severely damaging the microscope. Always be gentle when handling the stage arm.



3. The side-entry port requires a certain sequence of actions to allow the vacuum to pump and to insert the sample properly. Follow the instructions carefully to avoid damaging the vacuum/microscope.

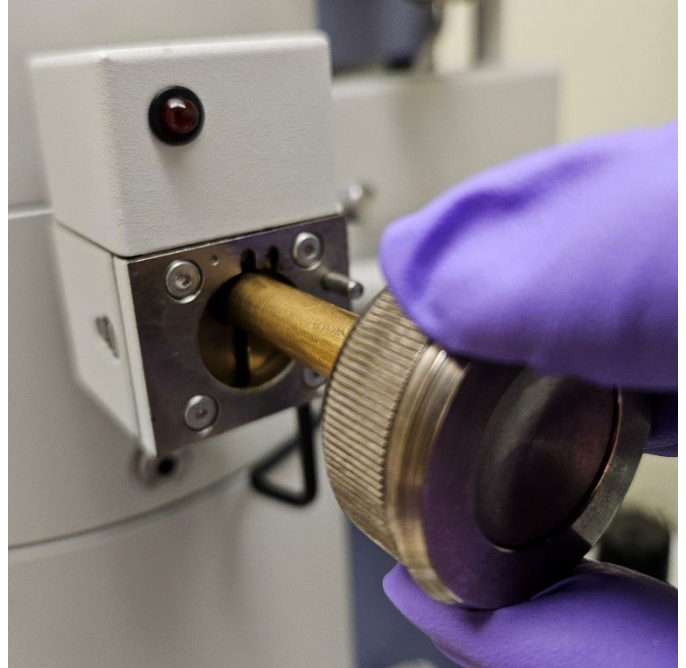
4. Insert the loading arm, placing the pin into the right notch (1 o'clock). The light will turn on and the vacuum pump will turn on.



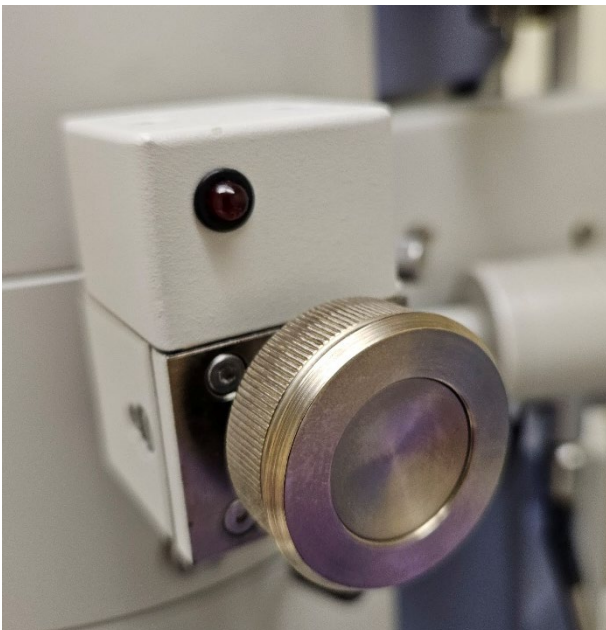
5. Once the vacuum starts, turn the arm counterclockwise using the silvery knob on the arm until the pin lines up with the left notch. You should feel the vacuum suck in the arm; guide and gently push it as it does.



6. Turn the arm counterclockwise until the pin faces 6 o'clock. The center of rotation is not the center of the arm, so it may feel a little weird.



6. When the pin is at 6 o'clock, you may feel the vacuum sucking in the arm. Gently guide the arm and push it into the microscope until the end of travel.



7. Press the button in the middle of the turning knob. This releases the head of the stage arm, which will stay inside the microscope during use. While keeping the button pressed, pull out the arm until the end of travel.



