FEI Tecnai F20 S/TEM: imaging in TEM mode

Nicholas G. Rudawski
ngr@ufl.edu
(805) 252-4916
(352) 392-3077

Last updated: 11/05/23

1. Filling the cold trap

    1.1. Prior to use, the cold trap needs to be filled with liquid N_2; due to how infrequently this instrument is used, this will be the responsibility of the user regardless of whether using the system is used during or outside of normal business hours.

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

    1.3. Guide the cold fingers into the trap while placing the dewar on the stand.

    1.4. Fill the cold trap with liquid N_2; after ~5 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. After filling the cold trap, it may be >15 min before the column vacuum reaches the highest level, but the system may be used immediately if the vacuum level is acceptable (described subsequently).

2. Removing the single-tilt holder from the column

    2.1. Log on to the TUMI system.

    2.2. Gloves must always be worn when handling the holders; this is to reduce the introduction of hydrocarbon contamination into the column.

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

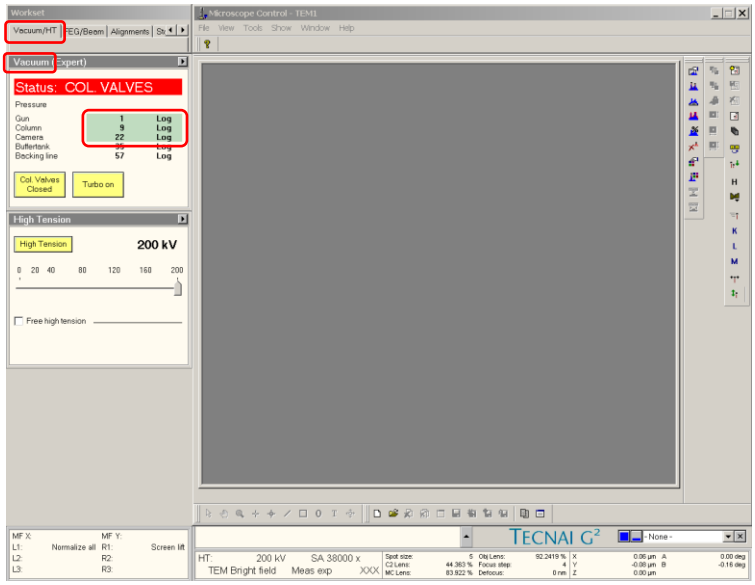
    2.4. Pull the holder straight out until it stops.

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

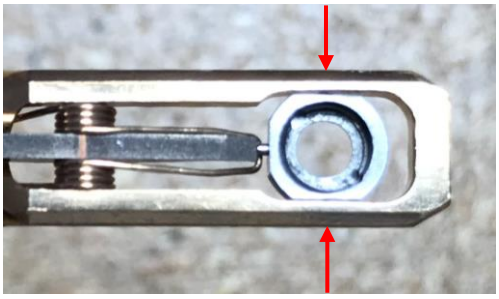
    2.6. Pull the holder straight out of the airlock (there will be some resistance).

3. Grid loading

3.1. In Microscope Control, select the Vacuum/HT tab and then the “Vacuum” control panel; verify the column vacuum is acceptable (green) before proceeding.



3.2. Load a grid into the desired holder; if using the double-tilt holder, use extreme caution when screwing/unscrewing the Be hex ring into/out of the basket; do not overtighten and/or apply excessive downward force as this may damage the sapphire screws (indicated by arrows) securing the basket in place (costing ~$3000 to fix).

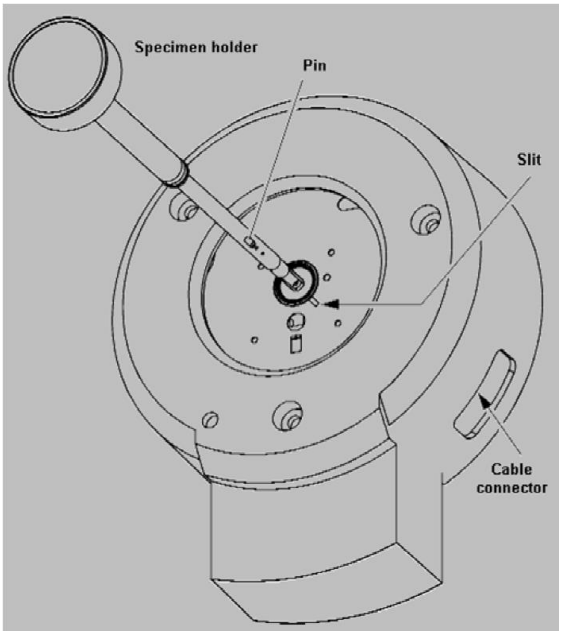


3.3. Please also be aware that the Be hex ring and anti-twist washer for the double-tilt holder are small, delicate parts and may easily be lost or damaged if mishandled and/or not properly secured in the double tilt-holder (costing ~$1000 each to replace).

3.4. One should (if permissible) plasma clean the holder and grid before inserting into the column; cleaning for 2 – 4 min is usually effective for most Omniprobe grids with non-carbonaceous lamella specimens.

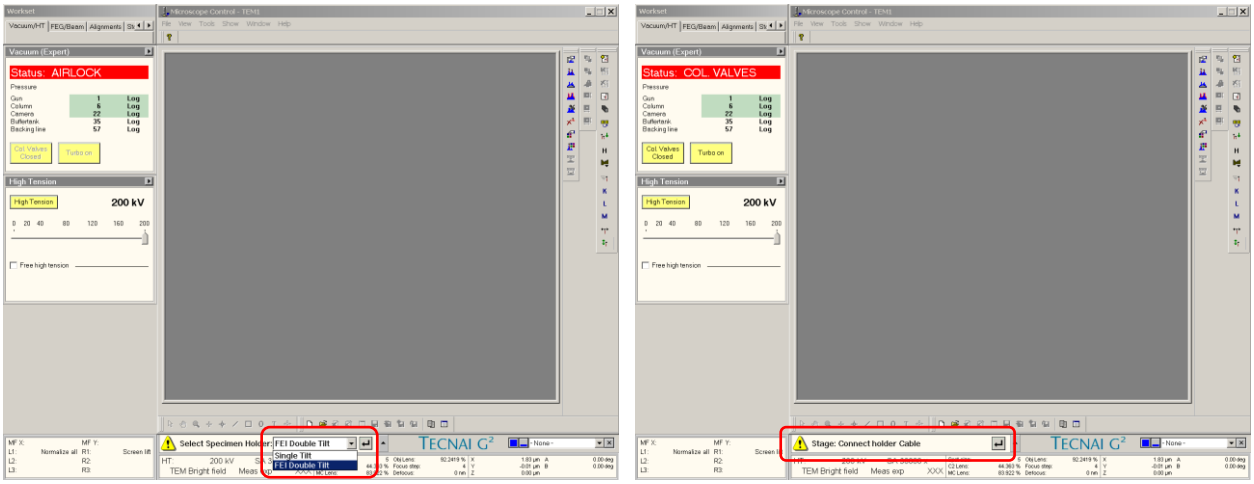
3.5. Please note that while plasma cleaning is usually very effective at mitigating hydrocarbon contamination, its effectiveness is not perfect, and some may still occur during analysis (particularly if no cleaning was performed prior to insertion). <u>The RSC is not responsible for hydrocarbon contamination to specimens occurring during S/TEM analysis.</u>

3.6. Line up the triggering pin on the shaft of the holder with the line at ~5 o'clock on the airlock opening; push the holder into the airlock until it firmly stops; if the pin was properly aligned, the holder should insert up to the tape mark indicated on the shaft.



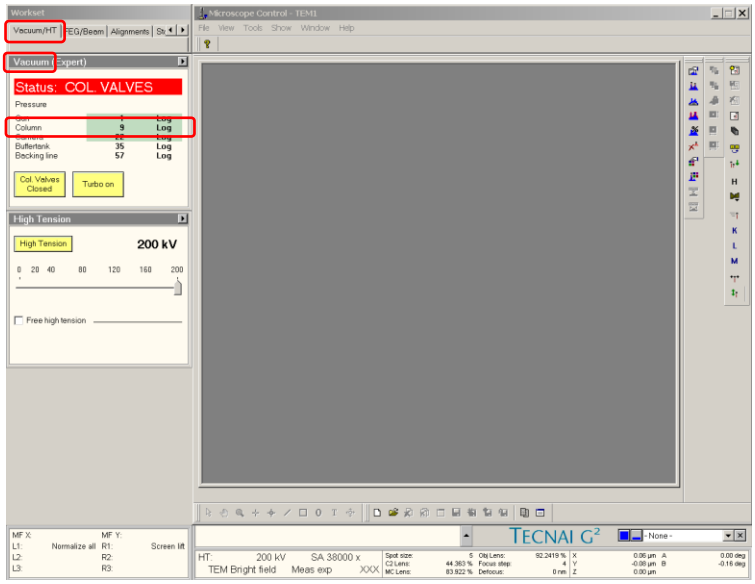
3.7. The red LED on the airlock will turn on; if the turbo pump is running at full speed, the airlock valve will open and airlock evacuation will begin (otherwise, the valve will open once the turbo pump is at full speed; this will take ~2 min).

