

FEI Themis Z S/TEM: TEM mode imaging
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ANALYSIS OF RADIOACTIVE SPECIMENS IS STRICTLY PROHIBITED

This document assumes the user has sufficient experience/competency in all the following areas related to use of the Talos F200i S/TEM and thus omits many related details/images for purposes of clarity:

- A. Basic TEM mode operation, including high-resolution imaging
- B. FluCam operation and manipulation
- C. The Microscope Control user interface
- D. TEM imaging in Velox

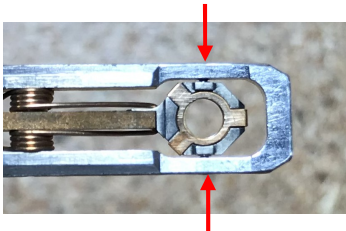
1. Instrument voltage

- 1.1. The default voltage setting is 200 kV. If needing to operate the instrument at a different voltage (60 or 300 kV), please notify staff (as far in advance as possible) for assistance.

2. Specimen loading/unloading

- 2.1. A special high visibility low background “soft-loc” double tilt holder is optimized for use with the Themis Z. Use extreme caution when loading/unloading the basket; do not apply excessive downward force as this may damage the sapphire screws (indicated by arrows) holding the basket in place.

- 2.1.1. This repair costs ~\$3000 and takes ~2 weeks to complete.



- 2.2. Holder loading/unloading into/out of the column is identical to as described for the Talos with the notable exception that a metal plug, rather than a holder, is stored in the column when the system is not actively in use.

- 2.2.1. Be sure to close the enclosure door once the holder is inserted and close the microscope room door upon leaving.

3. Base alignments, gun, and spot size settings

3.1. Select and apply the “200kV_RSC” alignment file (not shown; identical to as described for the Talos).

3.2. Select and apply the “200kV_uP” FEG register (not shown; identical to as described for the Talos).

3.3. Select the “Beam” tab and navigate to the “Beam Settings” control panel,

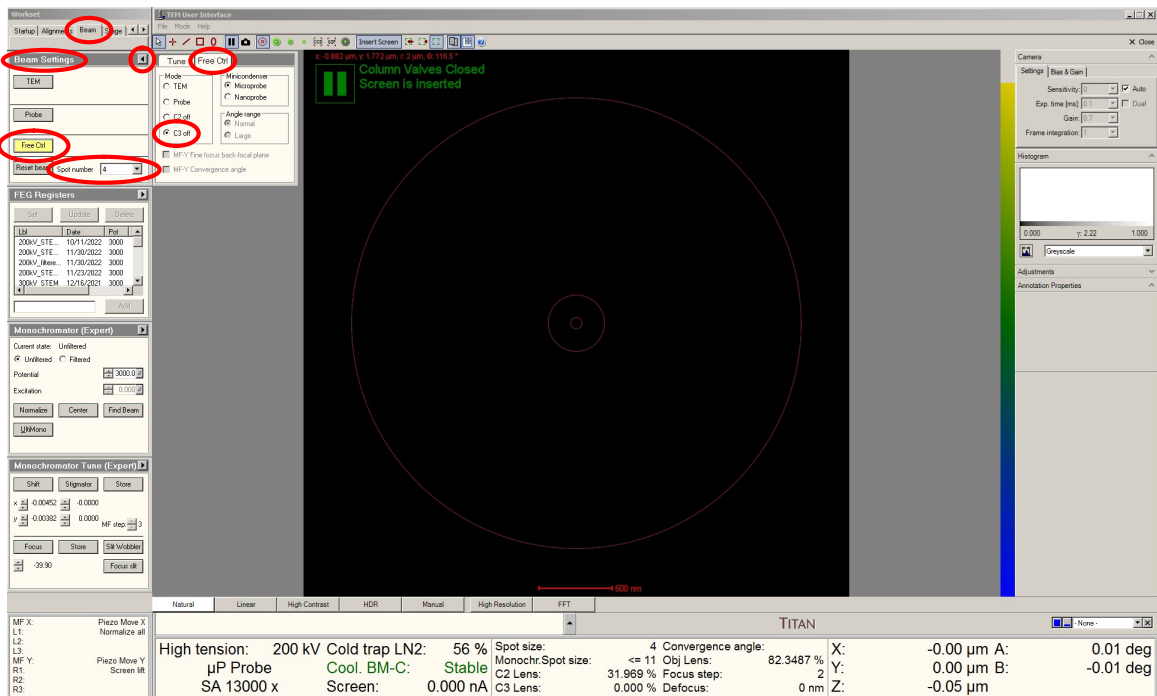
3.3.1. Select “Free Ctrl” and then the flap-out arrow.

