

FEI Osiris 200 keV TEM Standard Operating Procedure

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TEM sample requirements: dry solid samples (metals, semiconductors, oxides, thin films on substrates, nanoparticles) on TEM grids; stable in vacuum (no outgassing), during electron beam examination; samples must be conducting, clean of debris, free of oil and organics; no polymers, liquids, magnetics or bio-samples; all samples must be plasma cleaned prior to examination in TEM.

STARTING TEM SOFTWARE

On the TEM Main Computer, under Win XP log on into the TEM user account “temuser”. Start the TEM software using shortcuts on the desktop in the following sequence:

- TEM User Interface;
- Gatan Digital Micrograph;
- TEM Imaging and Analysis (TIA);
- FluCam Viewer.

You can also launch the software by selecting Start → “name of the program” in the order indicated above. **Wait until each individual software module loads before launching the next one. Make sure to acknowledge all popup error messages during software loading.** Launch of the software is complete when the FluCam viewer appears (initially a large black box on the left monitor).

Acknowledge all popup error messages, most of them are related to the now defunct EELS Filter Control.

Do not change the menus or desktop layout.

First, **check vacuum levels.**

Their values should look similar to

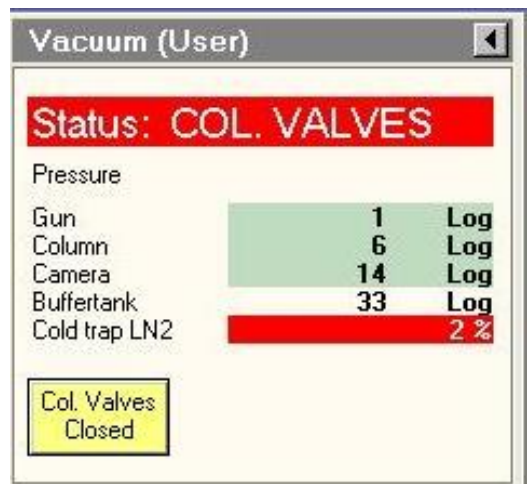
Ignore the cold trap LN warnings.

Log on to the local user account

“.\temuser”

“TEMuser01”

on the **TEM Support PC.**



TEM SOP

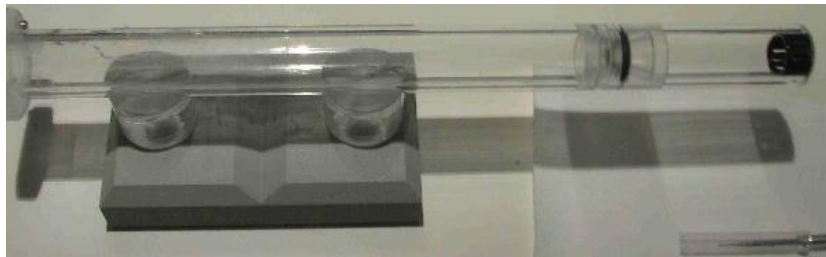
HANDLING TEM HOLDER

When handling the holder and **clamp lifting tool**, always wear clean gloves. The **clamp lifting tool** should be stored in the protective plastic sleeve.



Preparation:

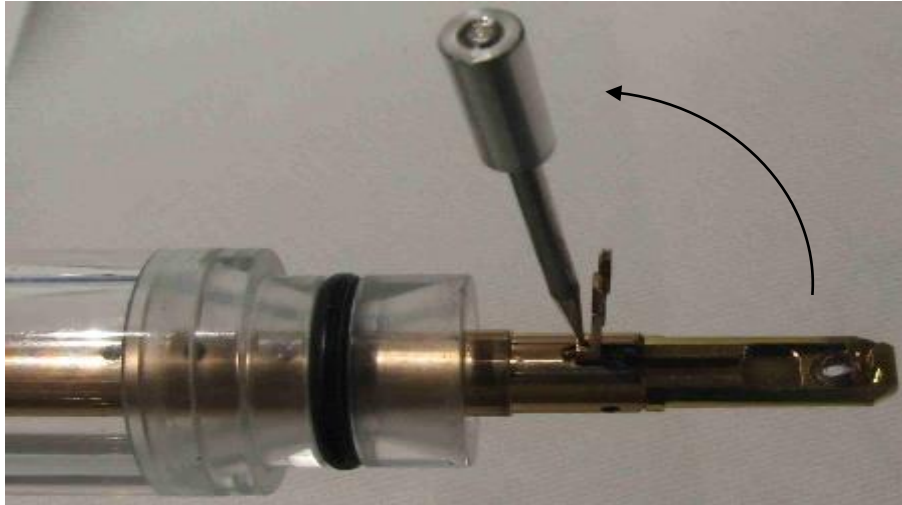
Dust off thoroughly the specimen loading stand with N2 gun prior to using it.



Put the sample holder into the **loading stand** on the specimen preparation table and inspect it for contamination. Turn on the monitor for the inspection camera on the sample preparation desk. Remove the cap at the end of the tube of the specimen holder cover. To ensure the holder cannot move out of the cover tube or rotate, keep one hand against the holder end opposite to where the sample sits. Remember not to touch any parts of the holder below the sealing O-ring with tweezers (use fresh sharpened wooden sticks to pick up visible debris, if you see any on the monitor screen).

Carefully fit the **lifting tool** into the hole in front of the clamp:





Remember that the clamp is spring loaded.

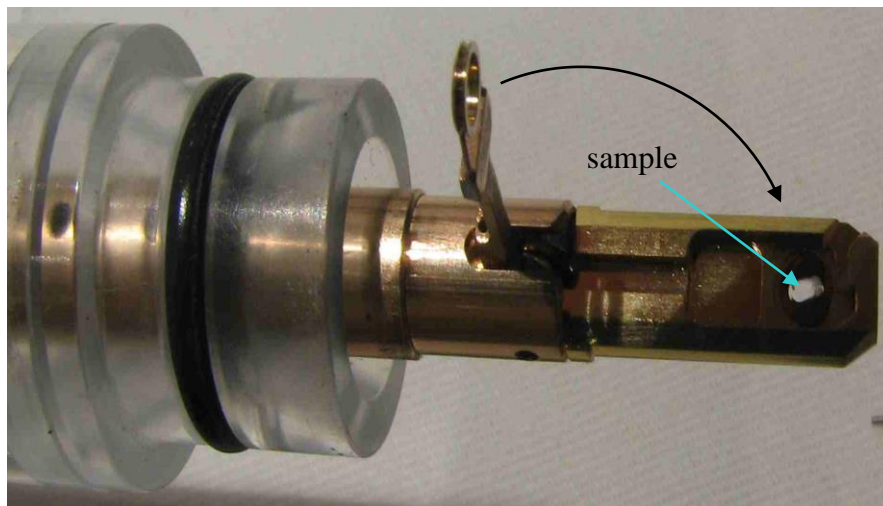
Remove the clamp lifting tool and use the camera to inspect the clamp for contamination using the camera monitor screen.