3.8. 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 outside of the airlock and confirm this in Microscope Control.



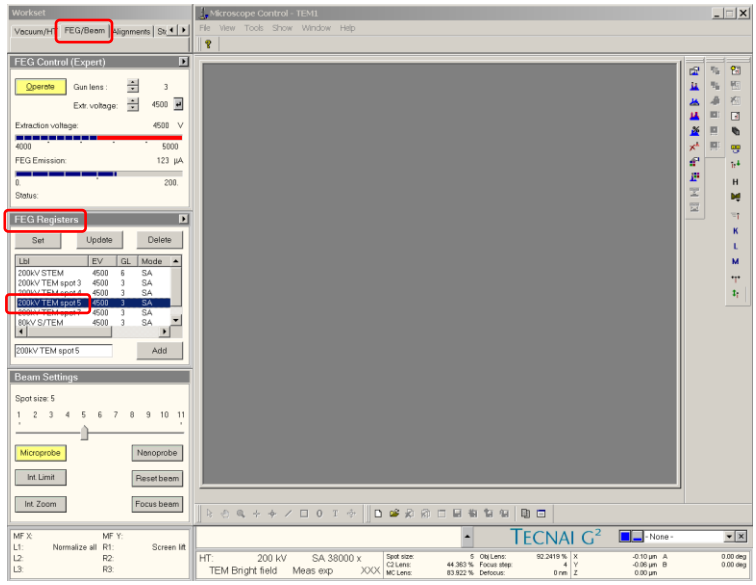
3.9. When evacuation is complete (~2 min), listen for the airlock valve to close and the red LED will turn off; if it appears that evacuation did not complete as expected, do not proceed further; contact RSC staff for advisement to prevent a possible vacuum crash. Next, slowly rotate the holder counterclockwise ~150° until a firm stop is felt; then maintain tension on the holder as the vacuum slowly pulls it all the way into the column (to prevent a collision and maintain the column vacuum).

3.10. Check the “Vacuum” control panel and verify that the “Column” vacuum is still acceptable (green); it may be higher than the initial value after insertion of the holder, but this is otherwise not of any concern.



4. FEG registers

4.1. In Microscope control, select the “FEG/beam” tab and then the “FEG Registers” control panel; select the “200kV TEM spot 5” setting and then “Set” to apply the gun alignment and set the spot size.



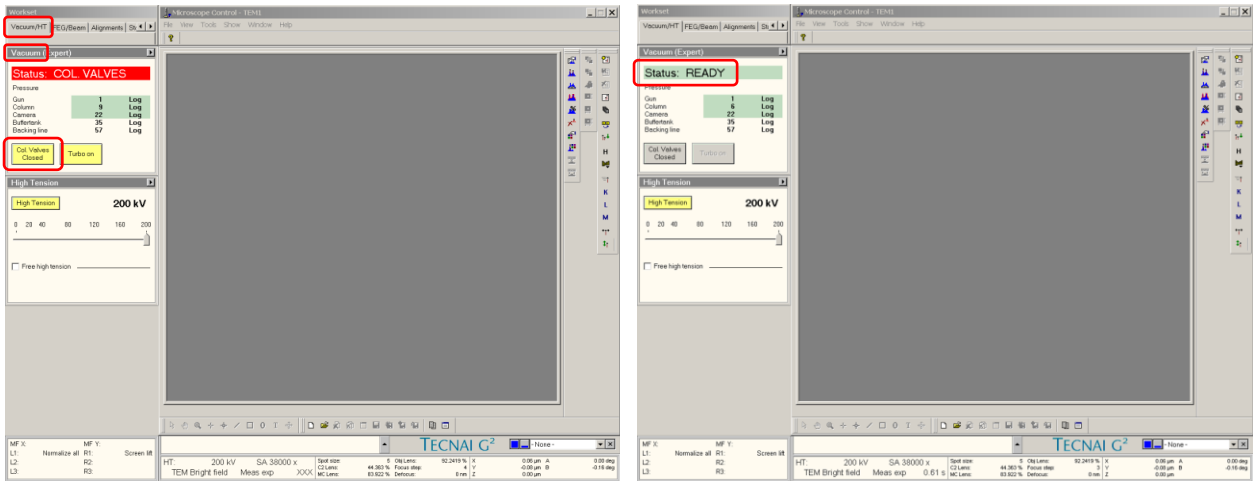
4.2. Spot size = 5 is the recommended starting point for obtaining sufficient beam current while keeping beam-induced damage to acceptable levels for inorganic, non-biological samples. However, this is not a guarantee that beam-induced damage will be avoided/minimized. It is the responsibility of the operator to recognize if the imaging conditions are damaging the specimen to the point adversely affecting the results and to adjust the imaging conditions accordingly (if possible). The RSC is not responsible for any beam-induced damage to specimens.

4.3. If the sample is thick, spot size = 3 or 4 may be used to increase the beam current (noting the increased risk of beam-induced damage); apply the “200 kV TEM spot 3” or “200 kV TEM spot 4” FEG registers accordingly.

4.4. Spot size = 3 is the lowest value that may be safely used for general TEM imaging; using spot size ≤ 2 results in a very high beam current that may damage the CCD camera.

5. Finding an area of interest

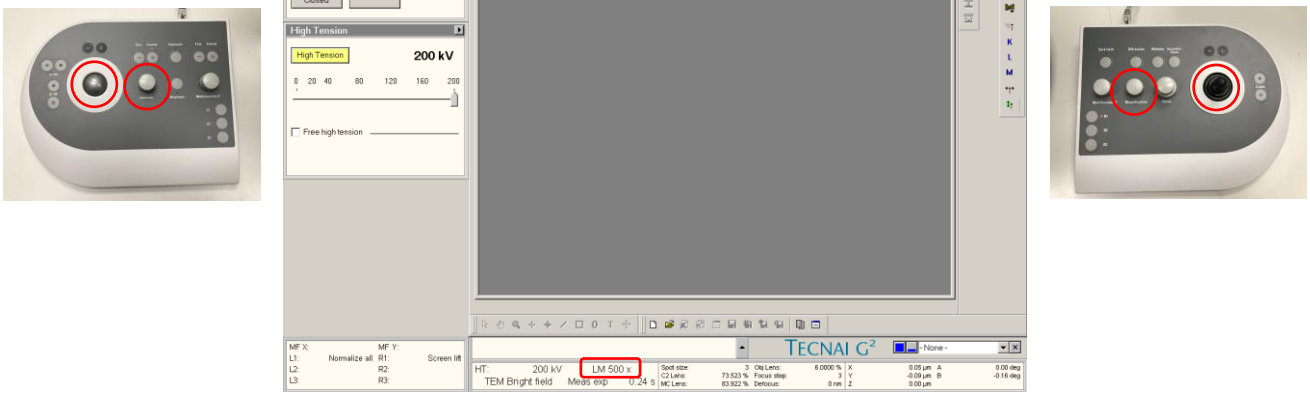
5.1. In Microscope Control, select the “Vacuum/HT” tab and then the “Vacuum” control panel; select “Col. Valves Closed” to open the column valves; the button will turn gray, the turbo pump will turn off, and status will read “READY”.



5.2. Adjust the “Magnification” knob (R control pad) until the microscope is in “LM” mode as verified in Microscope control in the bottom information panel.

5.3. Adjust the “Intensity” knob (L control pad) to expand/contract the beam and the trackball (L control pad) to shift the beam as needed.

5.4. Use the joystick (R 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 back in “SA” mode.



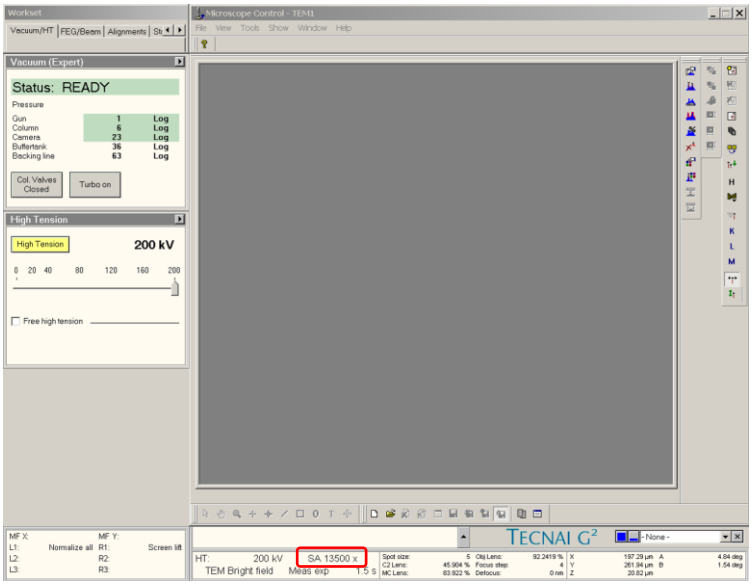
6. Setting eucentric height (coarse)

6.1. After switching from LM back to SA mode, the region of interest will likely have slightly shifted from the center of the viewing screen; recenter it using the joystick.