3.3.2. Select the “Free Ctrl” tab and select the “C3 off” radio button (C3 will be turned on again at later point during alignment).

3.4. Remaining in the “Beam Settings” control panel, set “Spot number” = 4.

3.4.1. This is a good general spot number that will work well for everything from microscale bright-field imaging to angstrom-resolution lattice imaging.

3.4.2. Every incremental decrease (increase) in spot number results in a beam current increase (decrease) of a factor of ~ 2 ($\frac{1}{2}$), all other factors being equal.

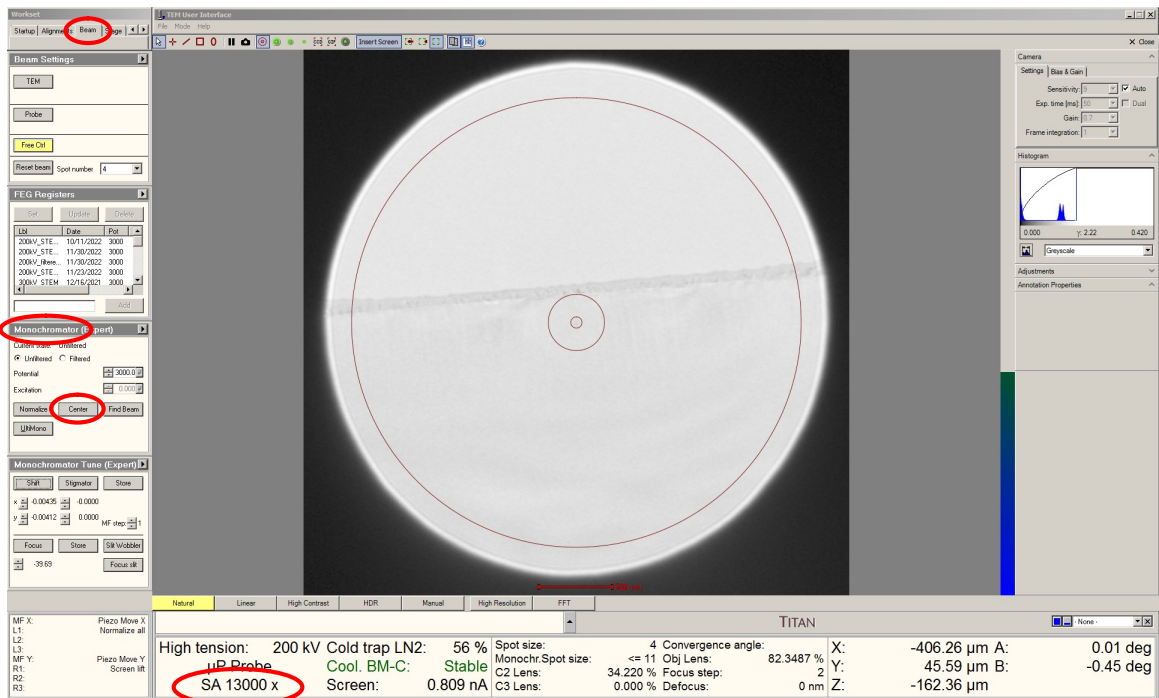


4. Finding an ROI, monochromator centering, setting eucentric height
 - 4.1. Open the column valves (not shown; identical to as described for the Talos).
 - 4.2. Adjust the indicated magnification as needed; expand/contract the beam and recenter/reposition the beam as needed (not shown; identical to as described for the Talos).
 - 4.3. Move the stage until an area of interest is found and centered on the FluCam; set the indicated magnification =13000× (not shown; identical to as described for the Talos).
 - 4.4. If possible, move the stage to retract the specimen from the field of view so the beam is over vacuum only.
 - 4.5. Expand the beam clockwise from crossover so it is approximately the same size as the 40 mm circle on the FluCam and center it (not shown; identical to as described for the Talos).
 - 4.5.1. The beam may look strange at this point; if so, this is likely due to the monochromator being uncentered, which will be subsequently addressed.

