FEI Tecnai F20 S/TEM: imaging in TEM mode
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1. Filling the cold trap (if needed)

    1.1. Prior to use, the cold trap needs to be filled with liquid N_2 and allowed to cool down to allow the column vacuum to reach acceptable levels (about 20 min total time); during normal business hours, this will be taken care of by RSC staff and the instrument should be ready for use by 9:00 am or earlier. However, if use is planned outside of normal business hours, the user will need to do this him or herself.

    1.2. Make sure the viewing screen is covered (exposure to liquid N_2 may cause it to crack).

    1.3. Place the cold trap onto the stand and guide the cold fingers into the trap.

    1.4. Fill the cold trap with liquid N_2; after ~1 min, top off the cold trap with more liquid N_2.

    1.5. Cover the top of the cold trap with the Styrofoam cap.

    1.6. Wait until the column vacuum reaches the necessary level before proceeding with instrument use (as described subsequently). After filling of the cold trap, it may take up to 20 min before the column vacuum reaches the necessary level.

2. Removing the single-tilt holder from the column

    2.1. Log on to the TUMI system.

    2.2. <u>Gloves are to be worn at all times when handling the holders</u> (no exceptions).

    2.3. The single-tilt holder is to be left inserted into the column when the TEM is not actively in use to help keep the load lock area and column clean.

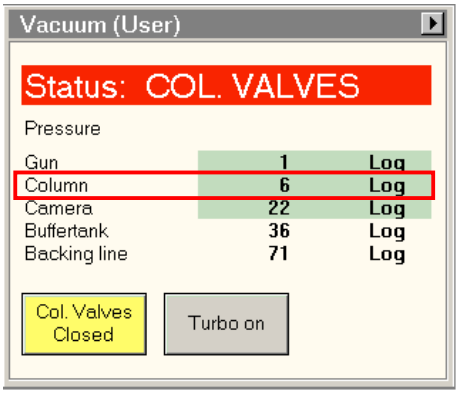
    2.4. Pull straight out until it stops.

    2.5. Rotate the holder clockwise ~150° until it stops.

    2.6. Pull the holder straight out of the load lock (there should be slight resistance).

3. Specimen loading

3.1. In Microscope Control, select the Startup tab and then find the "Vacuum" control panel. Verify that the column pressure is reading 6 (or less); *do not proceed further* unless this is the case.

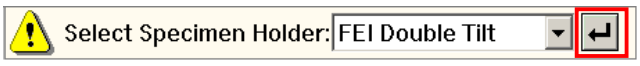


3.2. Load your specimen into the chosen holder; you should (if permissible) plasma clean the specimen and holder before loading the holder into the column; ask for a recommendation for how long to do this based on the type of specimen. *The RSC is not responsible for contamination to your specimen if you choose not to plasma clean it.*

3.3. Line up the pin on the shaft of the holder at ~5 o'clock on the load lock opening and firmly push in the holder; the holder should go in as far as the mark indicated on the shaft if the pin on the shaft was properly aligned.

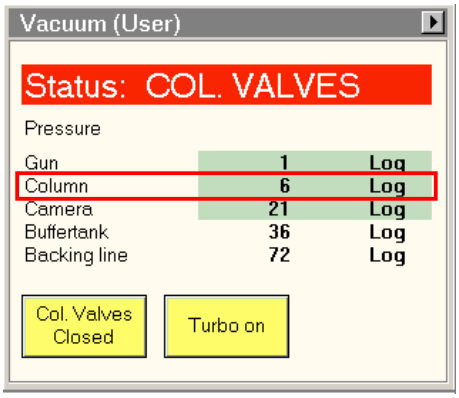
3.4. The red LED on the load lock will turn on; if the turbo pump is running at full speed, you should hear a valve open, indicating the load lock is being pumped down (otherwise, the valve will open once the turbo pump is at full speed).

3.5. In Microscope Control, select the holder being used in the bottom information panel; if using the double tilt holder, plug in the cable connection on the load lock.



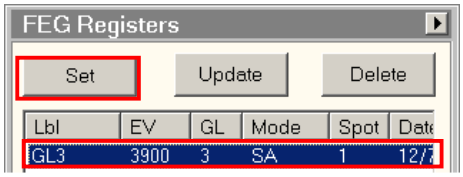
3.6. When evacuation is complete, you should hear the valve close and the red LED will turn off (~60 s); *if it appears that evacuation did not complete as expected, do not proceed further; contact RSC staff immediately for advisement*. Next, rotate the holder counterclockwise ~150° until the pin on the base of the holder is at 6 o'clock; maintain tension on the holder as the vacuum pulls it all the way into the column (to prevent a possible collision).