6.2. Set the indicated magnification to SA 13500× and press the “Eucentric focus” button (R control pad).

6.2.1. NOTE: “SA” mode is the standard TEM imaging mode and can effectively be used to image everything from micro- to atomic-scale features.

6.3. Use the “Z axis” buttons (R control pad) to adjust the sample height until the specimen is in focus (minimum contrast).



7. C2 aperture alignment

7.1. Check the C2 aperture assembly and verify that the #2 C2 aperture is inserted (it should be). 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 38000×.

7.3. 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 use the trackball to center the beam.

7.4. Turn the “Intensity” knob clockwise to expand the beam so it is nearly the same diameter as the viewing screen.

7.5. Use the positioning dials on the C2 aperture assembly to center the beam.

7.6. Turn the “Intensity” knob counterclockwise to return the beam back to slightly wider than the 40 mm circle and use the trackball to recenter the beam.

7.7. Repeat the last three steps until the beam remains centered on the viewing screen after expanding (usually 2 or 3 iterations is sufficient).

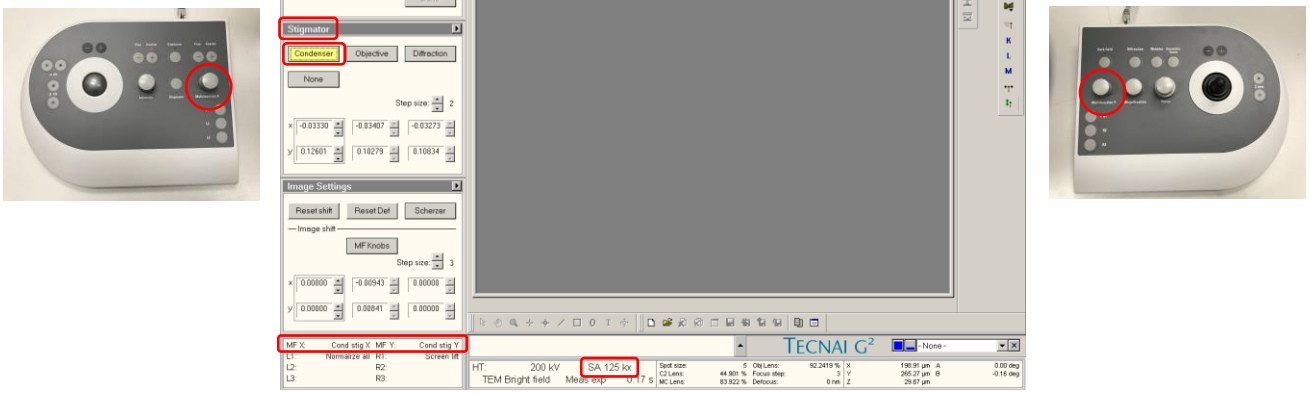
8. Condenser astigmatism correction

8.1. Use the joystick to temporarily move the specimen out of the way so the middle of the field of view is only vacuum (if possible).

8.2. Use the “Magnification” knob to set the indicated magnification = 125 k×.

8.3. Use the “Intensity” knob to expand the beam clockwise from crossover so it is slightly larger than the 5 mm circle on the viewing screen; use the trackball to center the beam.

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



9. Beam tilt pivot points; fine-tuning eucentric height

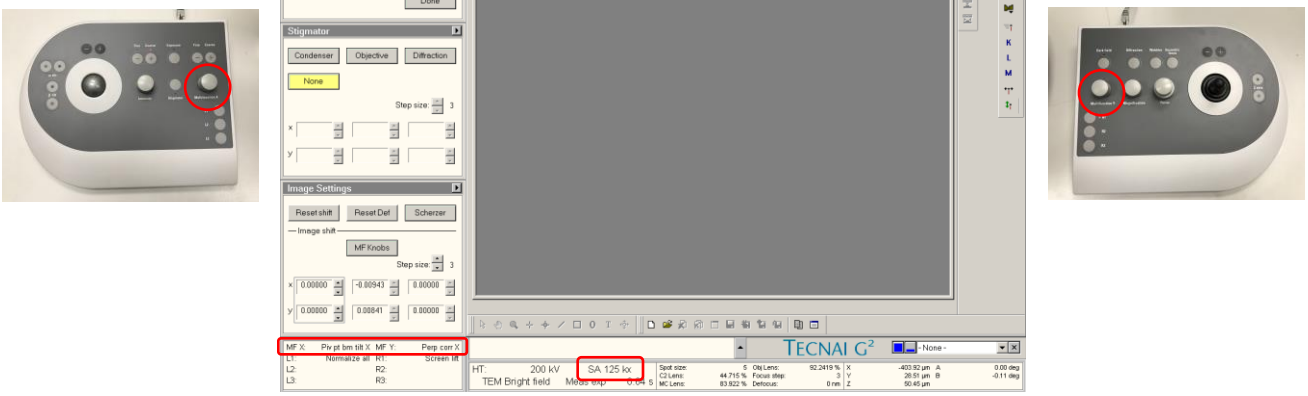
9.1. Use the joystick to center the area of interest on the viewing screen.

9.2. Leave the indicated magnification = 125 k×.

9.3. 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 use the trackball to center the beam.

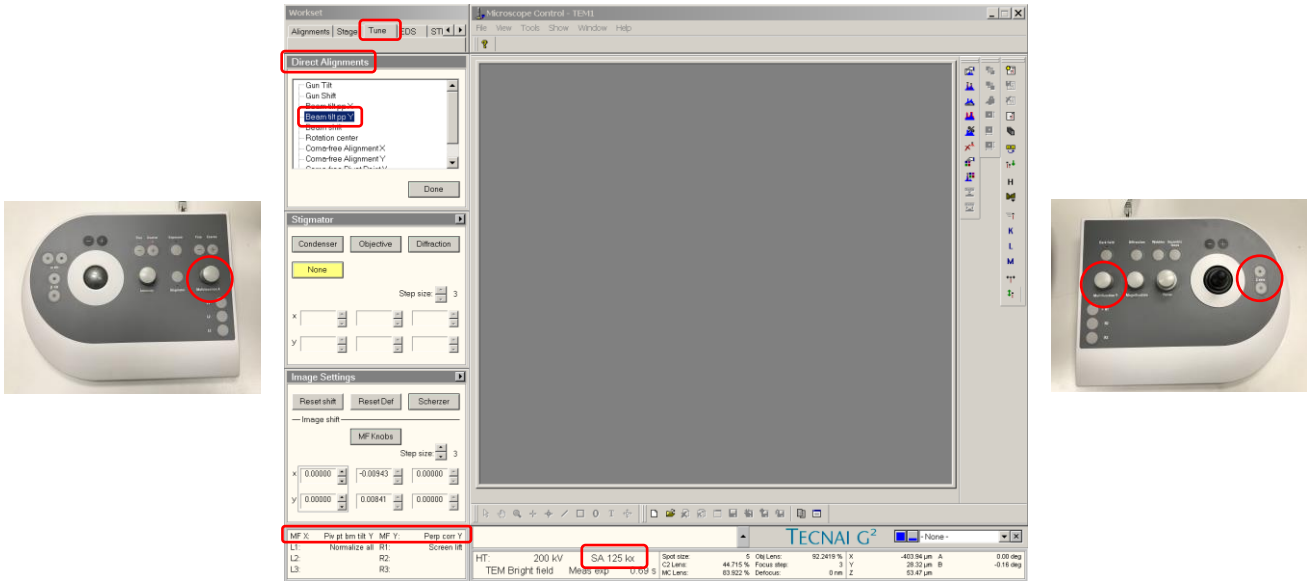
9.4. In Microscope Control, select the “Tune” tab and then the “Direct Alignments” control panel.

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. Next, select “Beam tilt pp Y” and then use the “Multifunction” knobs to eliminate separation of the beam; DO NOT press “Done” yet.

