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

This document assumes the user is familiar with basic operation of the Themis Z S/TEM in TEM mode.

This document also 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. STEM mode operation; particularly for *atomic-resolution* imaging
B. STEM imaging in Velox

1. Voltage, base alignments, and gun settings

1.1. The default voltage setting is 200 kV and will provide up to <80 pm STEM resolution. 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.

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

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

1.3.1. NOTE: the FEG register for STEM imaging is different than the FEG register for TEM imaging (just like when using the Talos).

2. Alignments before entering STEM mode

2.1. Turn off the C3 lens (described in TEM mode operation SOP).

2.2. Find a region of interest.

2.3. Perform monochromator centering (described in TEM mode operation SOP).

2.4. Set the region of interest at eucentric height.

2.5. With the C3 lens off, insert/center the 70 µm C2 aperture (described in TEM mode operation SOP).

2.5.1. If the C2 aperture is properly aligned in TEM mode (again, with the C3 lens off), it will be properly aligned in STEM mode.

2.6. Turn the C3 lens back on (described in TEM mode operation SOP).

2.7. If needed, enter diffraction mode and tilt the stage to crystallographically align the area of interest and then re-establish eucentric height.

2.7.1. At this stage, crystallographic alignment does not need to be very precise.

2.7.2. Precise adjustments to crystallographic alignment will be performed in STEM mode

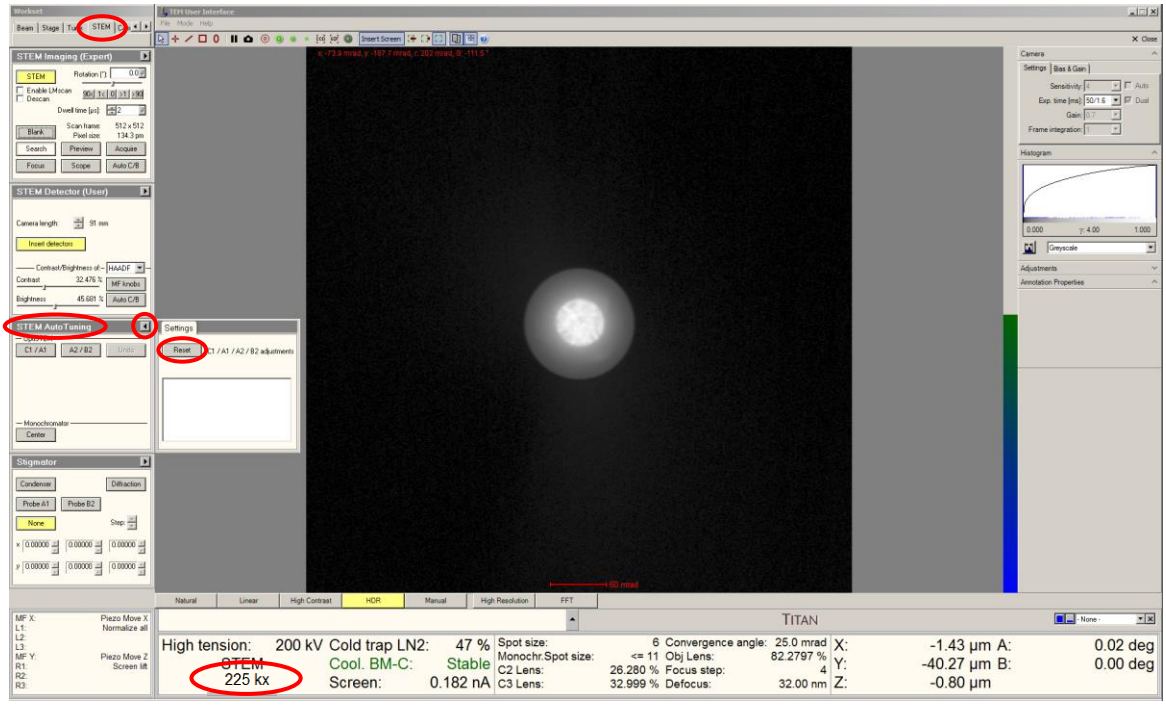
2.8. Verify the objective and SA apertures are both *retracted* (not shown; *identical to as described for the Talos*).