3.7. Go back to the “Vacuum” control panel and verify that the column vacuum is still in the green; it may be slightly higher than the initial value after insertion of the holder, but this is not of any concern.

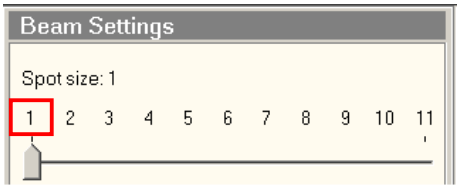


4. Gun alignment and spot size settings

4.1. In Microscope control, select the Beam tab; navigate down to the “FEG Registers” control panel and select the “GL3” setting; then select “Set” to apply the gun alignment.

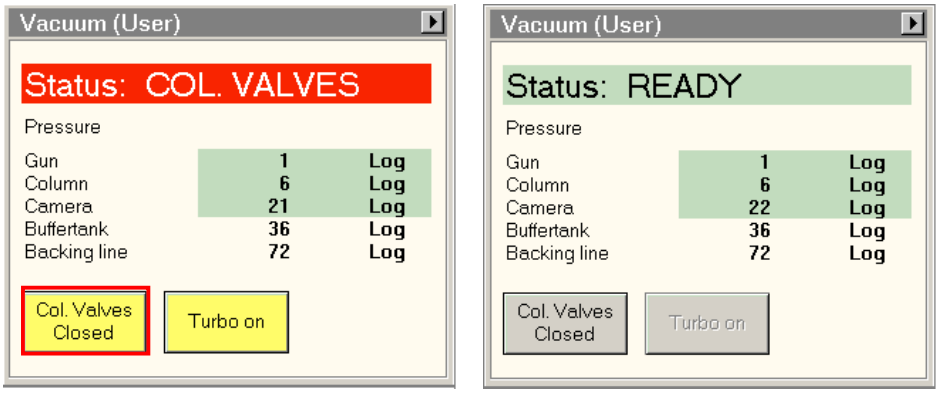


4.2. Use the “Beam Settings” control panel to set the spot size as desired. Spot size = 1 is recommended for most bright-field and high-resolution TEM imaging.



5. Finding an area of interest

5.1. In Microscope Control, open the Startup tab and go to the “Vacuum” control panel; select “Col. Valves Closed” to open the column valves; the button will turn gray, the turbo pump will turn off, and status will read “READY”.



5.2. Adjust the “Magnification” knob (right-hand control pad) until the microscope is in “LM” mode as verified in Microscope control in the bottom information panel; adjust the “Intensity” knob (left-hand control pad) to expand/contract the beam as needed.

5.3. Use the joystick (right-hand control pad) to move the stage until an area of interest is found and centered on the viewing screen, and then adjust the “Magnification” knob until the microscope is in “SA” mode.

6. Setting eucentric height

6.1. Set the magnification to SA 13500× and press the “Eucentric focus” button (right-hand control pad).

6.2. Use the “Z axis” buttons (right-hand control pad) to adjust the sample height until the specimen is in focus.

7. C2 aperture alignment

7.1. The #2 C2 aperture is left inserted by default and is usually sufficient for most TEM imaging. DO NOT use a different C2 aperture for TEM imaging without consulting with RSC staff first.

7.2. Use the “Magnification” knob to set the indicated magnification to SA 86000×.

7.3. Use the “Intensity” knob to expand the beam *clockwise* from crossover so it is approximately the same size as the 5 mm circle on the viewing screen; use the trackball (left-hand control pad) to center the beam on the screen.

7.4. Turn the “Intensity” knob *clockwise* to expand the beam so it is slightly larger than the 40 mm circle on the viewing screen.

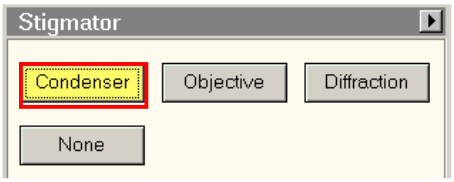
7.5. Use the C2 aperture shifting knobs to center the beam.