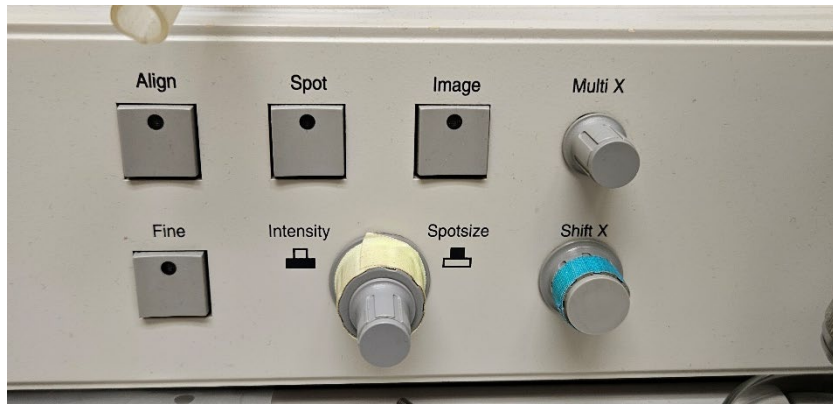
7. Turn the arm clockwise until the pin is back to 12 o'clock. Pull out the arm through this slot and place back onto the metal holder. Check that the head is no longer attached. If it is attached, you can reinsert and try again.



Using the microscope

1. Familiarize yourself with the control panel. It is split on the left and right of the microscope. The only buttons/knobs you will need for standard use are the Intensity (yellow), Shift X (medium blue), Shift Y (teal), Focus (pink), Focus Stepsize (blue), and Magnification (orange).

Left panel

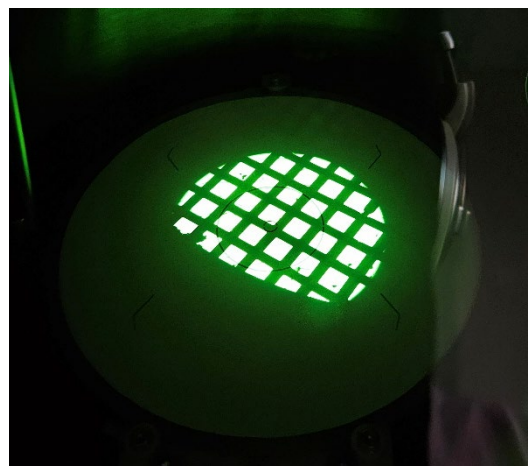
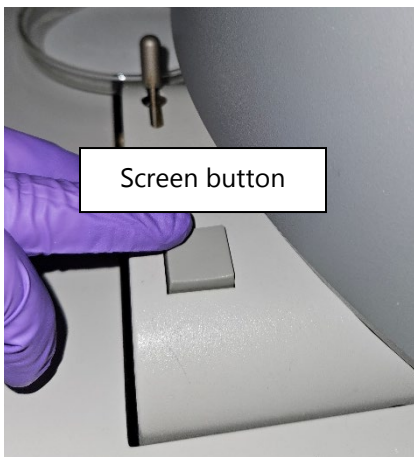


Right panel



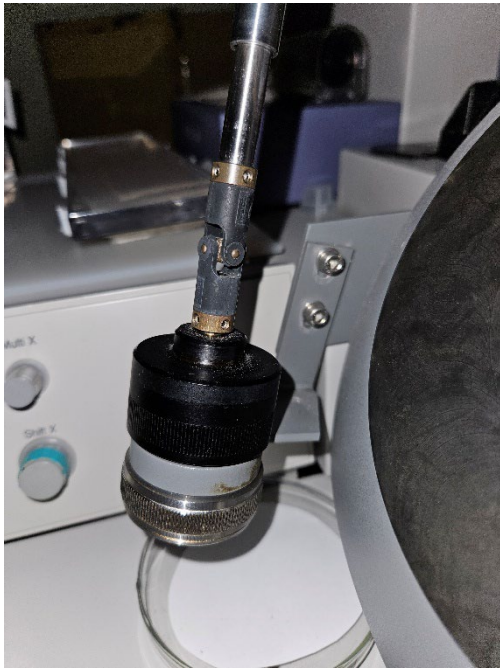
2. Shut off the light in the room. Too much light hitting the fluscreen can damage it. Take off the protective cover on the viewing window (black, rubber disc). If the fluscreen is up, press the "Screen" button next to the viewing window to lower it. Set the microscope to a low mag (e.g. 140x) to view the entire grid.