3. Entering STEM mode

3.1. Enter STEM mode and set the indicated magnification ≥ 225k× (not shown; *identical to as described for the Talos*).

3.2. Remain in the STEM tab and navigate to the “STEM AutoTuning” control panel.

3.2.1. Expand the flap-out arrow and select “Reset”.



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

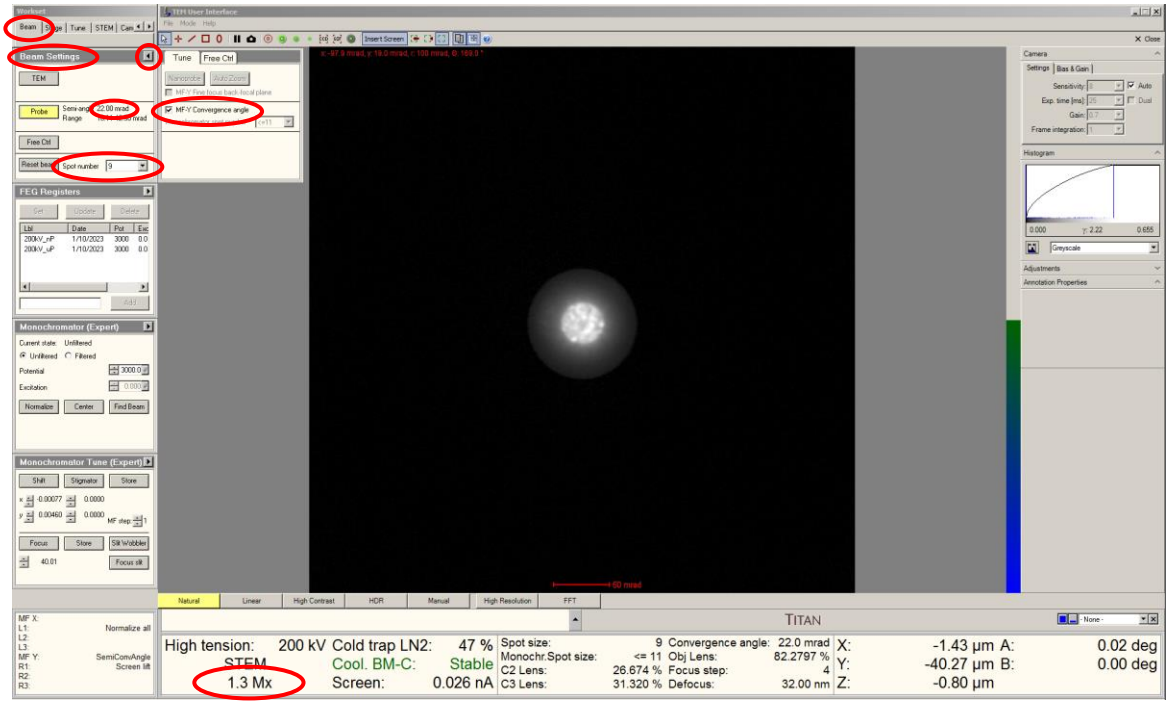
3.3.1. Set “Spot number” = 9.

3.3.2. Select the flap-out arrow and then the “Tune” tab.

3.3.3. Check “MF-Y Convergence angle” and use the “Multifunction Y” knob to set the convergence semi-angle as needed (22 mrad is recommended for the highest resolution).

3.3.4. Uncheck “MF-Y” Convergence angle” when finished.

3.3.5. Using the “200kV_nP” FEG register, this results in probe current ~25 pA, which is appropriate for the highest resolution STEM imaging.



3.4. Adjust the “Z axis” buttons (right-hand control pad) to “blow up” the Ronchigram and obtain a ballpark focus.

3.4.1. This will result in the sample deviating a few µm from eucentric height, but this is necessary to obtain proper STEM performance of this instrument.

4. Alignment

4.1. Set “Focus step” = 5.

4.2. Turn the “Focus” knob *clockwise* to observe an image of the specimen in the Ronchigram; if possible, move the stage so the beam is over vacuum; otherwise, move the stage to position the beam over an amorphous or partially amorphous area.