Lower the clamp down and proceed with plasma cleaning the holder **using a standard (short) plasma cleaner adapter** for at least 5 minutes. Prior to loading the holder into the plasma cleaner make sure to dust its entire length off with nitrogen gun. After finishing the plasma cleaning process you can load the sample onto the holder.

Loading sample onto the holder:

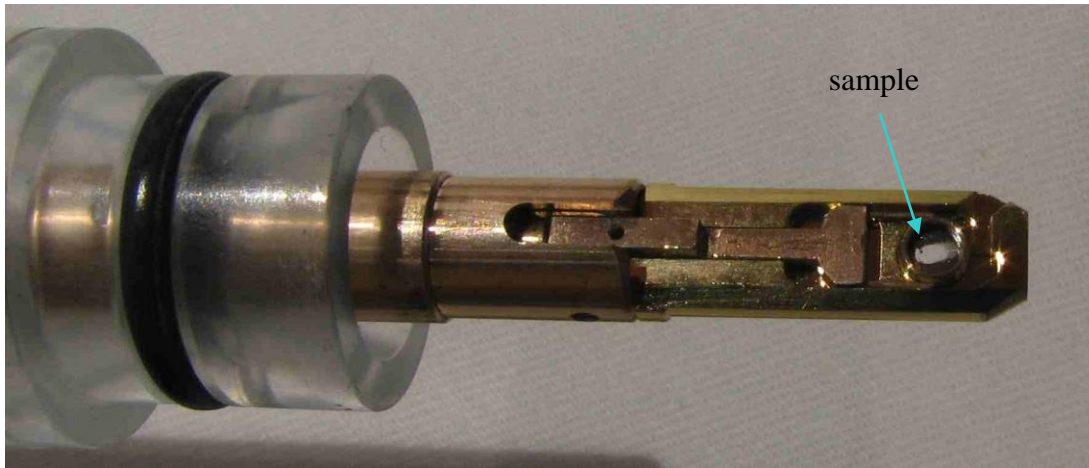
Repeat the steps described above to lift the clamp on the holder. Carefully remove the lifting tool without letting the clamp go back to the “close” position. If you do, you can destroy your sample, clamp and you can also damage the holder.

Place the specimen in the **circular recess** of the specimen holder tip. To position the sample properly, gently tap the holder, do not touch the holder end, where the sample sits, with any objects (including your tweezers).



TEM SOP

Very carefully lower the clamp with the tool onto the specimen. Rotate the holder and inspect the specimen under the camera. Gently tap on the holder end opposite to the sample area to verify the specimen is not moving and the clamp is keeping it in place.



If either of these items appears to be loose, stop, it is not safe to put the holder into TEM. Try repeating the specimen loading procedure.

Inspect the specimen under the camera using a higher magnification. Rotate the holder a few degrees each way and see how the specimen looks on the monitor screen. Do not forget to return the camera to the lowest magnification setting at the end.

Thoroughly inspect the holder and clean all visible debris (using fresh sharpened wooden sticks) paying extra attention to the holder O-ring.

Carefully dust the specimen holder off using the nitrogen gun. Clean the holder with the sample in the plasma cleaning unit for 1-2 minutes using a **standard (short) plasma cleaner adapter**. For cleaning sensitive samples (carbonated samples) please use a **shielded accessory (long adapter)** and clean samples for about 1-2 minutes. Prior to venting the plasma cleaner after finishing cleaning leave the holder under vacuum for a few more minutes to pump away the byproducts of plasma cleaning and to further stabilize the sample prior to loading into a high vacuum environment of the TEM column.

After plasma cleaning do not forget to inspect the holder with the sample and especially the holder O-ring under the camera. If you see any debris, make sure to remove it prior to proceeding with loading the holder into TEM. When finished, do not forget to turn off the monitor for the CCD camera on the specimen preparation table after finishing this step.

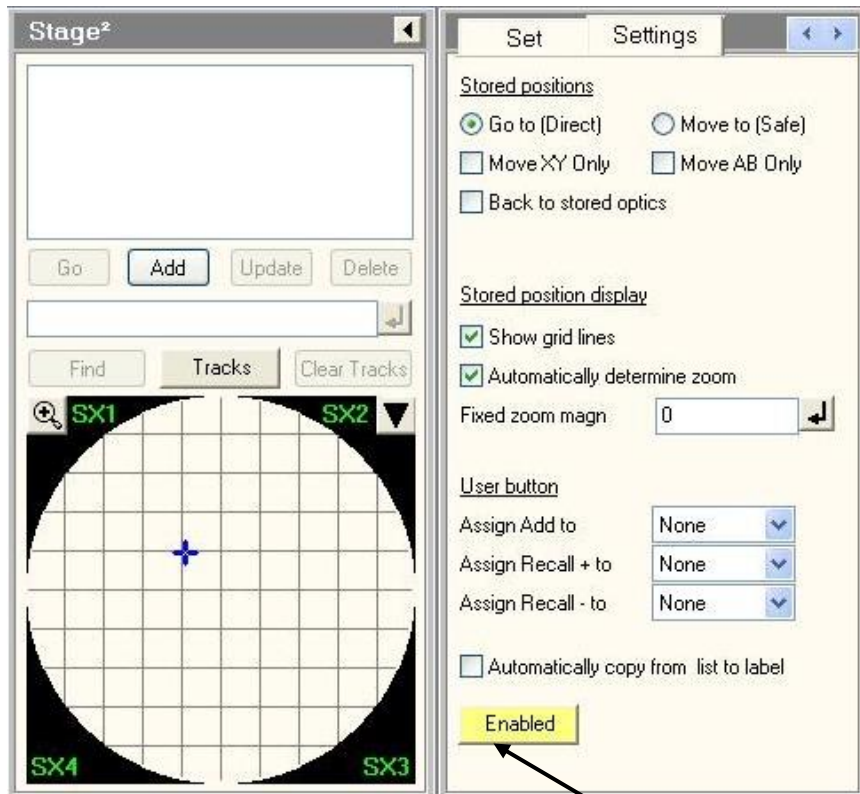
Note: While the sample holder is being plasma cleaned you can Home the CompuStage.