Screen button



- Adjust the stage to find an area you would like to image. The two actuators on the side of the microscope control the X and Y of the stage. Look for a section that is darker, indicating a lot of stain.

Left actuator

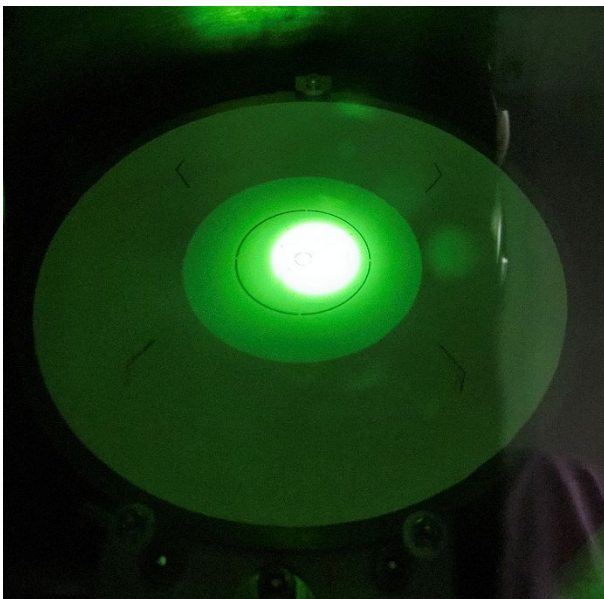


Right actuator

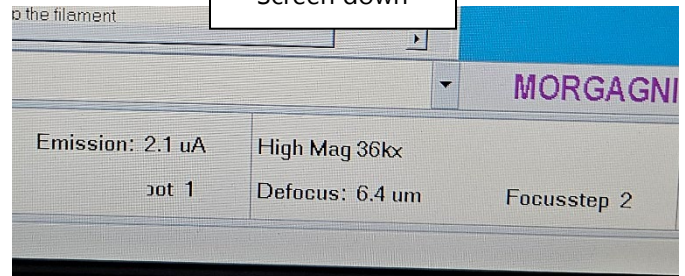


- Slowly increase the mag up to your desired magnification, all the while keeping the intensity as tight as possible. Bias the beam such that turning the intensity knob clockwise still tightens the beam. You may need to recenter the beam using the Shift X and Shift Y knobs.

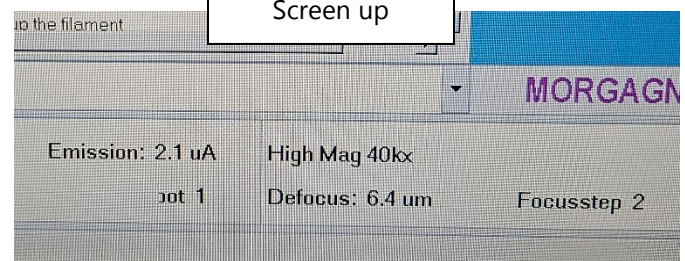
- Once you reach your desired mag, recenter the beam and lift the screen. Notice that the mag will change when the screen is lifted, as the camera sits lower in the imaging path.