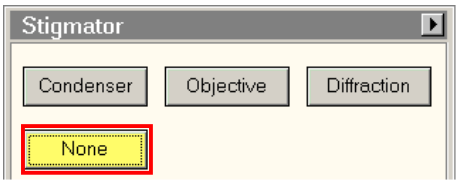
7.6. Repeat the previous three steps until the beam expands evenly (usually 2 or 3 iterations is sufficient).

8. Condenser astigmatism correction

8.1. Use the “Intensity” knob to expand the beam *clockwise* from crossover so it is approximately the same size as the 5 mm circle on the viewing screen; use the trackball to center the beam on the screen.

8.2. In Microscope Control, select the Tune tab and then navigate to the “Stigmator” control panel; select “Condenser” (the button will turn yellow) and then use the “Multifunction” knobs (both controls pads) to make the beam round; select “None” when finished.





9. Condenser deflector balancing

9.1. Use the “Magnification” knob to set the magnification as will be used during imaging. NOTE: to observe lattice fringes, it is usually necessary to use indicated magnifications ≥100 k× (200 – 500 k× is usually sufficient).

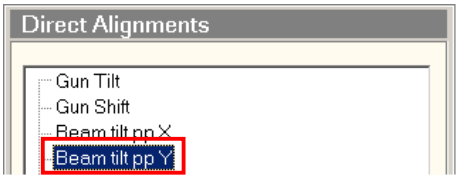
9.2. Use the “Intensity” knob to expand the beam *clockwise* from crossover so it is slightly larger than the 40 mm circle on the viewing screen; then center the beam on the screen using the trackball.

9.3. Use the “Focus” knob (right-hand control pad) to focus the image as best as possible.

9.4. In Microscope Control, select the Tune tab and navigate down to the “Direct Alignments” control panel.

9.5. Select “Beam tilt pp X” and then use the “Multifunction” knobs to eliminate separation of the beam; select Done when finished.

9.6. Select “Beam tilt pp Y” and then use the “Multifunction” knobs to eliminate separation of the beam; select Done when finished.

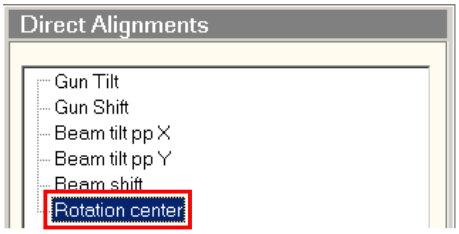


10. Rotation centering

10.1. Re-center the beam on the screen using the trackball.

10.2. Use the “Focus” knob to refocus the image (if needed).

10.3. In the “Direct Alignments” control panel, select “Rotation center” (the image will start oscillating); use the “Multifunction” knobs to minimize movement of the image; select Done when finished.



11. Specimen orientation adjustment (when working with large single crystals)

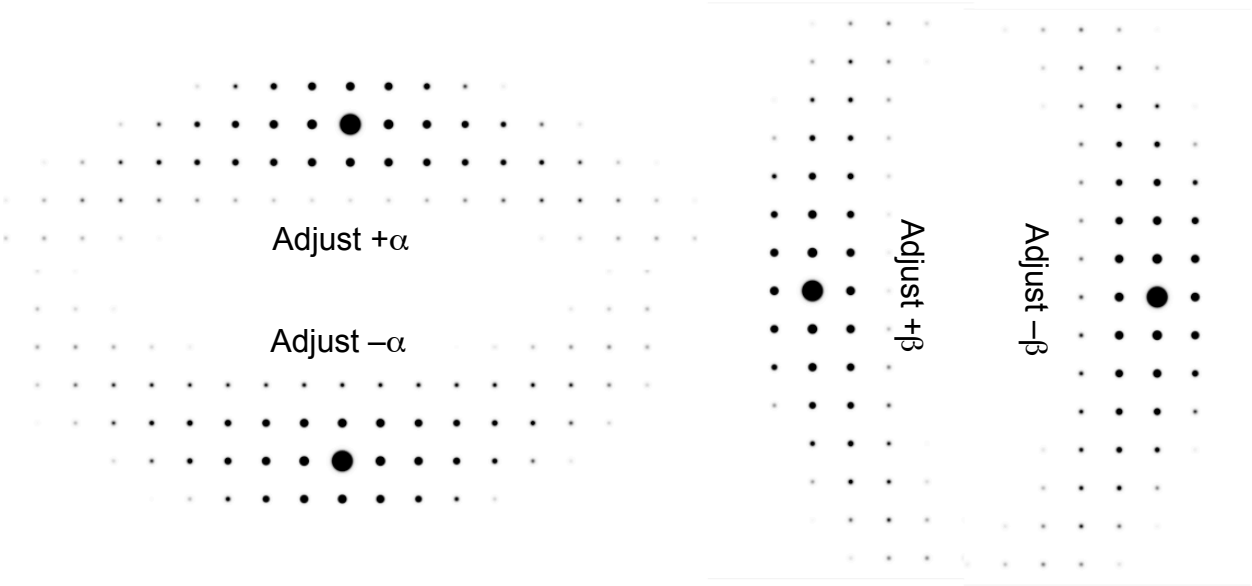
11.1. From crossover, turn the “Intensity” knob *clockwise* to expand the beam so it is slightly larger than the 40 mm circle on the viewing screen (this is the optimal beam size for collecting images with the digital camera).