4.6. Select the “Beam” tab and navigate to the “Monochromator” control panel.

4.6.1. Select “Center” to perform monochromator centering; the automated centering routine takes ~1 min to complete.

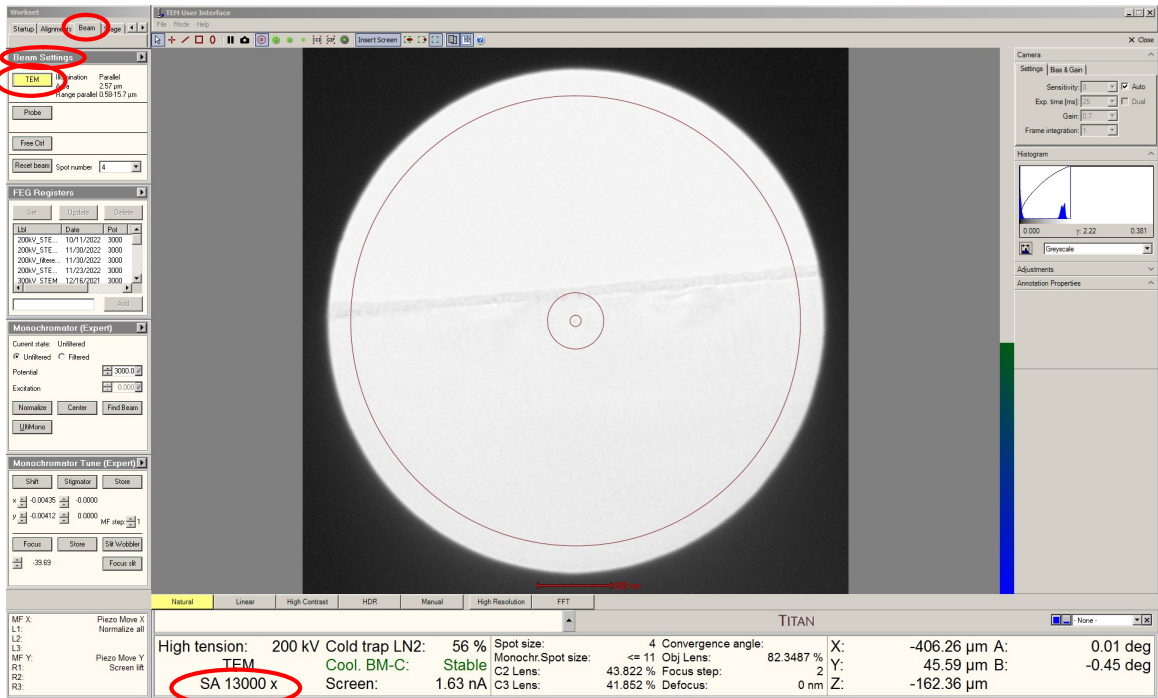
4.6.2. NOTE: if the specimen is not retracted from the field of view, the entire area illuminated by the beam must otherwise be transmissive for this to work.



4.7. Leave indicated magnification = 13000× and return the area of interest to the field of view (if necessary).

4.8. Press “Eucentric Focus” and set eucentric height (not shown; identical to as described for the Talos).

5. C2 aperture alignment, turning C3 back on
 - 5.1. Verify the 70 μm C2 aperture is selected; if it is not selected, then select it (not shown; identical to as described for the Talos).
 - 5.2. Leave the indicated magnification = 13000 \times and perform C2 aperture centering (not shown; identical to as described for the Talos).
 - 5.3. After the C2 aperture is centered, the C3 lens may be turned back on again. Select the “Beam” tab and navigate to the “Beam Settings” control panel.
 - 5.3.1. Select “TEM” to turn the C3 lens back on; this is the final configuration that will be used for imaging.
 - 5.3.2. The beam should always be expanded clockwise from crossover (just like when using the Talos).



6. Condenser astigmatism correction

6.1. Leave the indicated magnification = 13000 \times ; expand the beam clockwise from crossover to the size of the 1 mm circle on the FluCam and center it.

6.2. Activate the condenser stigmators and adjust to make the beam circular (not shown; identical to as described for the Talos).

7. Condenser centering (C2/C3 centering)

7.1. Leave the indicated magnification = 13000 \times ; expand the beam clockwise from crossover to the size of the 40 mm circle on the FluCam and center it.