Screen down

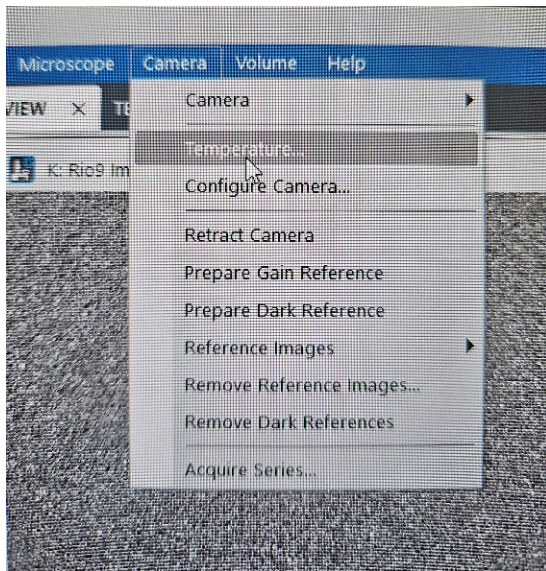


Screen up

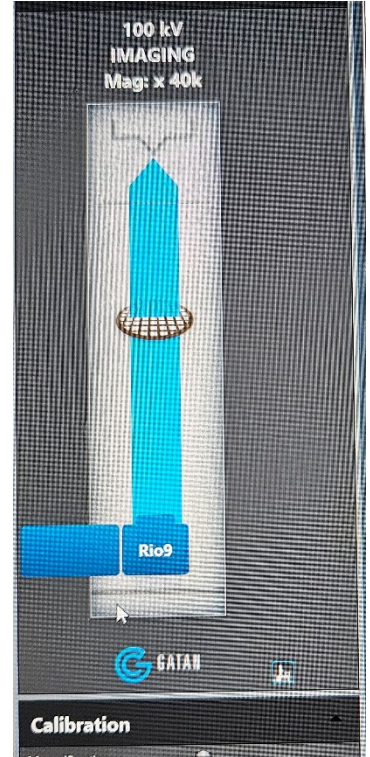
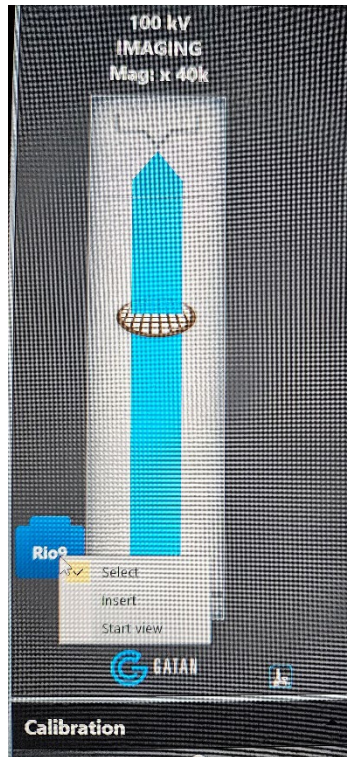


Taking an image (camera)

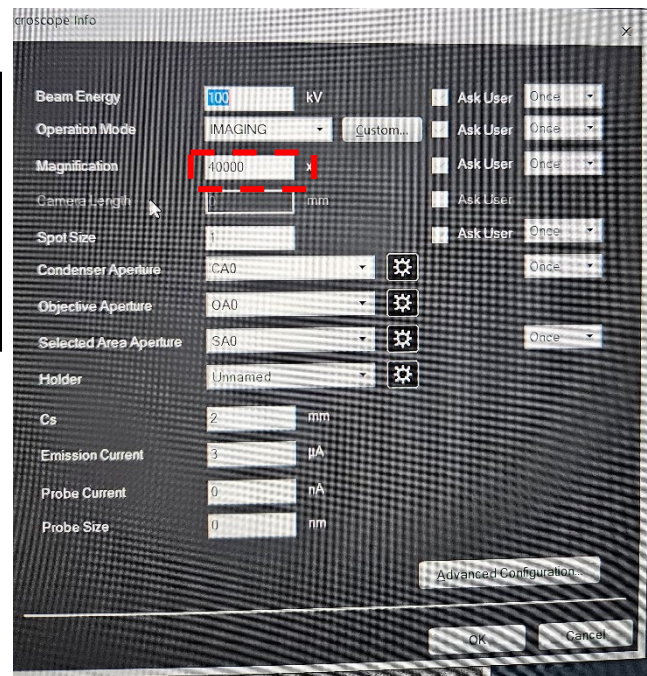
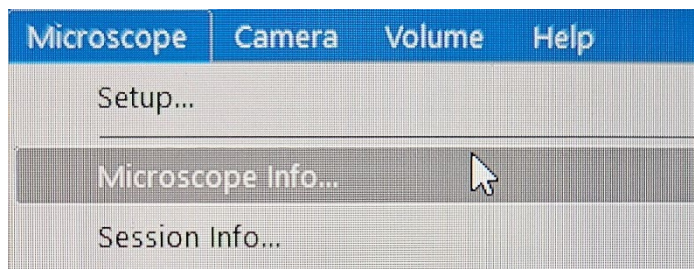
1. Camera should be at 20°C when not in use and 10°C when in use. Click the Camera at the top, select Temperature, and set to 10°C. Wait for it to reach temp before proceeding. Say yes to change temp, enter 10, and then hit "no" when it says to have the camera always be at 10 when GDM starts up.