HOMING COMPUSTAGE

Before the CompuStage is ready for use, it must be homed, a procedure in which it finds the zero positions.

Important: The procedure must be executed without a specimen holder.

Select **Search** → **Stage²** → **Settings Control Panel** → and press the **Enabled** button.



In the message box



Select Specimen Holder:

choose “No Specimen Holder” and click



The homing procedure will start as indicated by the red light on the stage. Wait until this process is completed, that is with all axes and angles becoming zeros and by red LED light on the CompuStage going off.

LOADING TEM HOLDER INTO TEM

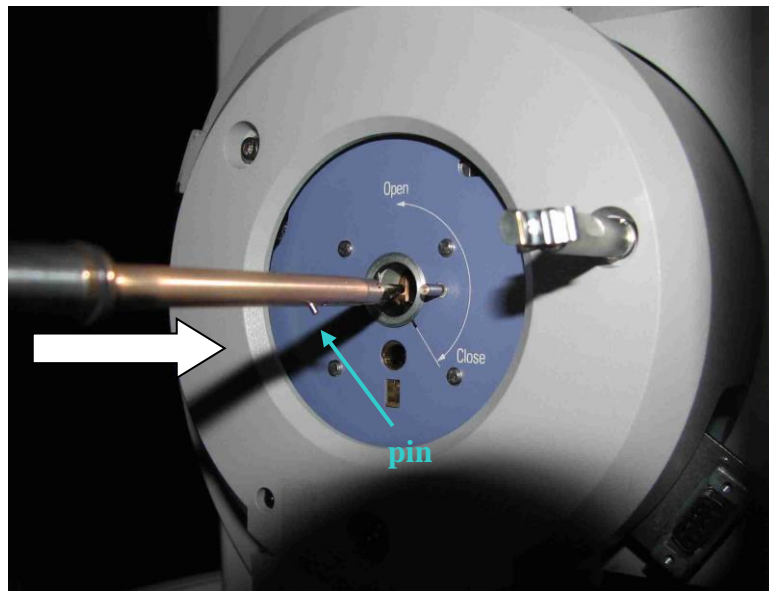
Important: Make sure the column valves are closed and the stage is at home settings (all zeros). If there is a red light on the loadlock, do not proceed with loading. Notify staff.

Always carry out the complete insertion procedure. If you do not want to insert the specimen holder after all (for example, if the airlock is not pumped properly), leave the airlock cycle to finish pumping (the red LED on the CompuStage going off) before removing the holder from the airlock.

Holder insertion

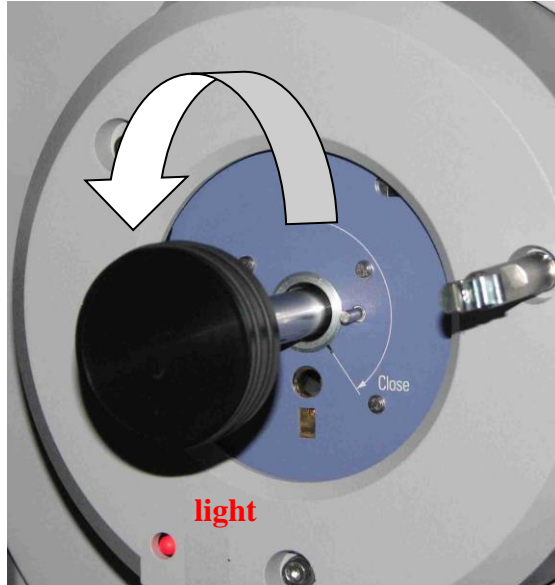
Hold the specimen holder with one hand with the airlock trigger pin parallel to the small slit in the CompuStage front plate (near the line labeled “Close”).

Put your other hand on the airlock. **Remember not to touch the part of the holder which goes into vacuum.**



Carefully insert the end of the specimen holder into the airlock cylinder and slide the holder in **until a stop is reached**. This will start the pump.

Keep holding the rod with one hand. Carefully wiggle the holder a few degrees CW/CCW to ensure the trigger pin is properly inserted.



Go over to the computer console and in the message box choose:



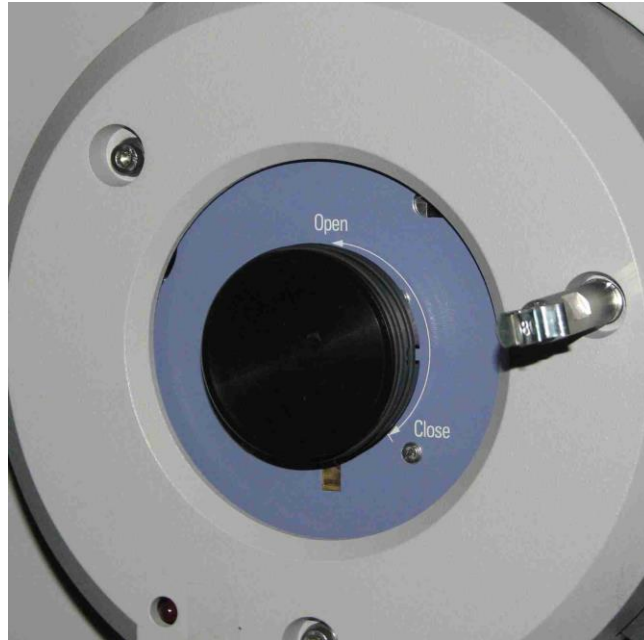
Wait until the red light on the CompuStage goes out. You will hear a click when the vacuum system switches over to the turbo pump. Soon after, the light will go out. When it does, place your left hand on the airlock while holding the holder with your right hand. **Carefully** rotate the holder about 120° **CCW** in the direction of the arrow pointing to “Open”. You will feel how the vacuum starts pulling the holder in. **Do not let the holder go.** Gently let it slide in without pushing. **DO NOT PUSH THE ROD.**

If you think you need to push the holder in or there is an audible hissing noise then you are doing it wrong. **DO NOT PUT THE HOLDER INTO THE TEM. GET HELP.**