4.3. Press “Eucentric Focus”.

4.4. Select “Diffraction” on the right-hand control panel (this will result in an image of the probe on the FluCam).

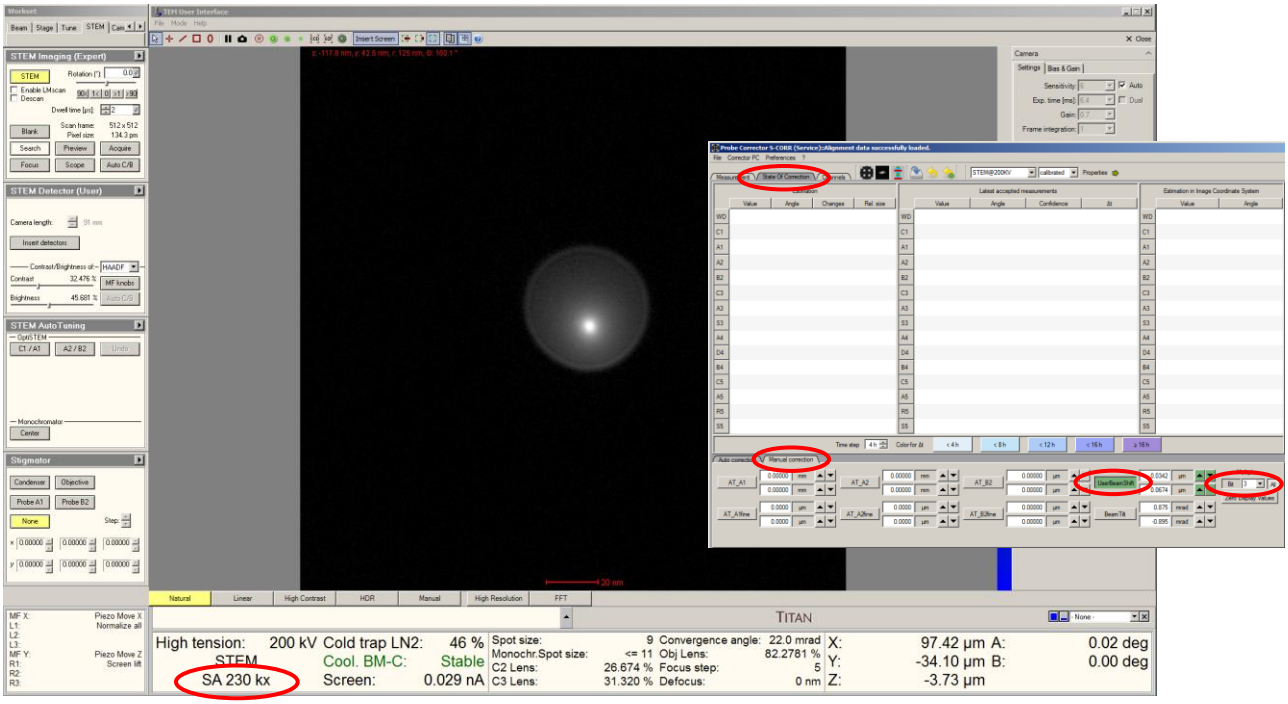
4.5. Set the indicated magnification = SA 230 k×