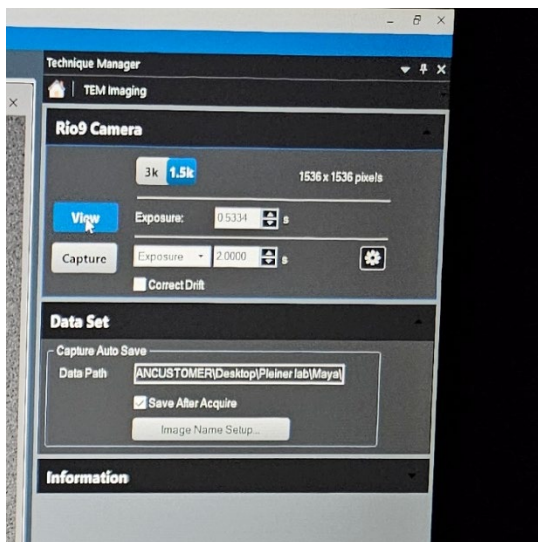
2. On the camera computer, in Gatan Digital Micrograph (GDM), you will see a graphic of the microscope and the camera. Right-click on the camera and select "insert". The camera should always be retracted when not in use.



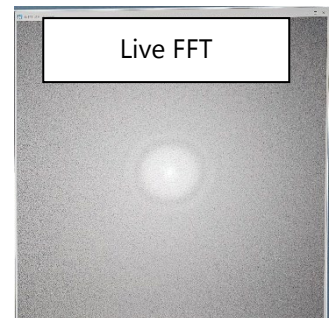
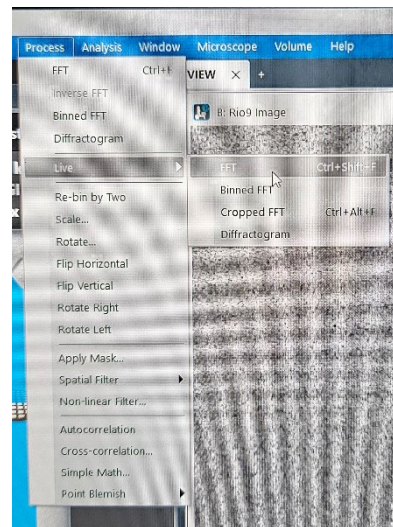
3. Set the microscope settings. Go to Microscope → Microscope Info... and a dialogue box will appear. You should only need to change the mag to the mag that it reads on the microscope PC.



- On the right, in the box that says Rio Camera, click View to see your image.