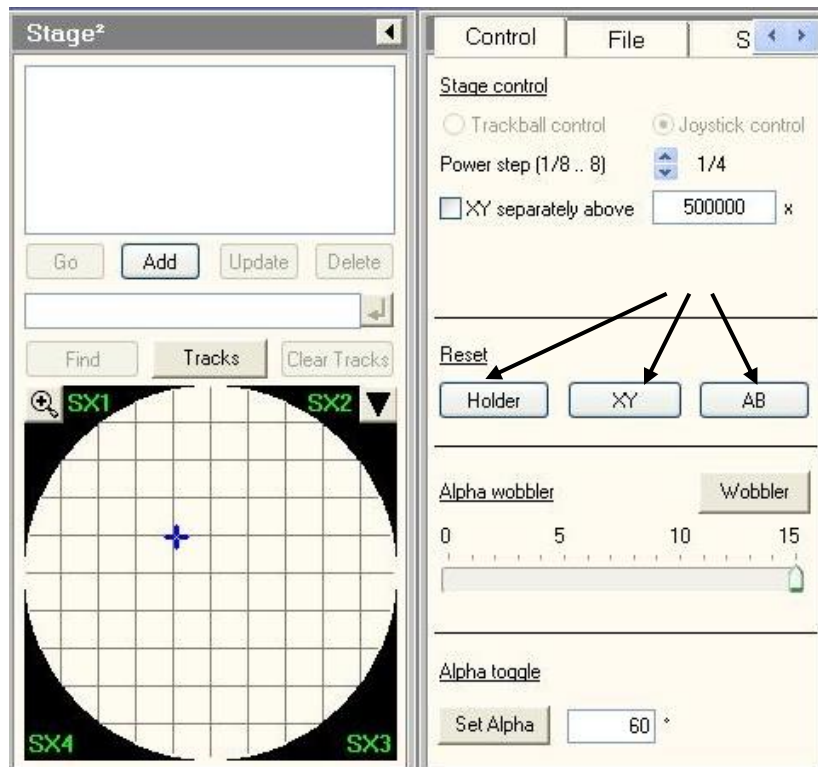
Wait for the light (next to the sample rod) **to go out.** You can think of the light as meaning “not ready”. As the holder moves in, gently wiggle it a little to make sure it is seated well. The pin near the rod handle should go into a small hole on the airlock.

Then tap on the holder cap twice. **Carefully** close the door on the panel cover. The loading procedure has been finished. Go over to the TEM console. Check vacuum levels.

TEM SOP



Go to Stage → Control → Holder → XY → AB.



Observe the numbers on the control panel changing to zero values. That ensures that your holder is fully controllable by the software.

TEM SOP

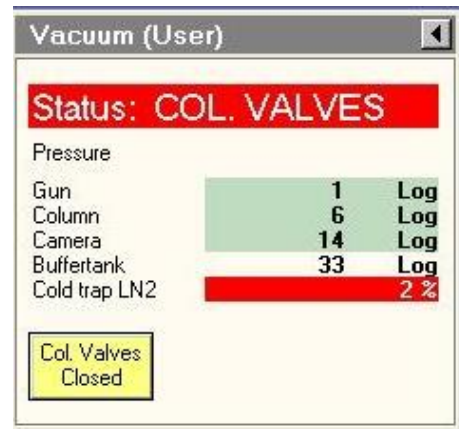
Note: When you begin loading the TEM holder into the airlock, there should be no audible hissing noise coming from the airlock area. If you hear the hissing noise appearing during the first few seconds immediately after you started loading, try inserting the holder a few more mm deeper into the airlock. You should be doing it very gently, without exerting any significant force on the holder. If the hissing noise persists, wait until the completion of the airlock pumping and then extract the holder. Check the message box on the software console for error messages. If you see the red light on the panel, then you cannot proceed with loading. Notify staff.

TURNING THE BEAM ON

Look over at the **Vacuum** panel. The vacuum levels need to be similar to these values:

Ignore the messages about the liquid nitrogen level.

Important: If the Column vacuum is high (~20 logs), it is not safe to turn the beam on. Wait until the level of vacuum in the column improves to at least 15 Logs or less.



Workset → Setup → Vacuum → Column Valves

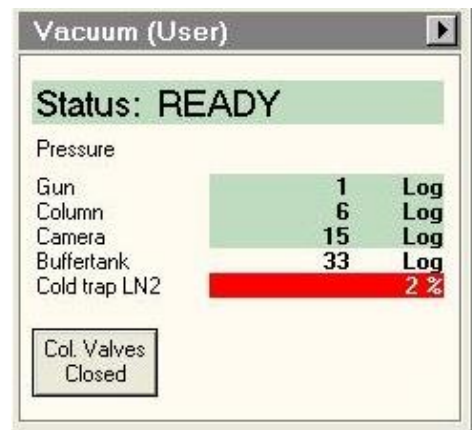
Yellow background on buttons means “this is the current state.”

Click to Open Column Valves.

Status:

Column Valves (in red)

→ Status: Ready (in green).



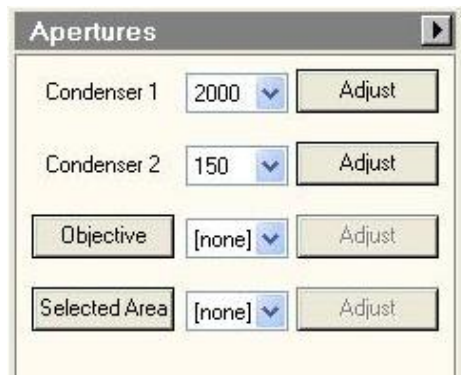
You should be able to see the beam on the FluCam monitor screen. Make sure the screen is inserted. If not, click Insert Screen on the FluCam viewer or press **R1** button. Use the trackball to center the beam on the screen.

Note: after you turn the beam on and after you just loaded the sample into the column there will be drift (even with good column vacuum). You may want to wait about 30 min letting the sample to stabilize before turning the beam on.