11.2. Select “Diffraction” (right-hand control pad) to enter diffraction mode.

11.3. Set the camera length by using the “Magnification” knob; a camera length of 300 – 400 mm is recommended for observing the diffraction pattern.

11.4. If needed, the diffraction pattern can be centered on the screen by using the “Multifunction” knobs.

11.5. Use the α and β tilt buttons (left-hand control pad) to orient the sample as desired. If the DP suddenly disappears during tilting, the specimen is probably no longer under the beam; press the “Diffraction” button to return to TEM mode and center the specimen and then press the “Diffraction” button to return to diffraction mode and resume adjusting the specimen orientation.



Above: tilt adjustments to align to zone axis when indicated camera length >200 mm

11.6. Afterwards, press the “Diffraction” button to return to TEM mode; re-establish eucentric height as described previously (this should be done *every* time after the specimen is tilted).

12. Objective aperture centering

12.1. Make sure the beam is centered on the viewing screen and expanded *clockwise* from crossover slightly larger than the 40 mm circle on the viewing screen.

12.2. Select the “Diffraction” button to enter diffraction mode; the diffraction pattern should be immediately evident on the viewing screen; use the “Magnification” knob to set the camera length as needed.

12.3. Move the focusing screen into the beam path and then look through the binoculars to observe the diffraction pattern; if the diffraction pattern isn't centered on the focusing screen, use the "Multifunction" knobs to center it.

12.4. Insert the desired objective aperture (at position 7 the aperture is retracted); aperture 4 should be used for bright-field imaging while aperture 2 is best for high-resolution imaging.

12.5. Center the objective aperture on the direct beam using the aperture shifting knobs.

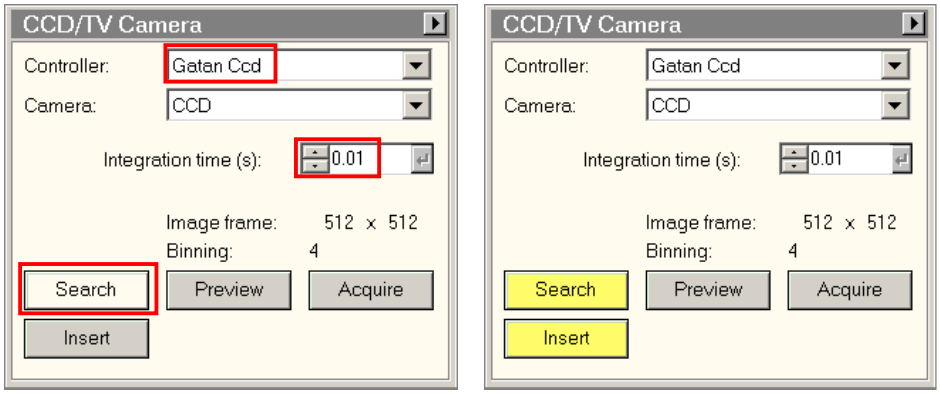
12.6. Select the "Diffraction" button to return to TEM mode.