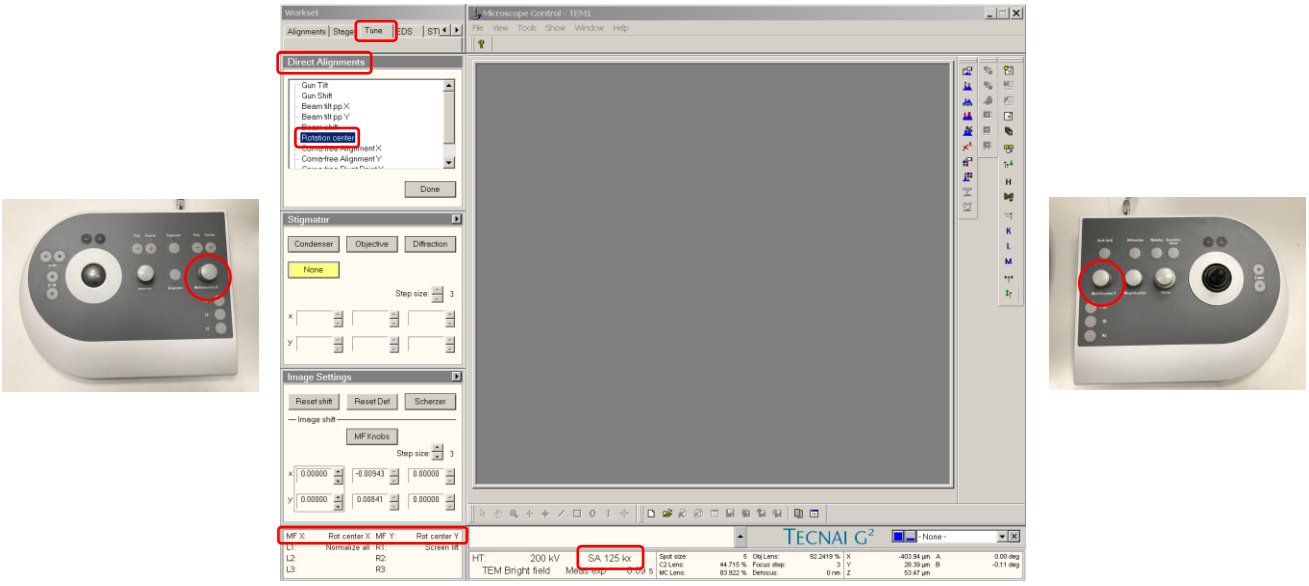
9.7. Once the beam is stationary, use the “Z axis” buttons to eliminate any separation of the image of the specimen; select “Done” when finished.



10. Rotation centering

10.1. Leave the indicated magnification = 125 k× and re-center the beam using the trackball.

10.2. In the “Direct Alignments” control panel, select “Rotation center”; the image will now oscillate from in to out of focus; use the “Multifunction” knobs to eliminate any shifting of the image; select “Done” when finished.



11. Specimen orientation adjustment (for single crystals or individual grains)

    11.1. Use the joystick to center the area of interest for zone axis alignment (a film/substrate interface, a specific grain, etc.) on the viewing screen.

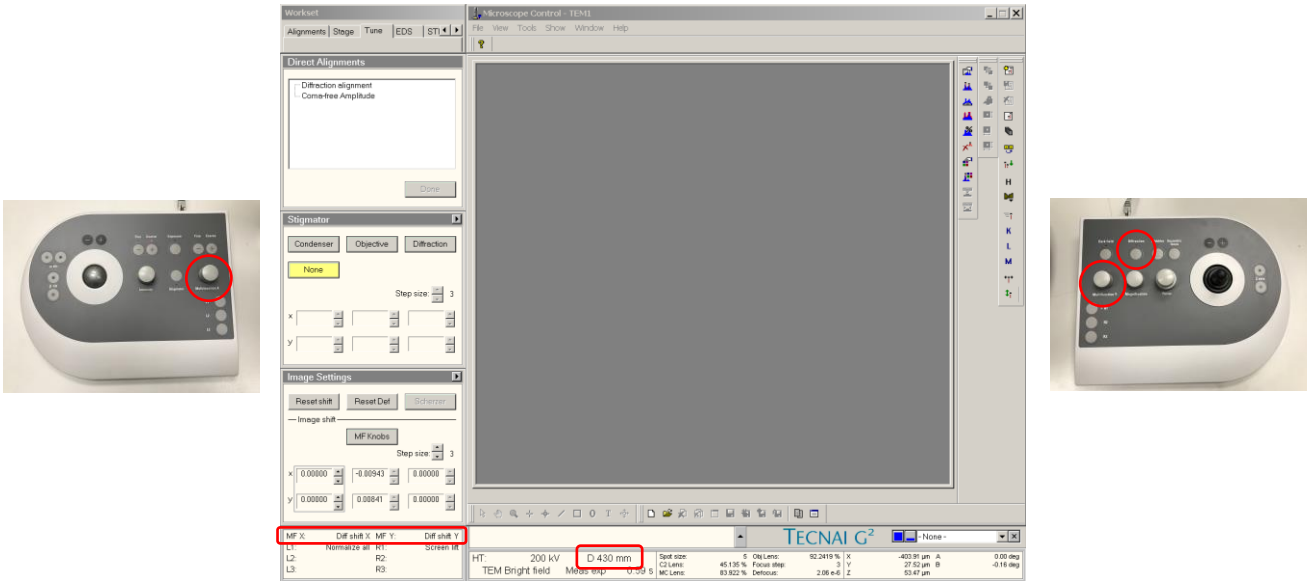
    11.2. Use the “Intensity” knob to expand the beam clockwise from crossover so it is slightly larger than the 40 mm circle on the viewing screen; then use the trackball to center the beam.

    11.3. Select “Diffraction” (R control pad) to enter diffraction mode; note that the area illuminated by the beam will be what is contributing to the observed diffraction pattern.

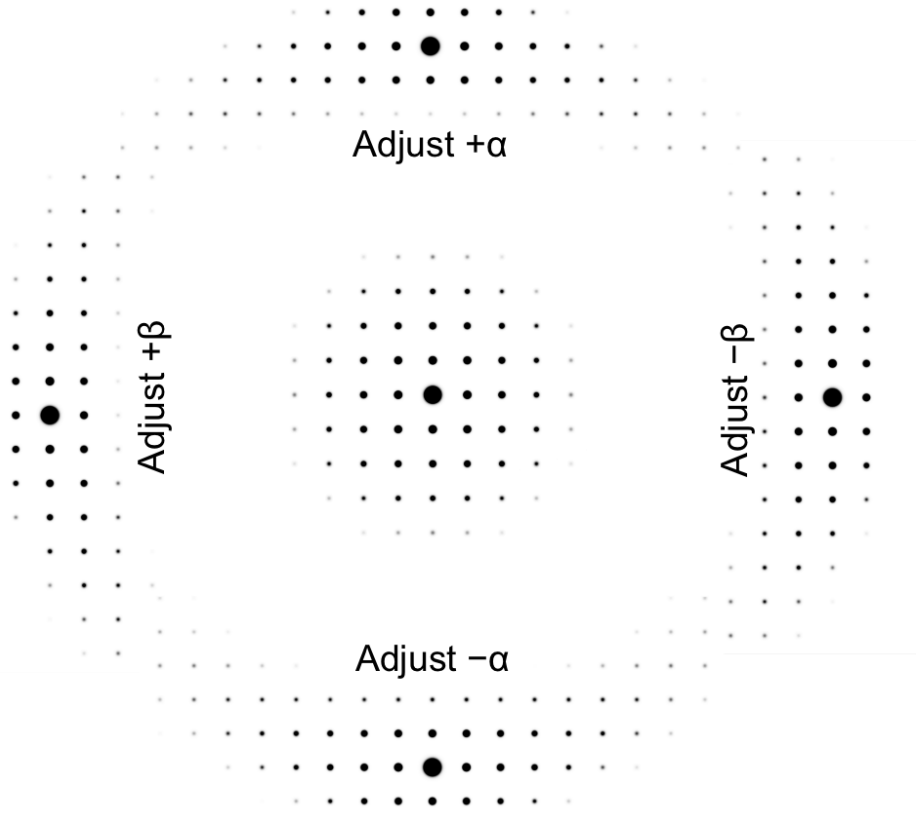
    11.4. Set the camera length by using the “Magnification” knob; an indicated camera length = 430 mm is recommended for observing the diffraction pattern.

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

        11.5.1. DO NOT use the trackball to shift the diffraction pattern.


Workset
Alignments
Stage
Tune
EDS
Direct Alignments
Diffraction alignment
Coma-free Amplitude
Done
Stigmator
Condenser
Objective
Diffraction
None
Step size
Image Settings
Reset shift
Reset Def
Scherzer
Image shift
MF Knobs
Microscope Control - TEM1
File
View
Tools
Show
Window
Help
TECNAI G²
HT: 200 kV
D 430 mm
TEM Bright field

11.6. Use the α and β tilt buttons (L 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 imaging mode and center the specimen, then press the “Diffraction” button to return to diffraction mode; resume adjusting the specimen orientation.



Above: zone axis tilt map when the indicated camera length is ≥ 200 mm

11.7. Afterwards, press the “Diffraction” button to return to imaging mode; re-establish eucentric height as described previously (this should be done <u>every</u> time after tilting the specimen).