7.2. Select the “Tune” tab and navigate to the “Direct Alignments” control panel.

7.2.1. Select “Condenser center TEM”; the beam will start expanding/contracting.

7.2.2. Adjust the “Intensity” knob to set the limits of expansion/contraction to roughly between the 40- and 1-mm circles on the FluCam.

7.2.3. Adjust the “Multifunction” knobs so the beam expands and contracts concentrically (the beam may shift while performing this alignment).

The screenshot displays the TITAN TEM User Interface. The 'Direct Alignments' panel on the left has 'Condenser center TEM' selected. The main view shows a circular beam with a 100 µm scale bar. The status bar at the bottom shows 'SA 13000 x' circled in red.

High tension:	200 kV	Cold trap LN2:	56 %	Spot size:	4	Convergence angle:	X:	-406.26 µm	A:	0.01 deg	
	TEM	Cool. BM-C:	Stable	Monochr. Spot size:	<= 11	Obj Lens:	82.3487 %	Y:	46.06 µm	B:	-0.45 deg
		Screen:	1.38 nA	C2 Lens:	40.481 %	Focus step:	2	Z:	-162.36 µm		
				C3 Lens:	52.930 %	Defocus:	0 nm				

8. Condenser deflector balancing

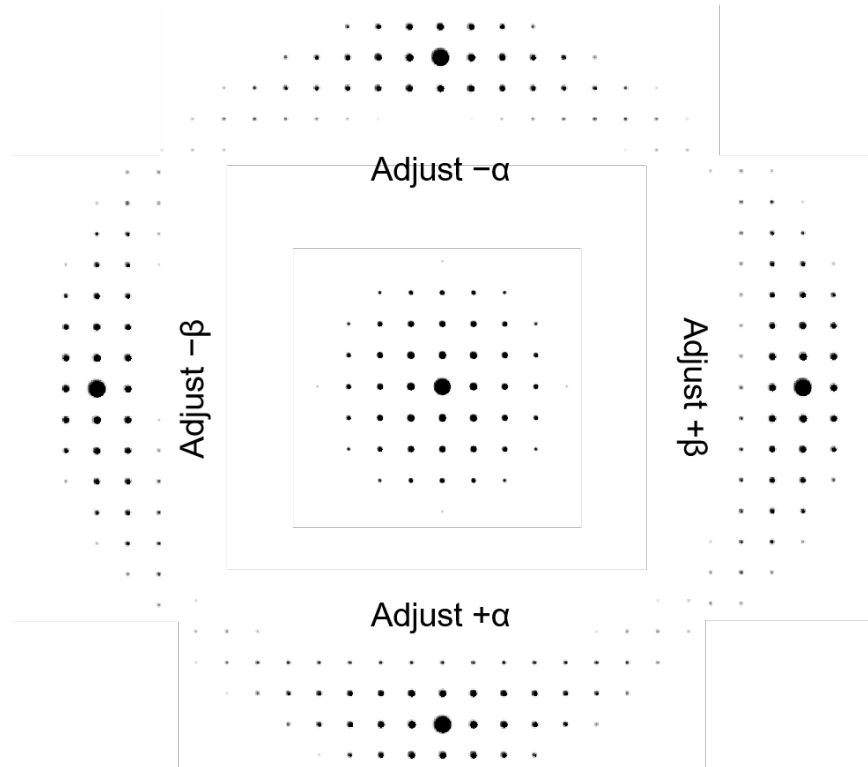
- 8.1. Set the indicated magnification as needed for imaging; NOTE: to effectively observe lattice fringes on the Themis, it is necessary to use indicated magnifications $\geq 115k\times$ (not shown; *identical to as described for the Talos*)
- 8.2. Expand the beam clockwise from crossover so it is approximately the same size as the 40 mm circle on the FluCam and center it (not shown; *identical to as described for the Talos*).
- 8.3. Finely focus the image (not shown; *identical to as described for the Talos*).
- 8.4. Perform the “Beam tilt pp X” direct alignment (not shown; *identical to as described for the Talos*).
- 8.5. Perform the “Beam tilt pp Y” direct alignment (not shown; *identical to as described for the Talos*).

9. Rotation centering

- 9.1. Center the beam and then expand clockwise from crossover so the beam is just outside the viewable area of the FluCam.
 - 9.1.1. If any fringes near the beam edge are still visible, continue expanding the beam until these are outside the viewable area.
- 9.2. Perform the “Rotation centering” direct alignment (not shown; *identical to as described for the Talos*).