TEM SOP

Check that there is no objective or selected area apertures inserted:

Search → Apertures



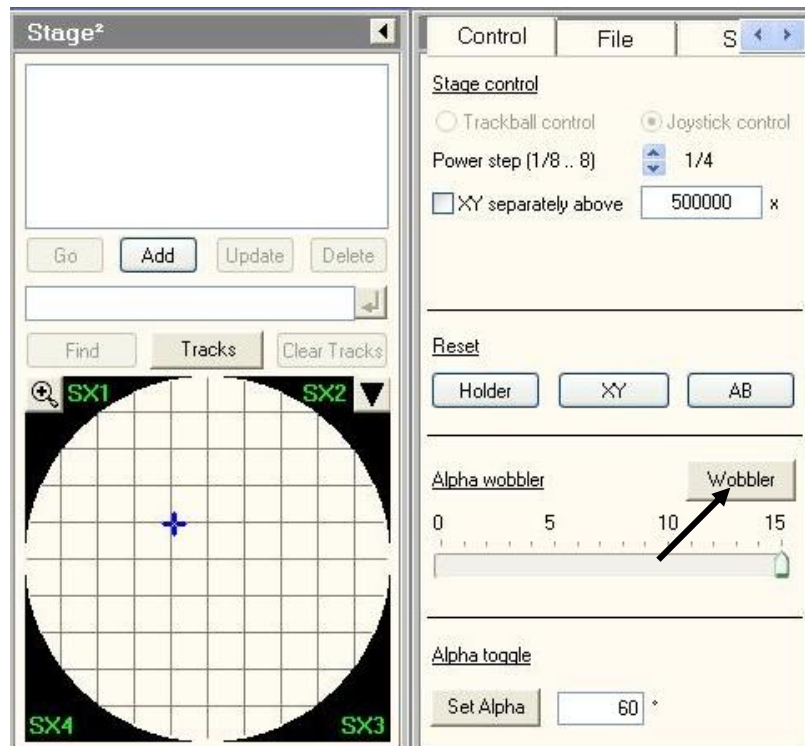
PUTTING HOLDER TO THE EUCENTRIC HEIGHT

Press the “**Eucentric Focus**” button on the right panel. Find the beam using the track ball.

Find your sample. **Try to work at the magnification in the SA range.** If the image appears black, try moving the joystick to move away from the copper grid lines/rim. You may need to lower magnification from SA range to M2300 or lower in the LM range. Inspect the whole sample on the grid. Find a recognizable feature on the specimen using a small SA range magnification at about 6300x-17500x. Pay attention to the value of exposure time and beam current, which should not exceed a few seconds (keep the beam current below 10 nA) to prevent damage to the screen/camera.

Set Eucentric height:

Workset → Search → Stage → Flap-out window → Wobbler



TEM SOP

Set amplitude to 5 or 10. Use the Z-axis button on the right panel to bring the stage up or down. Remember that the Z-axis button is pressure sensitive.

Minimize the image feature displacement on the monitor during wobbling. **Do not use joystick to move the stage along X- or Y-axes during wobbling.**

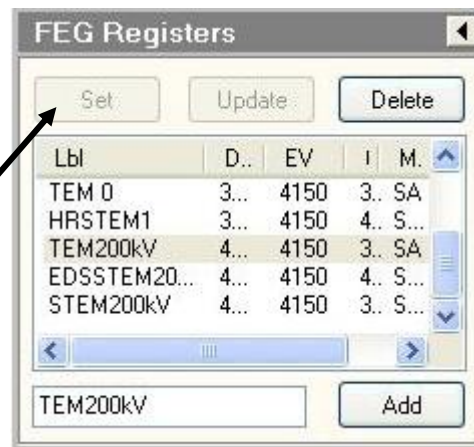
Turn the wobbler off and re-center the feature using the joystick X- and Y-controls.

Turn the wobbler on again, adjust the Z-axis height. Repeat several times until the visible displacement of the selected feature is minimized.

Note on using the FEG Registers:

The system is aligned and ready for imaging without resorting to the FEG Registers. If you need to load the FEG Registers, use the following sequence:

Workset → Setup → FEG Registers → **TEM200kV**.



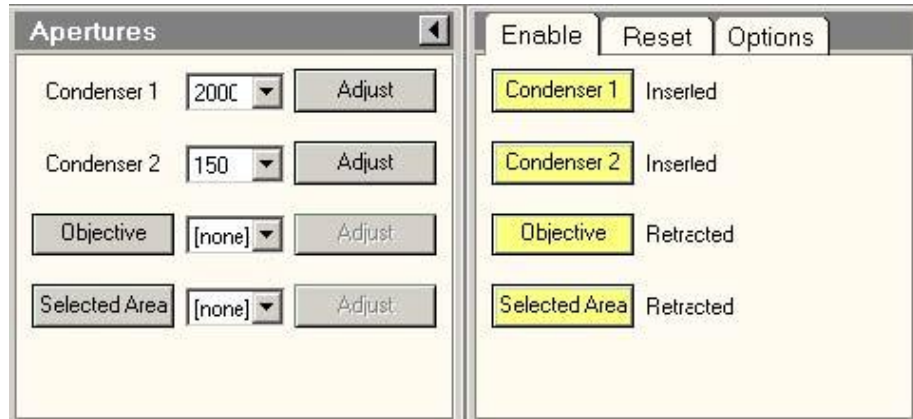
Then click on “Set”. **Do not click on “update”, “delete”, or “add”.** After loading the FEG Registers proceed with finding the eucentric height as described above.

BEAM ALIGNMENTS

While performing alignments always make sure the beam is centered on the screen with a track ball. Avoid rapid increases of magnification using the magnification knob without paying attention to the centering of the beam on the screen. Before going higher in magnification, condense the beam with the **Intensity** knob on the control panel. Once you increase the magnification, the beam will expand automatically highlighting a larger area of the screen. Pull out the objective aperture, if it is currently inserted.

By default, neither Objective nor SAD aperture should be left inserted:

Workset → Tune → Apertures → Objective → none and Selected Area → none

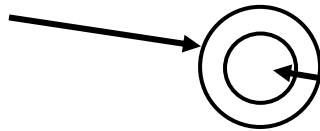


Look at the image at a low magnification (use **M range** or **SA range**). **Make sure that the beam is centered on the screen.** If necessary, re-center the circular illumination on the screen with the track ball. **This should be done frequently to optimize the imaging conditions.** Avoid rapid changes in magnification without having a beam centered on the screen first.

Look at features on the specimen. Increase magnification into the **SA range**. Set the focus to MINIMUM contrast.