12.7. Re-center the beam using the trackball.

13. Image acquisition and objective astigmatism correction

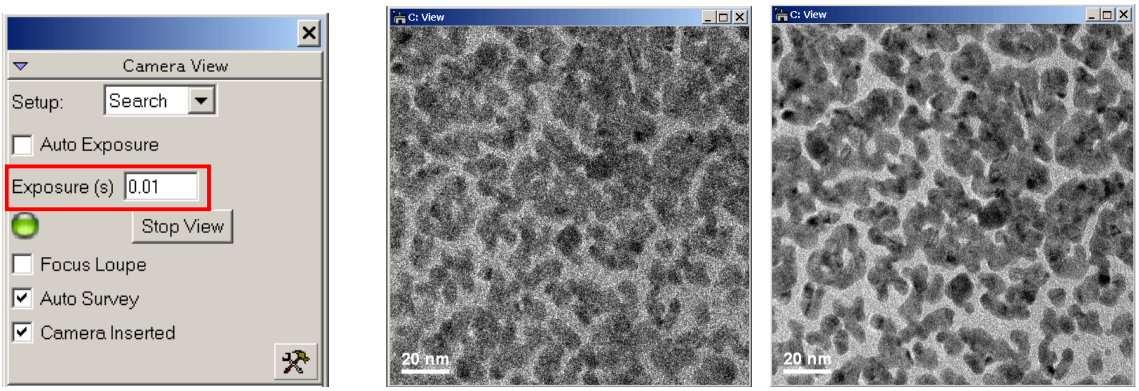
13.1. Cover the viewing screen.

13.2. In Microscope Control, select the Camera tab and navigate to the "CCD/TV Camera" control panel; under "Controller", make sure "Gatan CCD" is selected from the pull-down menu and set "Integration time (s)" = 0.01; then select "Search" (this button and the "Insert" button will turn yellow).



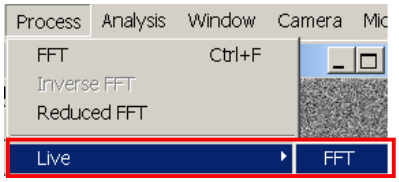
13.3. Listen for the camera to insert; once the camera is inserted, flip the viewing screen up by selecting the R1 button (right-hand control pad); a live image will appear in DigitalMicrograph once the camera is inserted.

13.4. In DigitalMicrograph, navigate to the “Camera View” tool; increase “Exposure (s)” as needed to improve signal to noise in the live image; keep in mind that increasing “Exposure (s)” will lead to a slower response of the live image if the sample is moved; it is usually not necessary to use values of “Exposure (s)” greater than 0.1 for observing a live image.



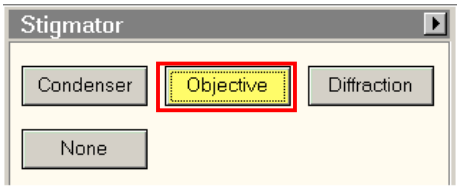
13.5. If some portion of the specimen is amorphous, this can be used to correct objective lens astigmatism. NOTE: the indicated magnification must be ≥100 k× to do this effectively; otherwise, DO NOT attempt to correct objective lens astigmatism.

13.6. In DigitalMicrograph, select “Process” from the pull down menu, then “Live”, and then “FFT”.

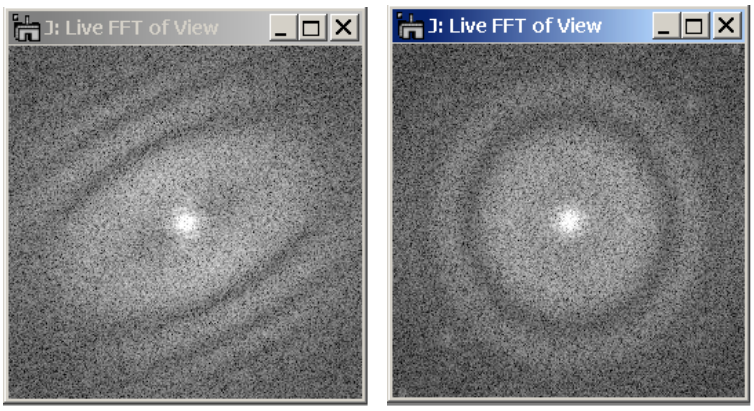


13.7. Slightly adjust the “Focus” knob to underfocus until rings are evident in the FFT.

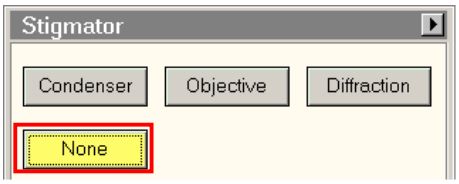
13.8. In Microscope Control, select the Camera tab; navigate down to the “Stigmator” control panel and select “Objective” (the button will turn yellow).