12. Objective aperture centering

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

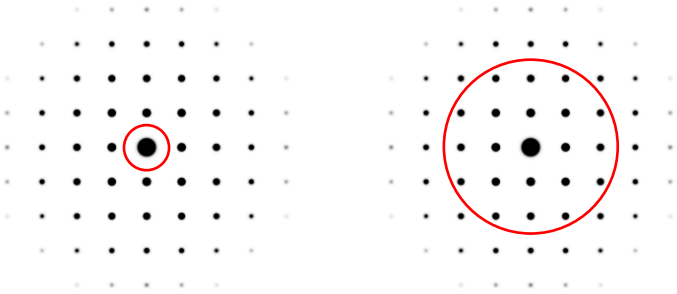
12.2. Select the “Diffraction” button to enter diffraction mode; the diffraction pattern should be immediately evident on the viewing screen; use the “Magnification” knob to set the indicated camera length = 430 mm.

12.3. Move the focusing screen into the beam path and then look through the binoculars to observe the diffraction pattern; use the “Multifunction” knobs to center the pattern.

12.3.1. DO NOT attempt to use the trackball to shift the diffraction pattern.

12.4. Turn the selecting knob on the objective aperture assembly to insert an objective aperture; aperture #4 should be used for BF-TEM imaging while aperture #2 should be used for HR-TEM imaging.

12.5. Use the objective aperture shifting dials on the objective aperture assembly to center the objective aperture on the direct beam.



Above: proper objective aperture centering for BF- (left) and HR- (right) TEM imaging

12.6. Select the “Diffraction” button to return to imaging mode; re-center the beam using the trackball.

12.7. The focus of the image may be slightly different due to the insertion of the objective aperture; use the “Z axis” buttons to focus the image as best as possible (minimum contrast).

13. Imaging in DigitalMicrograph; objective astigmatism correction

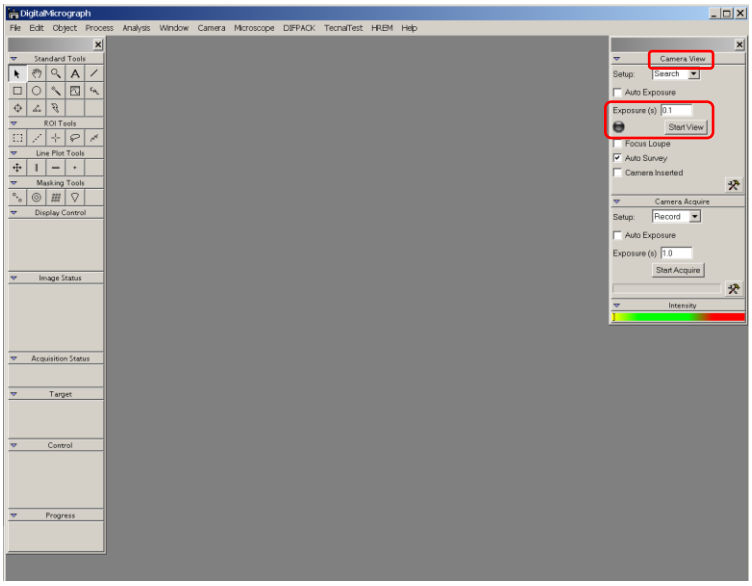
13.1. Set the indicated magnification as desired (remaining in SA mode).

13.2. 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 use the trackball to center the beam.

13.3. In DigitalMicrograph, navigate to the “Camera View” panel and set “Exposure (s)” = 0.1; do not exceed this value for live imaging with the CCD camera. Select “Start View” to start live imaging (affirming any messages asking to insert the camera).

13.4. Listen for the camera to insert; once the camera is inserted, flip the viewing screen up by selecting the “R1” button (R control pad); a live image should appear in DigitalMicrograph.

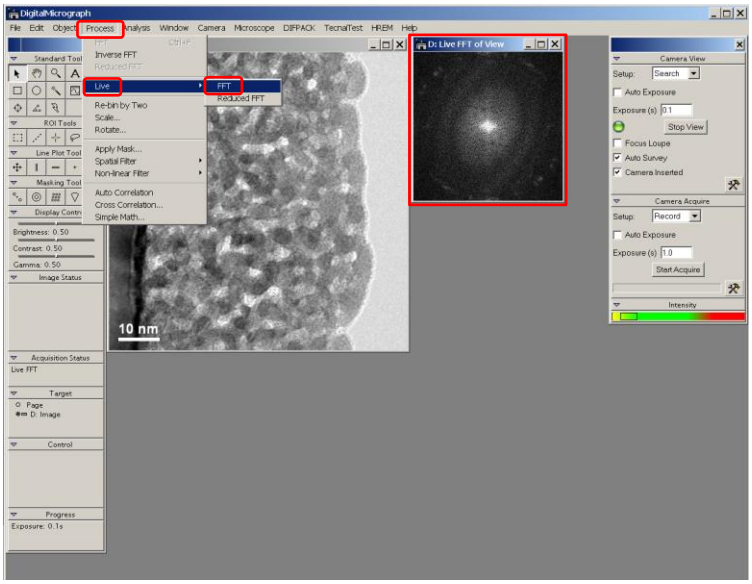
13.4.1. NOTE: the indicated magnification shown in Microscope Control will increase slightly due to the image plane of the CCD camera being deeper compared to that of the viewing screen.



13.5. If some portion of the specimen is amorphous, this can be used to correct objective lens astigmatism, but the indicated magnification must be ≥125000× to do this effectively; otherwise, DO NOT attempt to correct objective lens astigmatism.

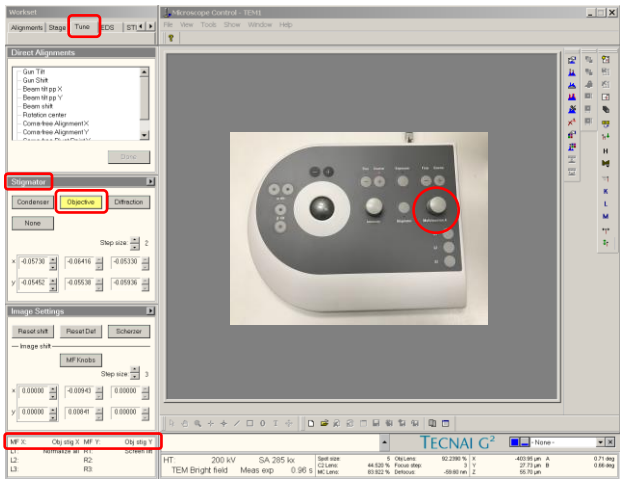
13.6. In DigitalMicrograph, select “Process” from the pull-down menu, then “Live”, and then “FFT”.

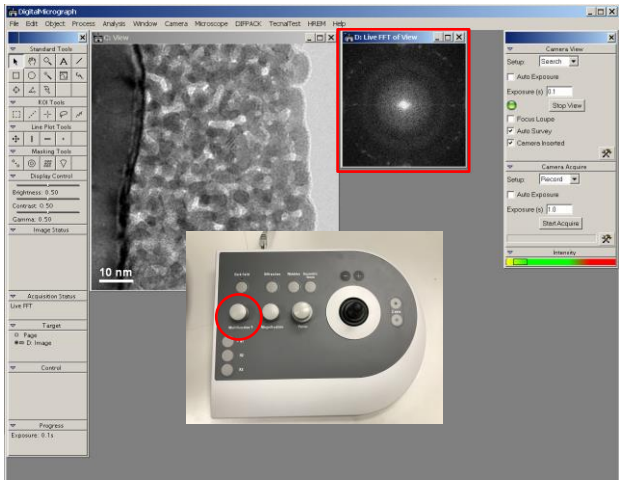
13.7. Adjust the “Focus” knob to obtain an under-focus condition where 1 – 2 diffuse rings are evident in the FFT.



13.8. In Microscope Control, select the “Tune” tab and then the “Stigmator” control panel; select “Objective” (the button will turn yellow).

13.9. Use the “Multifunction” knobs to adjust the live FFT so the diffuse rings are circular; select “None” when finished.

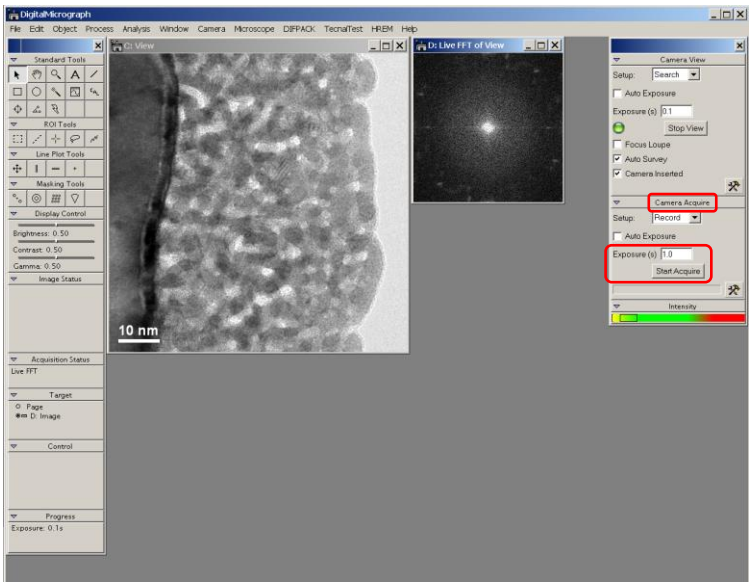




13.10. Use the “Focus” knob to precisely focus the image; for HR-TEM imaging, the optimal focal condition will correspond to slight under-focus.