On the right panel,

lower knob = focus step,



upper knob = focus

Check condenser centering

Use **Intensity** knob (counter-clockwise) to condense the beam to its minimum size.

Use the trackball to center the spot on the circular target.

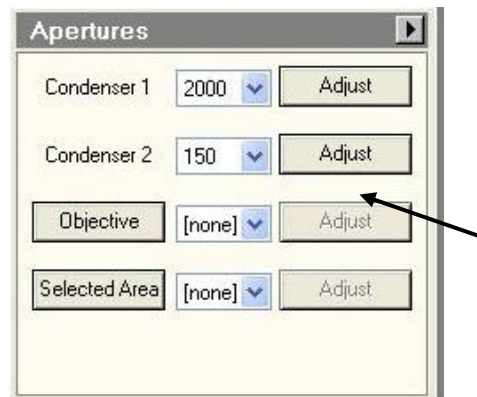
Expand the beam with the **Intensity** knob (clockwise) out to the larger circle.

If the beam does not meet the circle evenly, you should adjust the C2 aperture position.

Adjust the C2 condenser position by using

Workset → Tune → Apertures

Click on Condenser 2 Adjust.

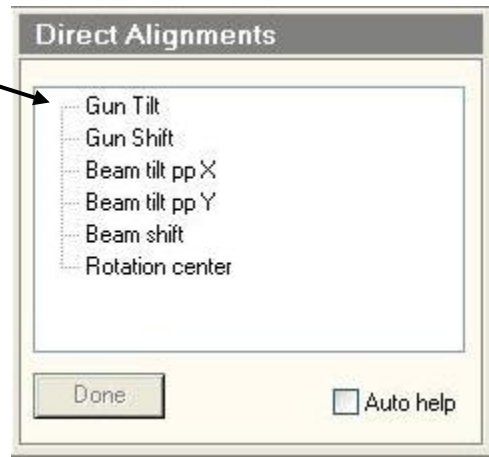


TEM SOP

The multifunction knobs X and Y will now control the condenser aperture position. Move the aperture so that the beam converges/expands **radially** with respect to the center.

If the image appears to be partially cut-off at the edges at low magnification (M range or at about 6300 x in the SA range), check **Gun Tilt** by going to

Tune → Direct Alignments → Gun Tilt



Do not touch Gun Tilt if you do not see the image partially cut-off at the edges.
Do not touch the Condenser 1 or any other settings under “Direct Alignments” tab as they do not need any adjustment.

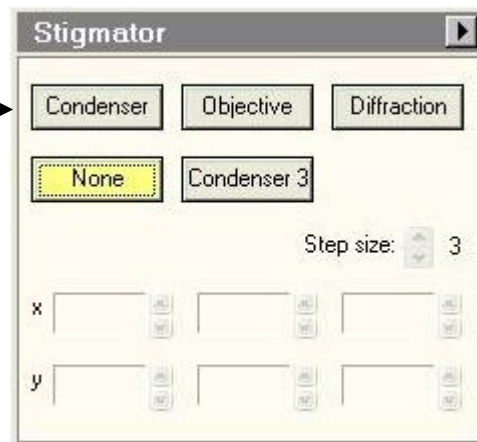
Remember that **Gun Tilt control is extremely sensitive** and may require only a tiny adjustment (that is **use the smallest amplitude** by pressing “-“ on the left control panel above the multifunction knobs). If not used with caution it can lead to the beam misalignment. Observe the image not being cut-off. When finished, click on “Done”.

Check condenser astigmatism

Look for a circular spot at the intensity crossover (use the Intensity knob). If the crossover appears as an ellipse rather than a circle, then you need to adjust the condenser stigmatism.

Choose

Workset → Tune → Stigmator → Condenser



TEM SOP

Then use the multifunction knobs to change the shape of the crossover so that it appears more or less circular. Pay attention to the amplitude you are using (“-“ or “+” above the Multifunction knobs).

When finished, click on “None” or press the “**Stigmator**” button on the left panel to release the multifunction knobs.

Important: Always leave the Intensity knob set so that the beam spreads out when the knob is turned clockwise.

Focus and Objective Stigmation

Make sure the screen is inserted (Insert Screen or press R1 button). Set the magnification into the SA range, about 26-39 kx. Set focus for minimum contrast.

Use the Intensity knob to condense the beam, and use the trackball to center it.

Spread the beam by turning the Intensity knob **clockwise**.

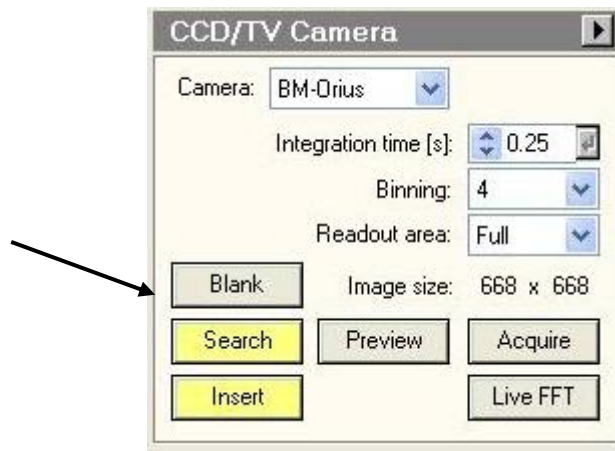
Find some amorphous material to view.

Imaging with TIA

Activate the software camera control with

Workset → Camera → CCD/TV → Search

Raise the screen with **R1**. Observe the image and click on Live FFT



Rock the Focus knob CW/CCW to see the rings in the FFT.

Condense the beam a bit (counter-clockwise), if the rings are unclear. The rings need to be circularly shaped. If they appear elliptical, you need to correct the astigmatism. Press the **Stigmator** button on the left panel, or find it on the **Tune** tab. The

TEM SOP

multifunction knobs now control the objective stigmatism. Look at the FFT. The best focus is when all the rings spread out and disappear. To check stigmatism, you need a few rings.

Use the multifunction knobs controlling the objective stigmatism to make the FFT rings more circular. Adjust the focus closer to ideal (no rings) and adjust the stigmatism again.

When you are done with stigmatism, click on “None”, so you will not accidentally change the stigmators by turning the multifunction knobs.

Taking images with camera

Find a region of interest, with the screen down (that is, using the FluCam viewer).

Lift the screen with **R1** button on the control panel.