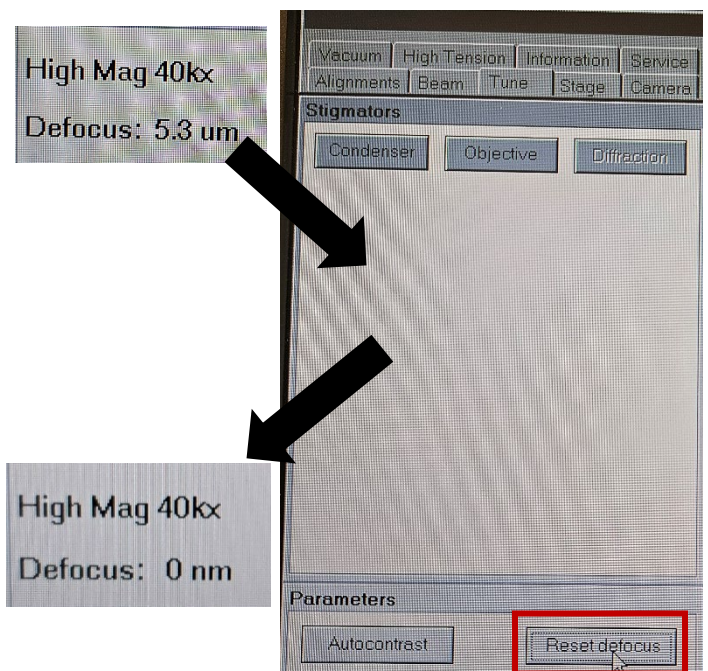


- Select Process → Live → FFT to see the live FFT, if it is not up already.

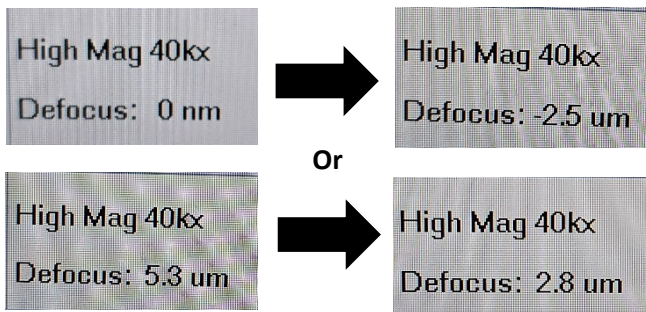


- We cannot move the stage to determine the eucentric height (wobble method). Therefore, use the focus knob to find the eucentric height and then apply underfocus. First, adjust focus until FFT and image look like below (no Thon rings in FFT).

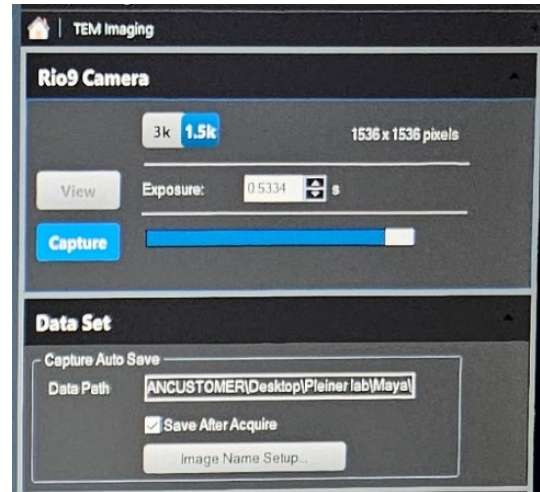
- Note what defocus is displayed. You can either reset the defocus value to zero (Tune → Reset Defocus) or just subtract the desired amount of defocus from this value in the next step.