13.11. In DigitalMicrograph, navigate to the “Camera Acquire” panel and set “Exposure (s)” = 1.0; if desired, “Exposure (s)” < 1.0 may be used to reduce blurring due to specimen instability/drift (at the expense of poorer signal), but do not exceed “Exposure (s)” = 1.0 as this may result in damage to the CCD camera.

13.12. Select “Start Acquire” to acquire the final, high-quality image.



13.13. If image collection at a different indicated magnification is desired:

13.13.1. Flip the viewing screen down (R1 button).

13.13.2. Adjust the “Magnification” knob accordingly.

13.13.3. 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 use the trackball to center the beam.

13.13.4. Flip the viewing screen back up (R1 button).

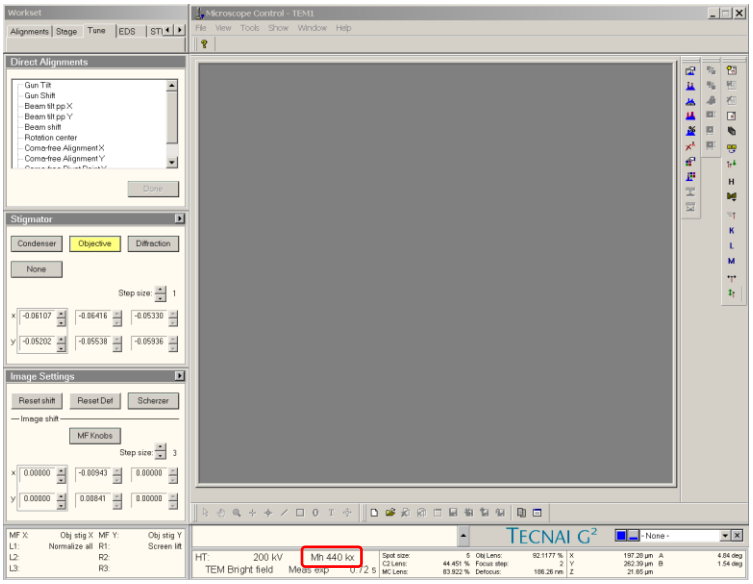
13.13.5. DO NOT adjust the indicated magnification, beam position, or beam size using the live image on the CCD camera.

13.13.6. Adjust the “Focus” knob and correct the objective astigmatism as needed (using the live FFT).

14. Mh mode

14.1. The highest indicated magnification possible in SA mode is 400k×; if the indicated magnification is adjusted higher than this, the instrument will switch into “Mh” mode (minimum indicated magnification = 440 k×).

14.2. Press the “Eucentric focus” button; use the “Z axis” buttons to focus the image as best as possible (minimum contrast).



14.3. Realign the beam tilt pivot points (step 9); reposition the beam with the trackball during these alignments (if needed).

14.4. 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 use the trackball to center the beam.

14.5. Resume live imaging in DigitalMicrograph; adjust the “Focus” knob and correct the objective astigmatism as needed (using the live FFT).

15. Acquiring diffraction patterns

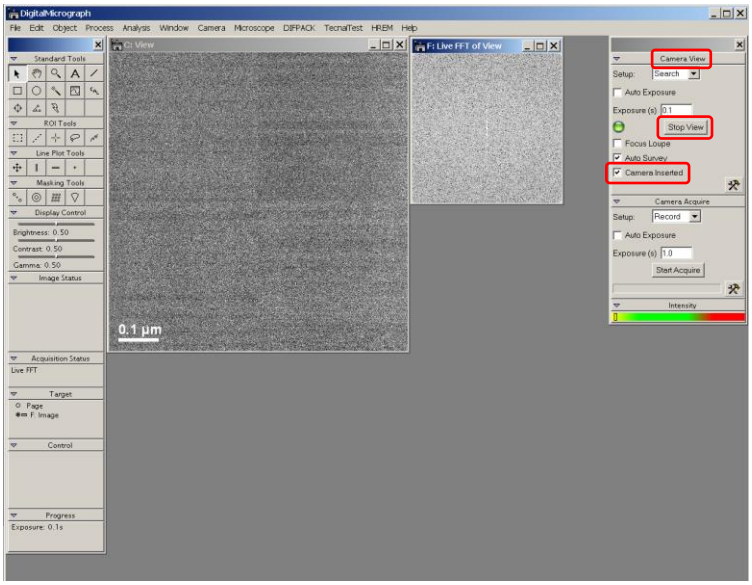
15.1. Acquiring diffraction patterns with the CCD camera is not covered in basic training; please submit a service request for “advanced training” if wanting this additional training; DO NOT attempt to record diffraction patterns with the CCD camera without being trained to safely do this; otherwise, severe damage to the camera could result.

16. Finishing with the CCD camera

16.1. The CCD camera should only be left inserted while it is actively being used; when it is time to exchange grids or to finish up the session, the camera should be retracted.

16.2. Flip the viewing screen down (R1 button).

16.3. In DigitalMicrograph, navigate to the “Camera View” panel; select “Stop View” to stop live imaging, uncheck “Camera Inserted”, and then listen for the camera to retract.



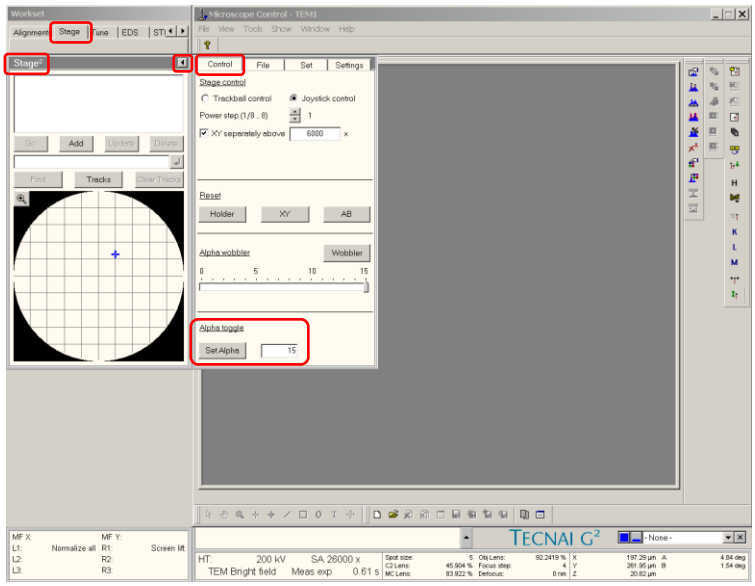
17. Collecting EDS spectra in TEM mode

17.1. EDS can be performed while in TEM (conventional, static beam) mode to obtain a general survey of the area illuminated by the beam; however, point analysis, linescans, and maps, must be performed in scanning mode (covered elsewhere).

17.2. Verify the microscope is in “SA” mode and that both the objective and SA apertures are retracted to prevent EDS detector overload and unwanted system X-ray signals.

17.3. If using the double-tilt holder, no adjustments to the stage tilt are needed to allow sufficient line of sight between the specimen and the EDS detector.

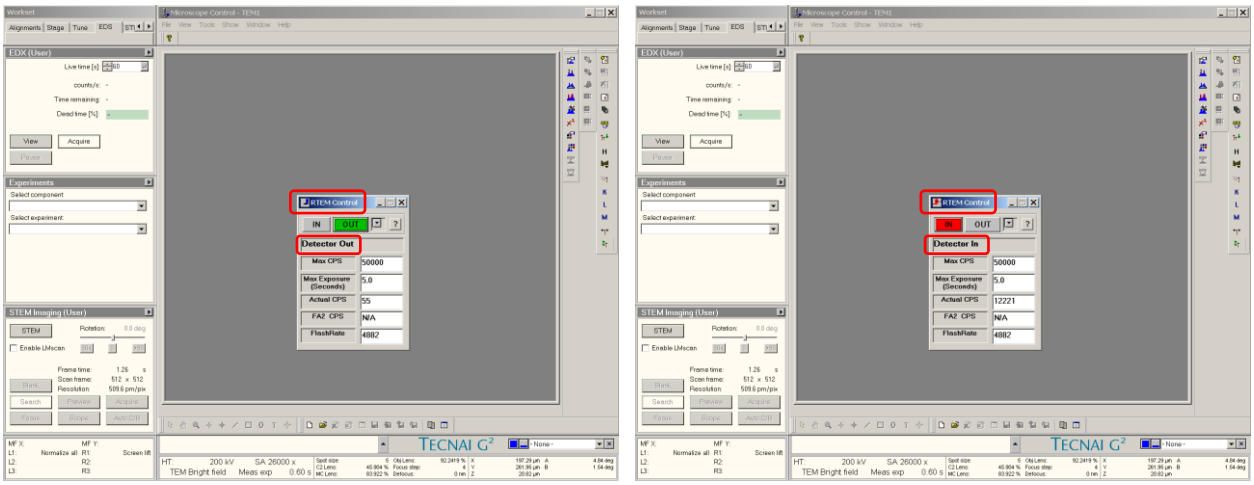
17.4. If using the single tilt holder, the α tilt must be ≥15° to allow sufficient line of sight between the specimen and the EDS detector. In Microscope Control, select the “Stage” tab and then the Stage^2 control panel; select the flap-out arrow and then the “Control” tab; under “Alpha toggle”, input 15°and select “Set Alpha”.