4.6. In SCORR, select the “State Of Correction” tab and then the “Manual Correction” tab.

4.6.1. Select “User Beam Shift” with Bit Multiplier = 3

4.6.2. Use the keyboard up/down left/right arrow keys to center the beam.

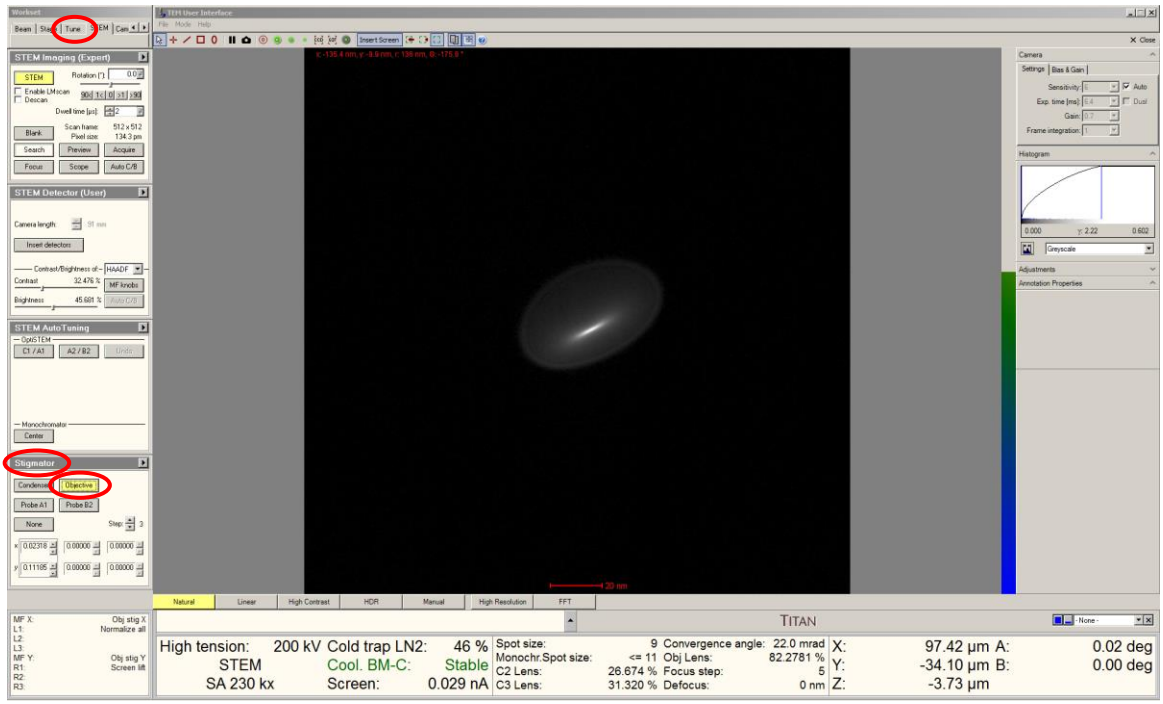
4.6.3. Center the beam as needed during alignment, but only using “User Beam Shift” inside SCORR.