8. Add -2.0 to -3.0 defocus to the value. The Thon rings should look like below.

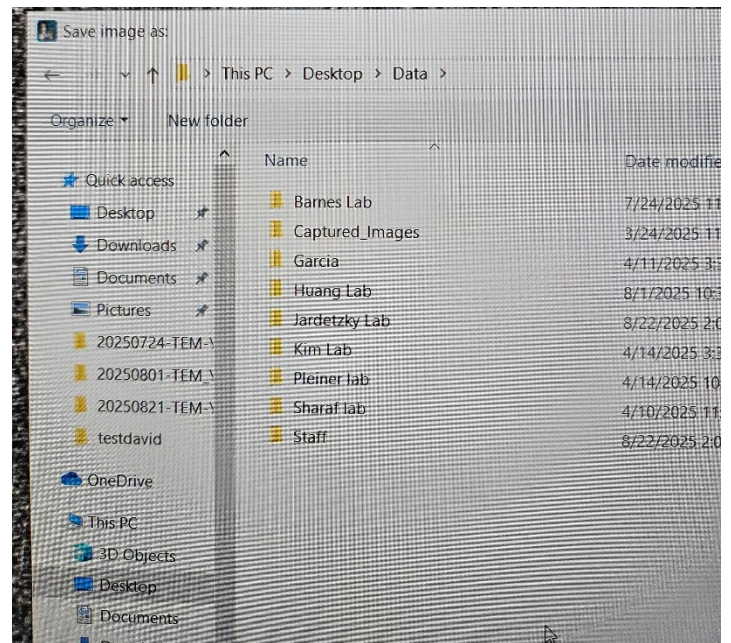
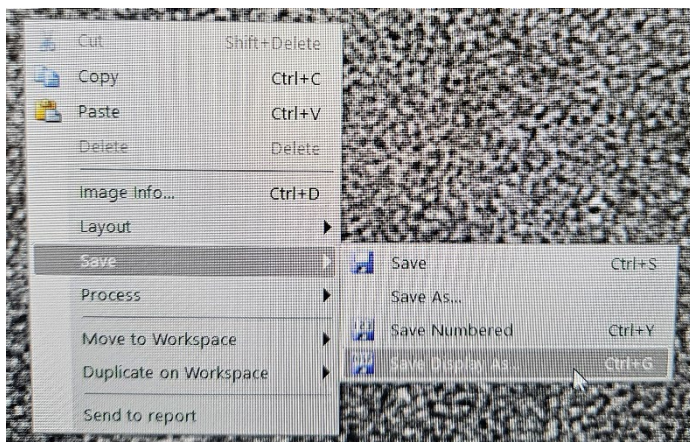


9. To take a picture, unselect View and set the exposure time to your desired duration (up to 2 seconds is usually fine). Select Capture.



10. The picture (and all subsequent) will appear in another tab. To save as an image file (e.g. .tif), right click on the picture and select Save → Save Display As...

11. Save all data (including image files) under Desktop\Data\Lab\User. In order to be able to open up the data in GDM again, right click and select Save. Save it as a .dm3 file.

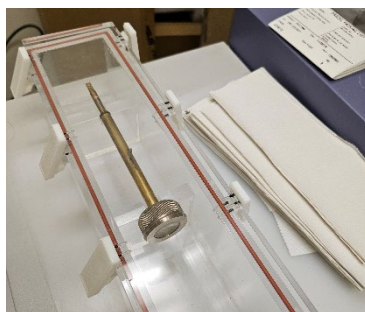
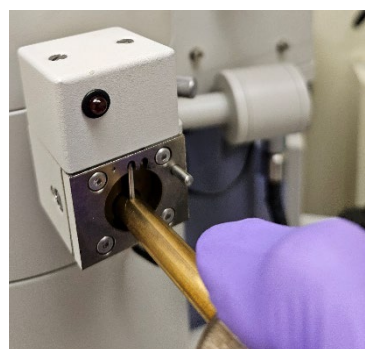


Finishing the session

1. When you are done with the sample, turn off the camera view in GDM and turn off the Heating on the filament. If you are done for the day, turn off the HT and reset it to 40 KV; otherwise, keep it on to load another grid.
2. Take out the loading arm. Insert the arm to activate the vacuum and rotate to 12 and then 6 o'clock like you did when you were loading.



3. This time, insert the arm with the button already pressed in order to grab the stage. Keep the button pressed until the end of travel, then release it. Draw it back, turn to 12 o'clock, and pull it all the way out. Put the arm back in the case and put away all tools if you are done, or load another grid.



4. At the end of your session reset the camera to 20°C.