Workset → Camera → Search then →Acquire

Look at the **integration time** for image acquisition. If the image is not drifting, then use 0.5-1-1.5 sec integration time. Take several images in succession using different exposures. If the image is drifting slightly, use short acquisition (0.25 seconds or so).

If the image is drifting rapidly, you might have to wait 30 minutes or longer for the sample to reach equilibrium.

Saving images

In TIA, right click on the image. Select “Export Data” and choose the path to your folder on the **TEM Support PC**:

TEM DATA → your folder

For the file extension, choose JPEG with a scale marker.

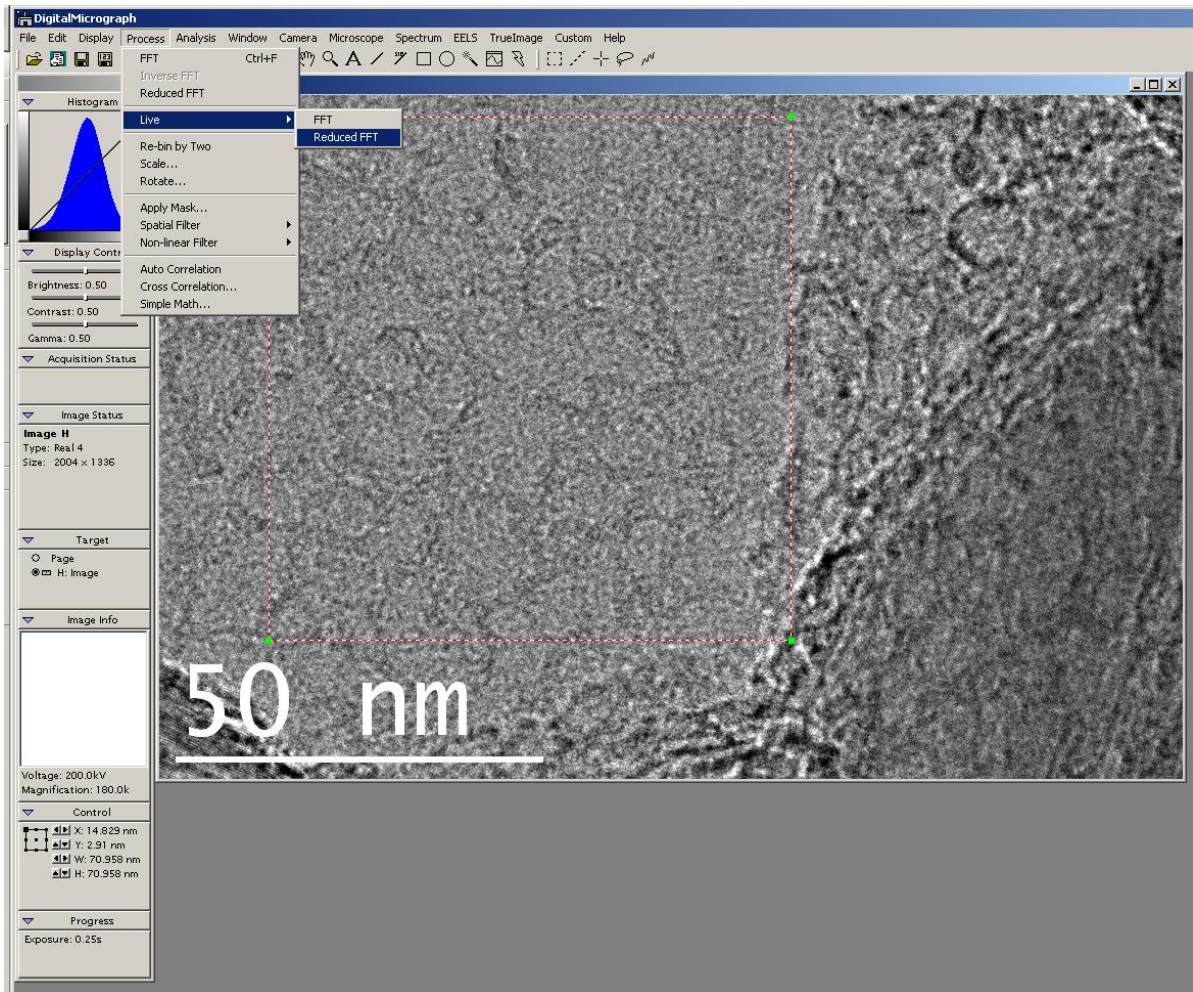
Select “Save as ...” to save image in the TIA format in your folder on the TEM Support PC.

TEM SOP

Alternatively you can use **Digital Micrograph** for taking images.

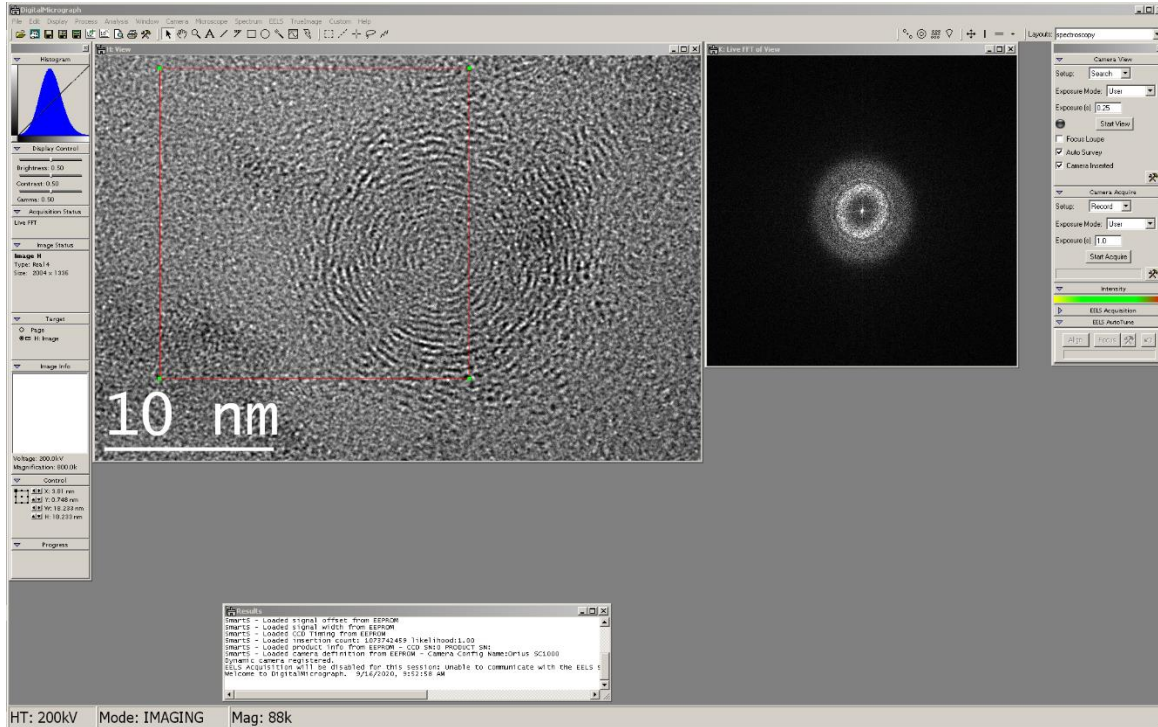
Use **Start View** under **Camera View** for finding ROI and **Start Acquire** under **Camera Acquire** for image capturing. Use 0.25 s exposure for Start View and 1 s or longer exposure for image acquisition.