17.5. After setting α = 15°, press “Eucentric Focus” and use the “Z axis” buttons to bring the sample into focus (minimum contrast).

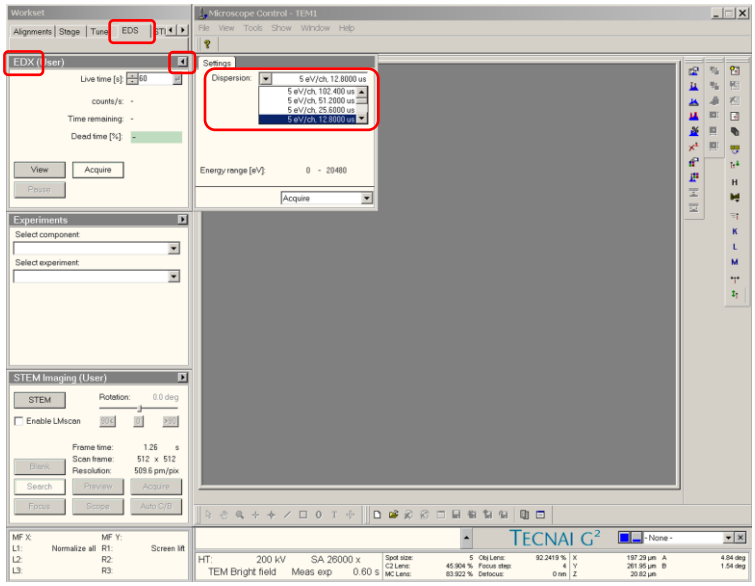
17.6. Set the magnification (remaining in SA mode) as needed and adjust the area of illumination as needed to cover the features/area of interest for EDS analysis; do not illuminate any part of the grid, as this will cause an overload to the EDS detector.

17.7. Maximize RTEM Control and select “IN” to insert the EDS detector; the “OUT” button will turn gray and the “IN” button will turn red.



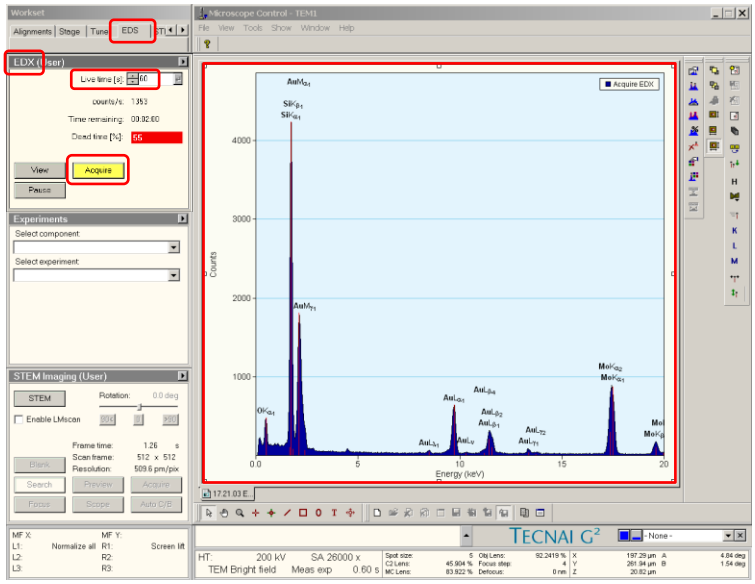
17.8. The beam may shift slightly from its initial position due to inserting the EDS detector; use the beam shift trackball to re-center it on the region of interest, if needed.

17.9. In Microscope Control, select the “EDS” tab and then the “EDX” control panel; select the flap out arrow to expand the panel. In the “Settings” tab, select from the pull-down list of available dispersions and process times.



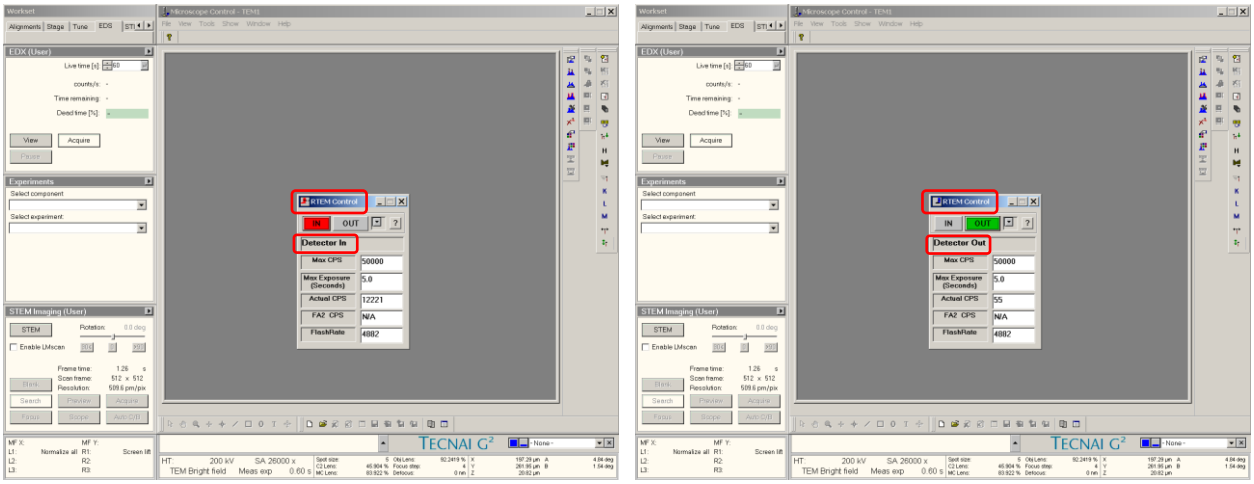
17.10. In the “EDX” control panel, input 30 – 60 s for “Live time (s)” and select “Acquire” (the button will turn yellow); a panel will open in TIA to display the live EDS spectrum; collection will stop automatically once the live time has elapsed.

17.10.1. NOTE: if the “Dead time [%]” is significantly higher than 50 %, consider reducing the process time and acquiring the spectrum again.

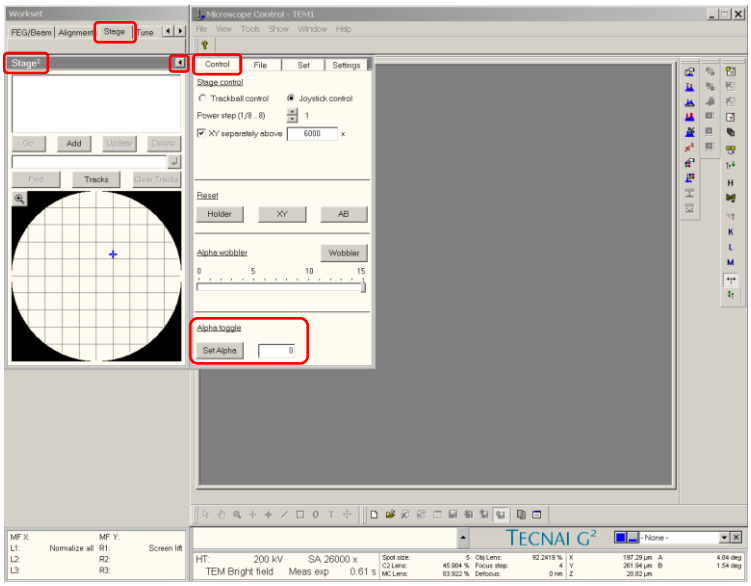


17.11. Further analysis of the collected EDS spectrum (peak identification, quantification, etc.) may be performed in TIA (not covered here).

17.12. When finished collecting EDS spectra, maximize RTEM Control and select “OUT” to retract the EDS detector; the “OUT” button will turn green, and the “IN” button will turn gray. <u>DO NOT leave the EDS detector inserted unless it is actively in use.</u>



17.13. If the single-tilt holder was used, reset the alpha tilt to 0°. In Microscope Control, select the “Stage” tab and then the “Stage^2” control panel; select the flap-out arrow to expand the panel and select the “Control” tab; under “Alpha toggle”, input α = 0° and select “Set Alpha”.