4.7. In Microscope control, select the “STEM” tab, and navigate to the “Stigmator” control panel.

4.7.1. Select “Objective”.

4.7.2. Use the “Multifunction” knobs to correct 2-fold astigmatism of the image of the probe (make image circular).

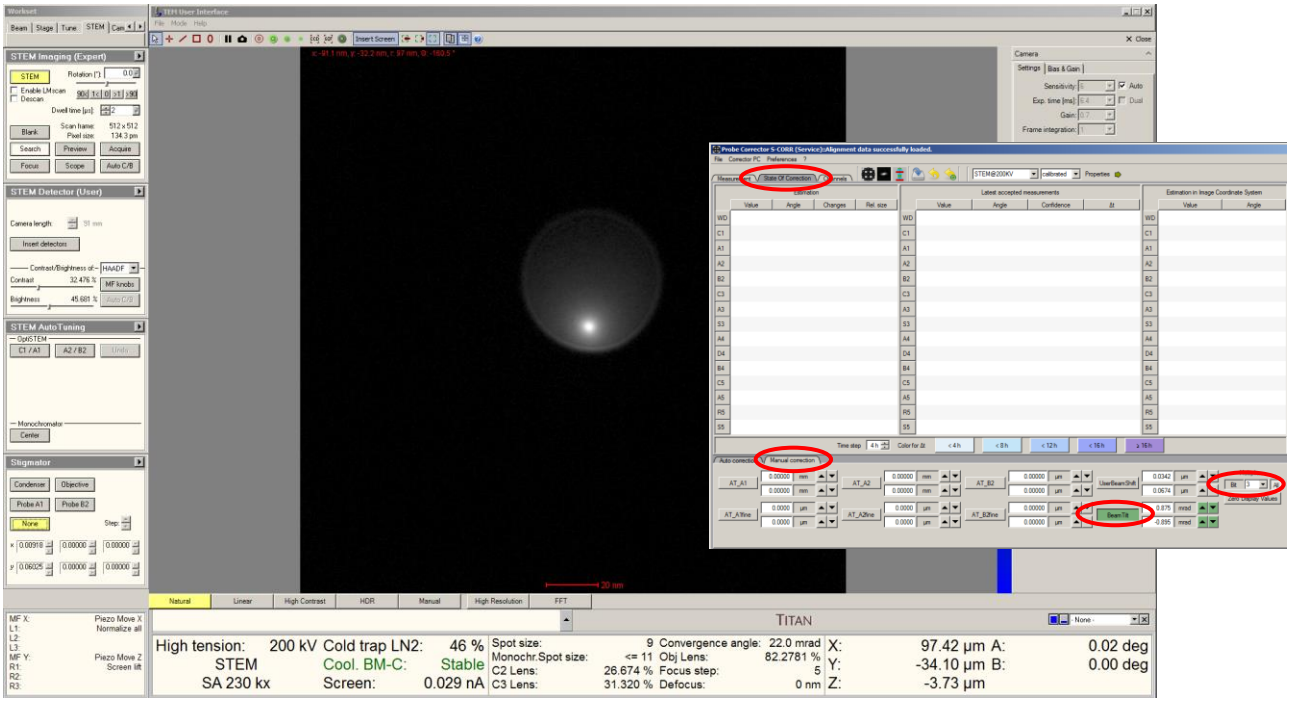


4.8. In SCORR, select the “State Of Correction” tab and then the “Manual Correction” tab.

4.8.1. Select “Beam Tilt” with Bit Multiplier = 3

4.8.2. Use the keyboard up/down left/right arrow keys to precisely center the caustic spot inside the diffuse area.

4.8.3. At “Eucentric Focus”, the caustic spot should remain stationary while the beam tilt is adjusted; if not, there may be a problem with the “Eucentric Focus” lens settings and staff should be notified.



4.9. Press “Eucentric Focus” again to set the proper settings for STEM.

4.10. Select “Diffraction” on the right-hand control panel to see the Ronchigram again.

4.11. Retract the HAADF detector and set the indicated “Camera length” as needed to facilitate easier viewing of the of Ronchigram (not shown; *identical to as described for the Talos*).

4.12. Return to Microscope Control, select the “Tune” tab, and navigate to the “Direct Alignments” control panel.

4.13. Use the “Diffraction Shift” direct alignment to *precisely* center the Ronchigram on the FluCam (not shown; *identical to as described for the Talos*).

4.13.1. This is important as this will serve as the reference position when the C2 aperture is subsequently changed.

4.14. Insert the 150 µm C2 aperture and center it on the FluCam (not shown; *identical to as described for the Talos*).

4.15. As needed, turn the “Focus” knob *clockwise* to defocus the Ronchigram to observe an image of the specimen and then move to an area (amorphous or partially amorphous) suitable for alignment.

4.15.1. After moving to a new area, press “Eucentric Focus”; then adjust the “Z axis” buttons to obtain a ballpark “blowup” condition.

4.15.2. After pressing “Eucentric Focus”, the Ronchigram may shift from the center of the FluCam; if so, recenter with “Diffraction Shift”.

4.16. In Microscope Control, select the “STEM” tab, and navigate to the “Stigmator” control panel.

4.17. Select “Probe B2”; the Ronchigram will start pulsing in and out of focus.

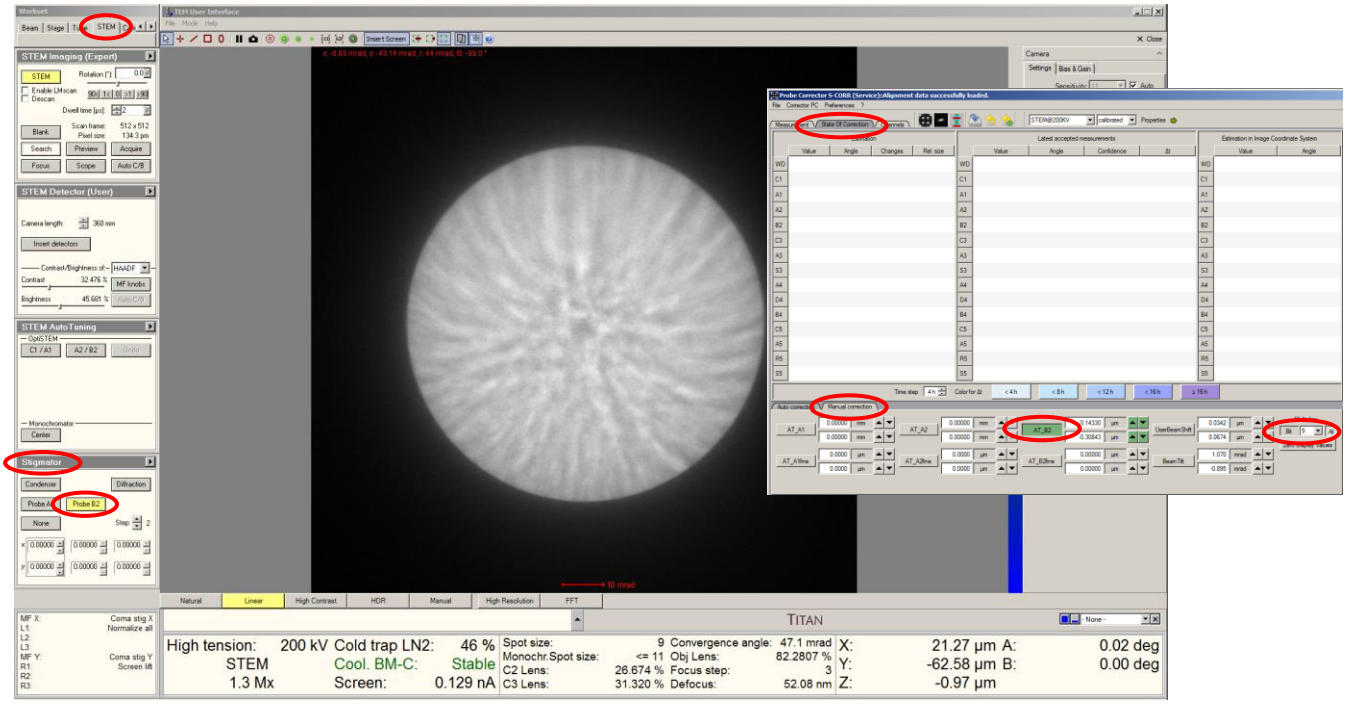
4.17.1. DO NOT adjust the “Multifunction” knobs.