10. Zone axis alignment (when working with large single crystals)

- 10.1. Center the area of interest on the FluCam.
- 10.2. Enter diffraction mode and set the indicated camera length as desired (not shown; identical to as described for the Talos).
- 10.3. Tilt the stage (see zone axis tilt map below; valid for any camera length) to orient the sample toward the zone axis.



- 10.4. Return to TEM mode.
- 10.5. Recenter the area of interest on the FluCam.
- 10.6. Refocus the image using the Z axis stage control (not shown; identical to as described for the Talos).
- 10.7. The area of interest may move when tilted (particularly if the β tilt is adjusted), so you should return to diffraction mode and verify the specimen orientation is as desired where imaging is intended.
 - 10.7.1. If the orientation is still not as desired, then repeat the previous steps iteratively until the desired orientation is obtained.

11. Objective aperture centering

11.1. Enter diffraction mode and select the camera length as needed (not shown; identical to as described for the Talos).

11.1.1. L = 460 mm is a good setting for objective aperture alignment.

11.2. Select, insert, and center an appropriate objective aperture (not shown; identical to as described for the Talos).

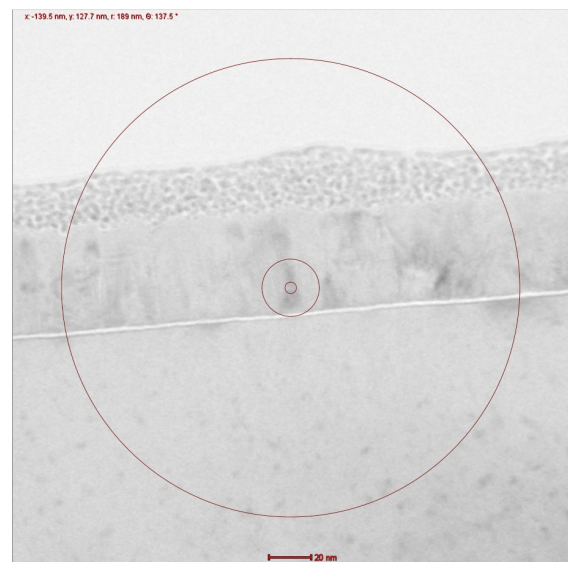
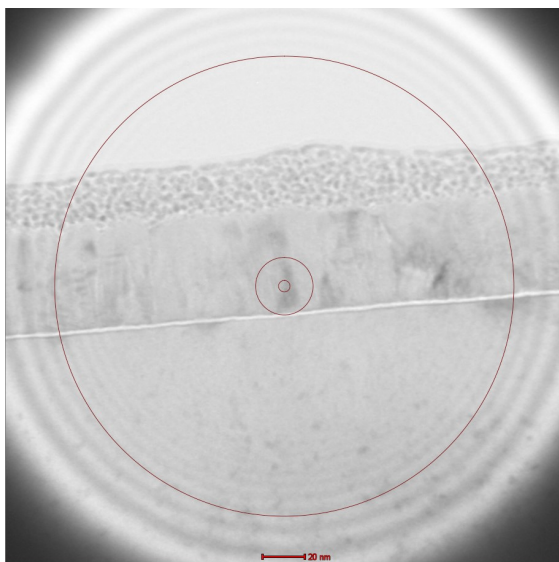
11.2.1. The 40 μm objective aperture is the optimal aperture for high-resolution (lattice) imaging at 200 kV.

11.2.2. The 10 μm objective aperture is appropriate for low magnification bright-field imaging.

11.3. Return to TEM mode.

11.4. Center the beam and then expand clockwise from crossover so the beam is just outside the viewable area of the FluCam.

11.4.1. If any fringes near the beam edge are still visible in the viewable area (but may be faint), continue expanding the beam until these are no longer observed.