13.9. Use the “Multifunction” knobs to adjust the live FFT so the rings are round.



13.10. In the “Stigmator” control panel, select “None” when finished.



13.11. Use the “Focus” knob to finely focus the live image in DigitalMicrograph.

13.12. In DigitalMicrograph, navigate to the “Camera Acquire” tool and input an appropriate value for “Exposure (s)” to acquire a high-quality image; increasing “Exposure (s)” will improve the signal to noise of the image, but will make the image more susceptible to blurring due to drift; for a sufficiently thin specimen, it is usually not necessary to use a value for “Exposure (s)” greater than 1.0 – 2.0 s.

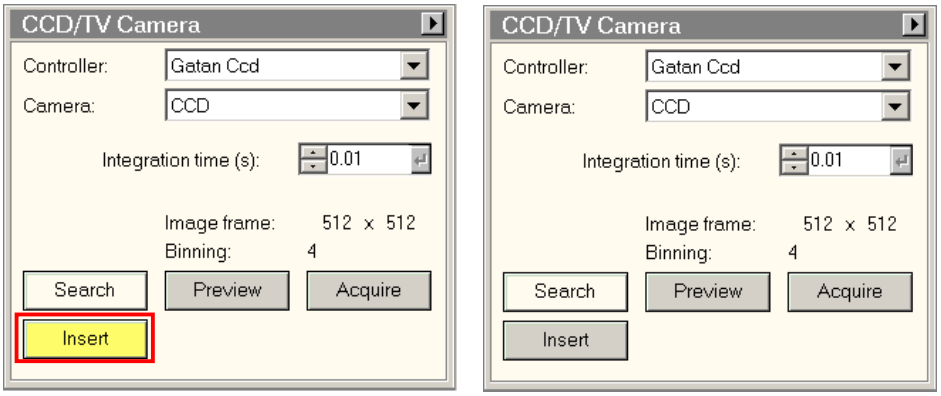
13.13. Select “Start Acquire” to acquire a high-quality image.

13.14. If image collection at a different magnification is desired, flip the viewing screen down, adjust the “Magnification” knob, expand the beam *clockwise* from crossover using the “Intensity” knob so it is slightly larger than the 40 mm circle on the viewing screen, and re-center the beam with the trackball.

13.15. **If you are performing high-resolution imaging and you wish to increase the indicated magnification, it is usually a good idea to recheck the alignment**: retract the objective aperture, focus the image, balance the condenser deflectors, perform rotation centering, reinsert and center the objective aperture (in diffraction mode), and corrective objective astigmatism (using the live FFT).

13.16. When finished using the digital camera, flip the viewing screen down by selecting the R1 button (right-hand control pad).

13.17. In Microscope Control, select the Camera tab; in the "CCD/TV Camera" control panel, select "Insert" to retract the camera (the button will turn gray).



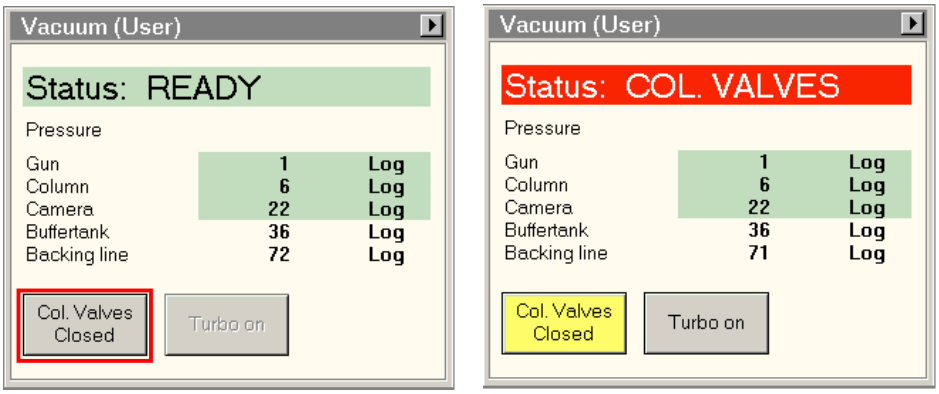
14. Acquiring diffraction patterns

14.1. See separate application note on using the digital camera to record diffraction patterns; DO NOT attempt to record a diffraction pattern without being shown the proper method for doing this; otherwise, severe damage to the camera scintillator could result.