4.18. Adjust the “Focus” ring *counterclockwise* to reduce the strength of the focusing oscillation as needed.

4.19. In SCORR, select the “State Of Correction” tab and then the “Manual Correction” tab.

4.19.1. Select “AT_B2” with Bit Multiplier = 9.

4.19.2. Adjust the keyboard up/down and left/right arrow keys to correct axial coma (B2).



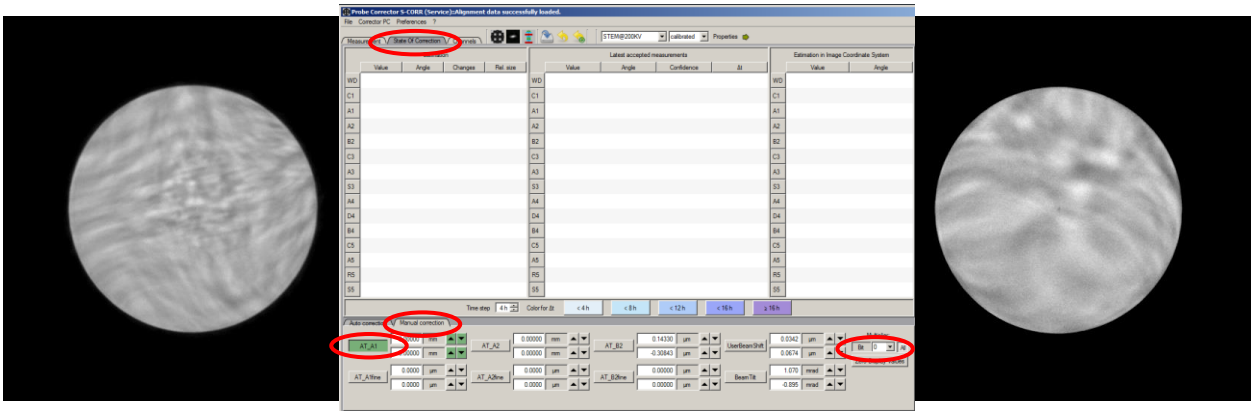
4.20. When finished correcting B2, return to Microscope Control, select the “STEM” tab, navigate to the “Stigmator” control panel, and select “None” (not shown; *identical to as described for the Talos*).

4.21. Remain In SCORR, select the “State Of Correction” tab and then the “Manual Correction” tab.

4.21.1. Select “AT_A1” with Bit Multiplier = 0.

4.21.2. Use the keyboard up/down left/right arrow keys to correct 2-fold astigmatism (A1); periodically adjust “Focus” as needed.

4.21.3. As A1 is corrected, this may also result in the Ronchigram continuing to “blow up”.



Correcting AT_A1: left = before correction and right = after correction.

4.22. Re-insert and precisely center the 70 µm C2 aperture on the FluCam.

4.23. Turn off diffraction mode and correct “Beam Tilt” using SCORR (again) as described previously.

4.23.1. Use “User Beam Shift” in SCORR to re-center the probe as needed.

4.23.2. If the probe image looks astigmatic again (likely), use the objective stigmators to correct this.

4.24. Turn on diffraction mode again, re-insert and re-center, the 150 µm C2 aperture and correct B2 and A1 using AT_B2 and AT_A1 in SCORR (again) as described previously.