Left: beam not expanded enough; fringes near beam edge observed in viewable area
Right: beam expanded properly; no fringes near beam edge observed in viewable area

12. Image acquisition and objective astigmatism correction using Velox
 - 12.1. Correction of objective lens astigmatism should only be attempted if the indicated magnification is $\geq 115 \text{ k}\times$; do not attempt otherwise.
 - 12.2. Retract the main screen and start acquiring a live image in Velox (not shown; identical to as described for the Talos).
 - 12.3. Observe the FFT of the live image and adjust "Focus" to obtain a slightly underfocused image with 1 or 2 diffuse rings evident in the FFT (not shown; identical to as described for the Talos).
 - 12.4. Activate the objective stigmators and adjust accordingly to make the rings in the FFT circular (not shown; identical to as described for the Talos).
 - 12.5. Adjust "Focus" as needed and acquire the final image (not shown; identical to as described for the Talos).
 - 12.6. If image collection at a different magnification is needed
 - 12.6.1. Reinsert the viewing screen and adjust the magnification
 - 12.6.2. Resize and recenter the beam as specified previously.
 - 12.6.3. Resuming live imaging in Velox; refocus and correct the objective astigmatism (will likely be necessary when changing magnifications).
 - 12.7. Adjustments when switching from SA (380k \times maximum) to Mh (460k \times minimum) mode:
 - 12.7.1. Retract the objective aperture
 - 12.7.2. Focus the image
 - 12.7.3. Perform condenser deflector balancing
 - 12.7.4. Perform rotation centering
 - 12.7.5. Reinsert/center objective aperture
 - 12.7.6. Resume live imaging in Velox; adjust "Focus" and correct objective astigmatism.
 - 12.8. When finished imaging with the Ceta camera, reinsert the viewing screen and retract the Ceta camera (not shown; identical to as described for the Talos).

13. Using the piezo controller

13.1. At the upper limit of SA mode and in Mh mode, it can be useful to use the piezo controller of the stage to enable fine positioning of the specimen with nm precision prior to image acquisition.

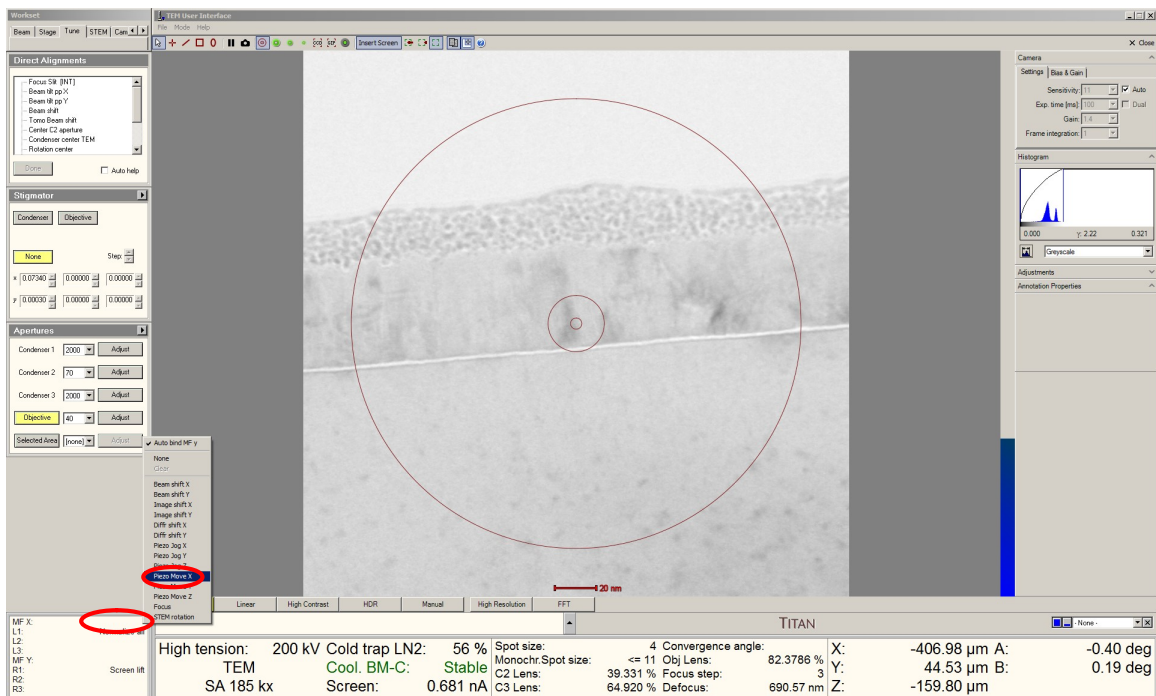
13.1.1. The piezo controller can range several hundred nm in both positive and negative X and Y directions.