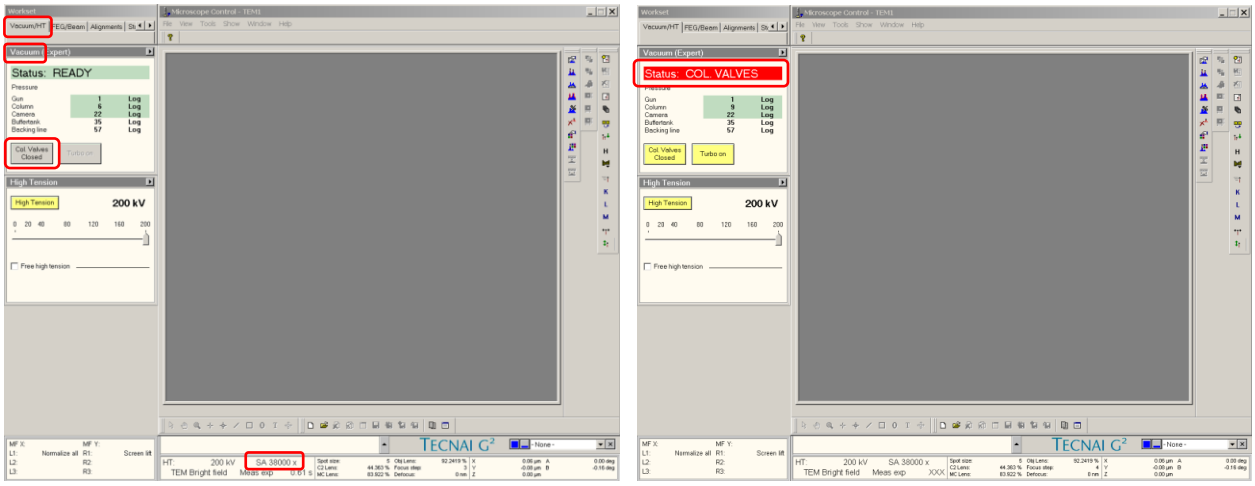


18. Grid exchanges; finishing the session

18.1. When finished with the current grid, return to Microscope Control; leave the instrument in SA mode as verified in the bottom information panel.

18.1.1. DO NOT leave the instrument in LM, M, Mh, diffraction, or any other mode besides SA.

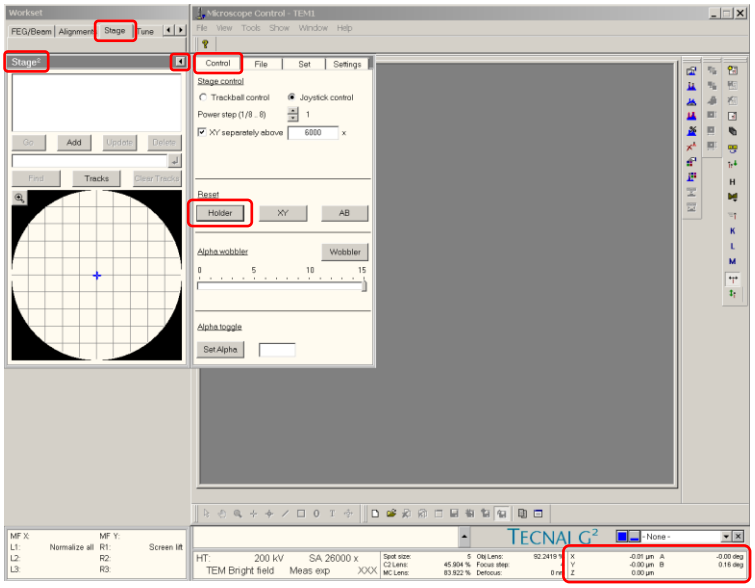
18.2. Select the “Vacuum/HT” tab and then the “Vacuum” control panel; select “Col. Valves Closed” to close the column valves; (the button will turn yellow), and status will now read “Status: COL. VALVES”.



18.3. Retract the objective (set to “OUT”) and SA (bar flipped to the right) apertures (if still inserted).

18.4. In Microscope Control, select the “Stage” tab and then the “Stage^2” control panel; select the flap-out arrow to expand the panel and select the “Control” tab; under “Reset”, select “Holder” to home the stage. Verify that X, Y, and Z positions and α and β tilt values are all ~0 before proceeding.

18.4.1. <u>DO NOT initiate homing unless the objective aperture is retracted</u>; otherwise, damage to the objective aperture blade, holder, or stage may result.



18.5. If using the double-tilt holder, disconnect the β tilt control; then remove the holder from the column (described previously) and remove the grid from the specimen holder.

18.6. If another grid is to be imaged, load it into the holder (cleaning it as appropriate), and insert the holder back into the column.

18.7. The FEG register should be reloaded after a grid exchange.

18.8. Eucentric height must be reset after a grid exchange.

18.9. It is best practice to perform all other alignments again (except for C2 aperture alignment) after a grid exchange; particularly if wanting to obtain the best performance from the instrument.

18.10. If finished with the session, insert 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 and turn off (but do not vent) the plasma cleaner.

19. Cryo cycling (if needed)

    19.1. Cryo cycling the instrument is necessary at the end of each day or after the session; due to the infrequency of use of this instrument, it will be the responsibility of the last user of the day to perform this task.

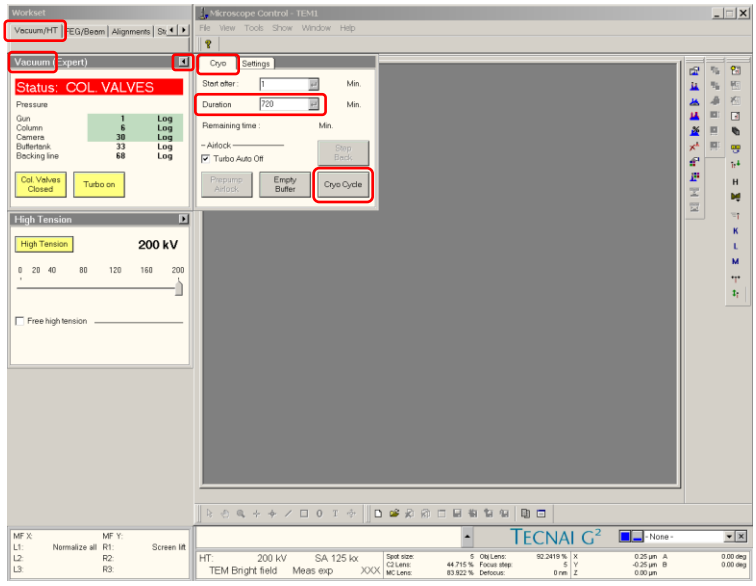
    19.2. Verify that the single tilt holder is inserted into the column; this must be done before initiating the cryo cycle.

    19.3. Cover the projection chamber.

    19.4. Completely remove the cold trap dewar from the enclosure, if there this any LN_2 remaining in the cold trap dewar, pour it back into the storage dewar.

    19.5. In Microscope Control, select the “Vacuum/HT” tab and then the “Vacuum” control panel; select the flap-out arrow to expand the panel and then select the “Cryo” tab.

    19.6. Set “Duration” = 720 min (if not already set accordingly); DO NOT adjust any of the other cryo cycle settings. Select “Cryo Cycle” to initiate the cryo cycle.



20. Logging off

    20.1. Logging off the TUMI system may be done after unloading the (last) specimen from the selected holder, inserting the single tilt holder back into the column (and, if used, the double tilt holder back into the plasma cleaner), and collecting all needed data from the microscope computer.

Appendix: shorthand procedure for imaging in TEM mode

1. Fill cold trap with LN_2
2. Remove single-tilt holder from column
3. Load grid in desired holder
4. Verify column vacuum level is adequate
5. Load holder into column
6. Apply desired FEG register (do not go lower than spot size = 3)
7. Open column valves
8. Adjust magnification to enter LM mode
9. Use intensity knob to expand/contract beam
10. Use trackball to shift beam
11. Use joystick to navigate to area of interest
12. Return to SA mode
13. Alignment
    a. Press “Eucentric focus” and focus with Z (13500×)
    b. Align C2 aperture (38000×)
    c. Correct condenser astigmatism (125000×)
    d. Expand beam clockwise from crossover to slightly larger than 40 mm circle and center beam
    e. Align beam tilt pivot points; fine focus with Z (125000×)
    f. Align rotation center (125000×)
    g. Enter diffraction mode and tilt specimen to zone axis (if applicable)
    h. If specimen tilted, return to imaging mode and refocus with Z
    i. Return to diffraction mode and continue tilting sample as needed
    j. Insert/center appropriate objective aperture
    k. Return to imaging mode
14. Set magnification as needed; fine focus with Z
15. Resize and recenter beam on viewing screen (as needed)
16. Insert CCD camera, flip screen up, acquire live image in DigitalMicrograph
17. Correct objective astigmatism using live FFT (≥125000×)
18. Precisely focus and acquire final image
19. Flip screen down, retract CCD camera
20. Close column valves
21. Retract objective and SA apertures
22. Home stage
23. Retract holder from column and unload grid from holder
24. Insert single-tilt holder back into column