15. Specimen unloading

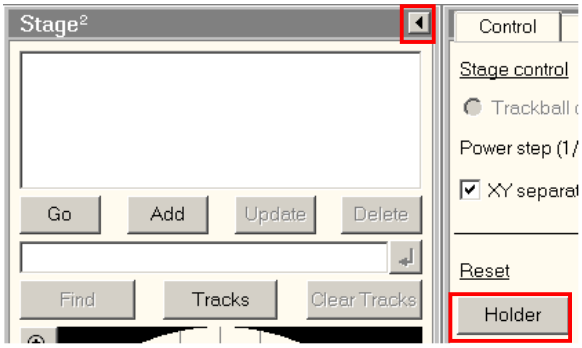
15.1. Leave the instrument in SA mode as verified in the bottom information panel.

15.2. In Microscope Control, select the Startup tab and go to the “Vacuum” control panel; select “Col. Valves Closed” to the column valves; the button will turn yellow, and status will now read “COL. VALVES”.

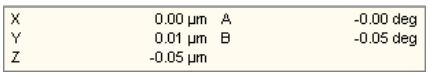


15.3. Retract the objective (set to position 7) and SA (bar flipped to the right) apertures (if still inserted).

15.4. In Microscope Control, select the Stage tab; in the “Stage^2” control panel, select the flap-out arrow to expand the panel; under “Reset” in the expanded panel, select “Holder” to reset the holder; DO NOT initiate resetting the holder unless the objective aperture is retracted.



15.5. In Microscope Control, observe the bottom information panel and verify the X, Y, and Z positions and α and β tilt values are all ~0 before proceeding; DO NOT attempt to remove the holder with the stage in any other state as this may cause damage to the holder and/or the stage.



15.6. If using the double-tilt holder, disconnect the β tilt control.

15.7. Remove the holder from the column as described previously.

15.8. Remove the sample from the specimen holder.

15.9. In Microscope Control, select the Startup tab and navigate to the "Vacuum" control panel; if the turbo pump isn't running, then turn it on as described previously (button will be yellow).

15.10. If another specimen is to be imaged, load it into the holder and load the holder back into the column.

15.11. If no further imaging is to be performed, load the single tilt holder back into the column (if the double-tilt holder was used, insert it back into the plasma cleaner and place it back under vacuum).

16. Cryo cycling (if needed)

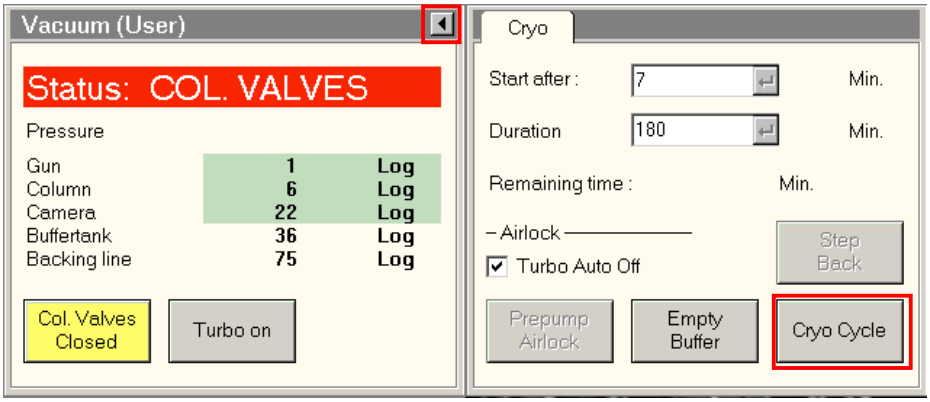
16.1. Cryo cycling the TEM will only be necessary if the instrument is being used after hours; during regular business hours, RSC staff will take care of doing this at the end of the day (around 5:00 PM).

16.2. Insert the single tilt holder into the column; this must be done ***before*** initiating the cryo cycle.

16.3. Cover the viewing screen.

16.4. Remove the cold trap and place a cloth on the support underneath the cold fingers.

16.5. In Microscope Control, select the “Startup” tab; in the “Vacuum” control panel, select the flap-out arrow to expand the panel, and then select the “Cryo” panel; then select “Cryo Cycle” to initiate the cryo cycle. Note that once the cryo cycle has been started, it cannot be undone and the microscope will be unusable for ~6 h.



17. Logging off

17.1. You may log off the TUMI system *after* you unload your specimen from the selected holder, insert the single tilt holder back into the column (and, if used, the double tilt holder back into the plasma cleaner), and collect all your needed data from the microscope computer.