13.2. If nothing else is being controlled by the “Multifunction” knobs, these should default to “Piezo Move X” and “Piezo Move Y”.

13.2.1. Simply use the “Multifunction” knobs to position the specimen as needed using the piezo controller.

13.3. Otherwise, navigate to the bottom left information panel, find the “MF X:” line, right click on the right side of the line, and select “Piezo Move X”.

13.3.1. the “MF Y:” line will automatically set to “Piezo Move Y”.

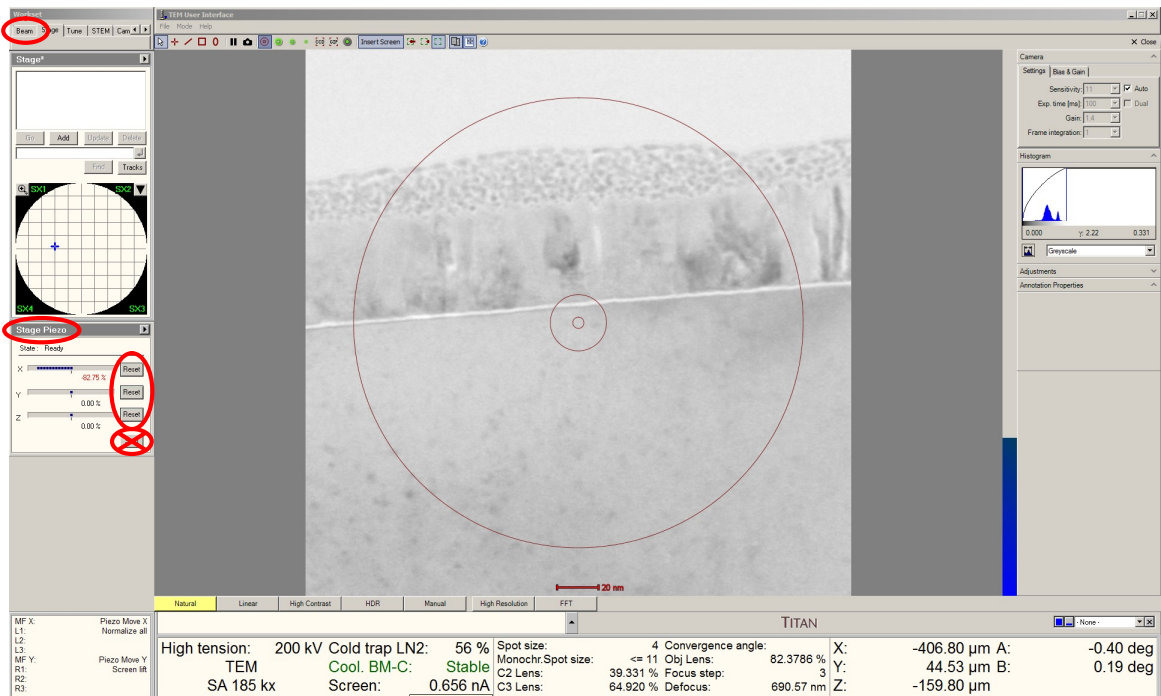


13.4. If the limit of one of the axes is being approached, a warning message will appear and the piezo controller axis in question should then be reset. Select the “Stage” tab and navigate to the “Stage Piezo” control panel.

13.4.1. Select “reset” next to the axis in question.

13.4.2. The specimen should remain basically stationary after resetting of the piezo controller.

13.4.3. DO NOT stop the piezo controller as this will turn it off (resulting in less stable performance when it is needed).



13.5. After resetting, resume normal use of the piezo controller again (as needed).

14. Finishing the session

- 14.1. Verify the Ceta camera is retracted.
- 14.2. Verify the SA and objective apertures are retracted.
- 14.3. Place the instrument in SA mode (do not leave in LM, Mh, or diffraction mode).
- 14.4. Close the column valves.
- 14.5. Reset the stage and verify all coordinates are returned to ~0.
 - 14.5.1. Additionally, be sure to reset each axis of the piezo controller (described earlier).
 - 14.5.2. Again, DO NOT stop the piezo controller as this will turn it off.
- 14.6. Retract the holder and insert the metal plug into the airlock.
 - 14.6.1. The metal plug has no airlock triggering pin and thus may be inserted at any orientation.