In order to see FFT in Digital Micrograph, select and drag to create a squared area with an ROI tool on the image *while keeping the Alt button pressed*.



The select **Process** → **Live** → **Reduced FFT**:

TEM SOP



NOTE: You cannot use Camera search or Preview in TIA and Camera Live View in Digital Micrograph running at the same time. If you encounter a sluggish program response during live view, double-check that the Live View is not running in both programs.

Save files as Gatan *.dm3 format and use “Save display as” option to save *.JPEG files on the TEM Support PC.

When you are finished with collecting data on the instrument **CLOSE THE COLUMN VALVES. Never leave the instrument unattended with Column Valves Open.**

At the end of your session copy do not forget to backup your data from the TEM Support PC. **The TEM Support PC is NOT backed up.**

AT THE END OF YOUR SESSION

1. If you are in STEM mode, go back to TEM mode by selecting Microprobe and Spot Size 3.

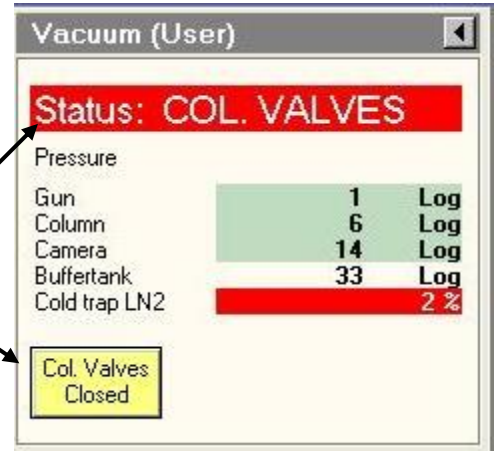
2. Remove objective and SAD apertures, if applicable.

3. **Put down the screen with R1.**

4. Leave the magnification in the SA range.

5. **Close the column valves**

Workset → Setup → Vacuum → Column Valves Closed



6. **Reset the stage**

Search → Stage → Control → Holder → XY → AB.

7. Remove the sample rod (see below).

8. Remove your sample.

9. Inspect the holder and the sample holding clamp for potential contamination using the camera on the specimen preparation table. Then **plasma clean the holder for 5 minutes** using the short adapter.

10. Home the CompuStage.

11. Following the plasma cleaning of the holder inspect it under the camera and then load back into TEM.

12. Leave the clamp lifting tool, clip and washer in the plastic box near the camera on the sample preparation table.



11. Shut down the operating software in the order reverse to the starting one (see the first page).

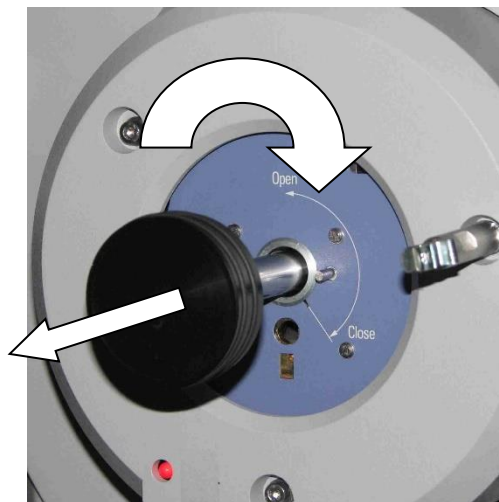
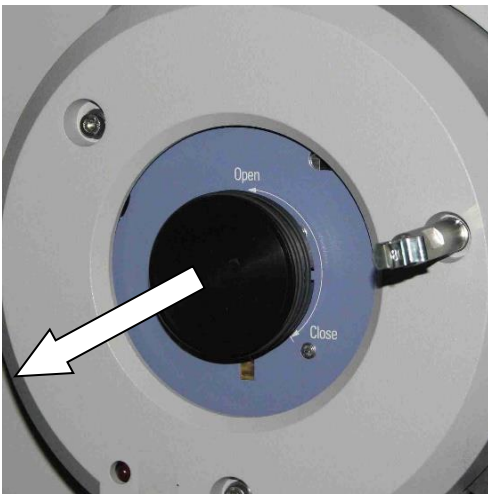
12. Log out of the TEM user account on the Main TEM PC. **Do NOT restart the Main TEM computer!** Turn off PC monitors on Main and Support TEM PCs.

Put down into the TEM logbook your name, group, description of the work performed (samples and experiments), and describe issues encountered, if any.

REMOVING SAMPLE ROD

TO BE PERFORMED WITH CLOSED COLUMN VALVES!

1. Reset the stage Search → Stage → Control → Holder → XY → AB.
2. **Slowly** pull the rod out **with one hand** until it stops, keep the other hand on the airlock to prevent the rod from sliding back in.



3. **Carefully** rotate the rod about 120° CW from **Open** to **Close position** until the rod stops.
4. **Very gently** pull the rod out **with one hand** until it pops loose from the vacuum. Keep the rod **horizontal** to avoid damage to the holder and the airlock.
5. Pull the rod out completely. **Remember not to touch the part of the rod which goes into vacuum.** Wear gloves.
6. Put the holder into the stand on the specimen prep table. Remove your sample.
7. Plasma clean the holder and put it back into TEM. **Again, remember to keep the column valves closed.**