4.25. Set the indicated “Camera length” = 115 mm for HAADF imaging (not shown; *identical to as described for the Talos*).

4.26. Press “Eucentric Focus”.

4.27. Use the “Diffraction Shift” direct alignment to center the Ronchigram inside the inner rim of the HAADF detector (not shown; *identical to as described for the Talos*).

5. HAADF imaging using Velox

5.1. Deviations from the “Eucentric Focus” setting should be kept as minimal as possible to ensure optimal performance; thus, ballpark focusing should be accomplished via the “Z axis” buttons while either observing the live STEM image or the Ronchigram (blow up condition) with a stationary beam.

5.1.1. If there is any concern about the deviations being excessive, simply press “Eucentric Focus”.

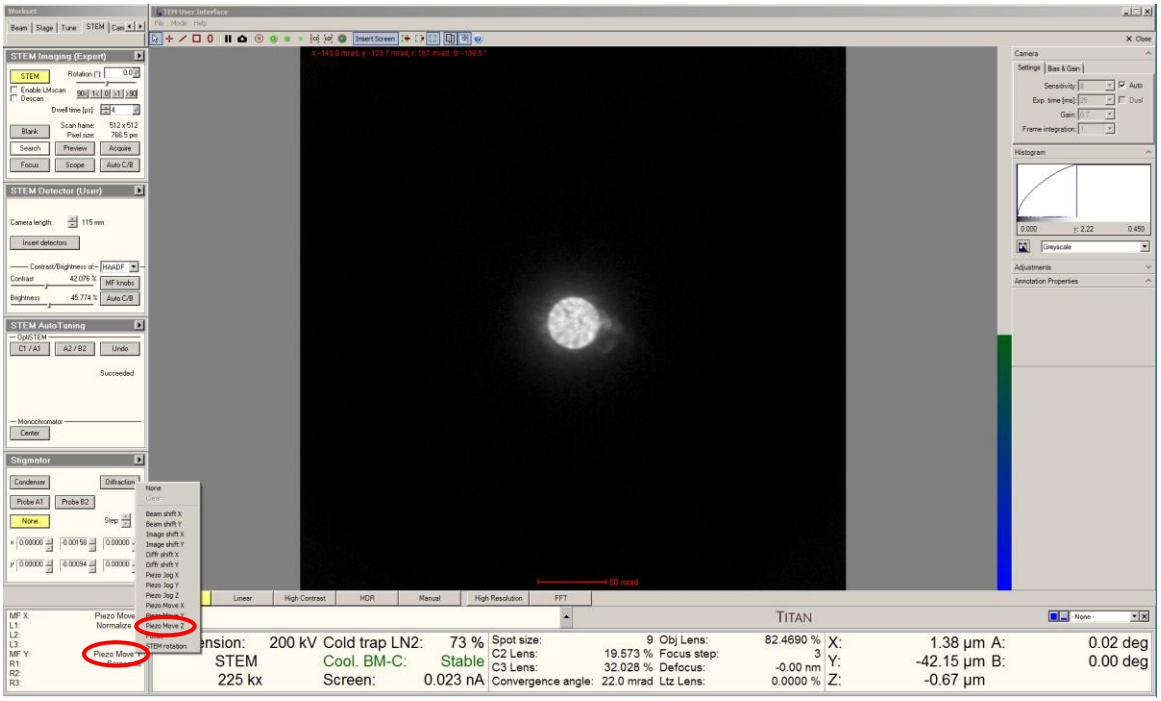
5.1.2. This may also result in a shift in the CBED pattern; simply recenter with “Diffraction alignment” as needed.

5.2. Use the piezo controller as needed to assist in accurate positioning at very high magnifications (as described in the TEM mode SOP).

5.3. After ballpark focus is obtained, focusing with atomic-level precision may be accomplished using the piezo controller. Navigate to the bottom left information panel, find the “MF Y” line, right click on the right side of the line, and select “Piezo Move Z”.

5.3.1. While live imaging, use the “Multifunction Y” knob to precisely focus the image; final focusing may be done with the “Focus” knob.

5.3.2. If the limit of an axis of the piezo controller is reached, be sure to reset it and then ballpark adjust using the joystick and/or Z axis buttons (as described in the TEM mode SOP).



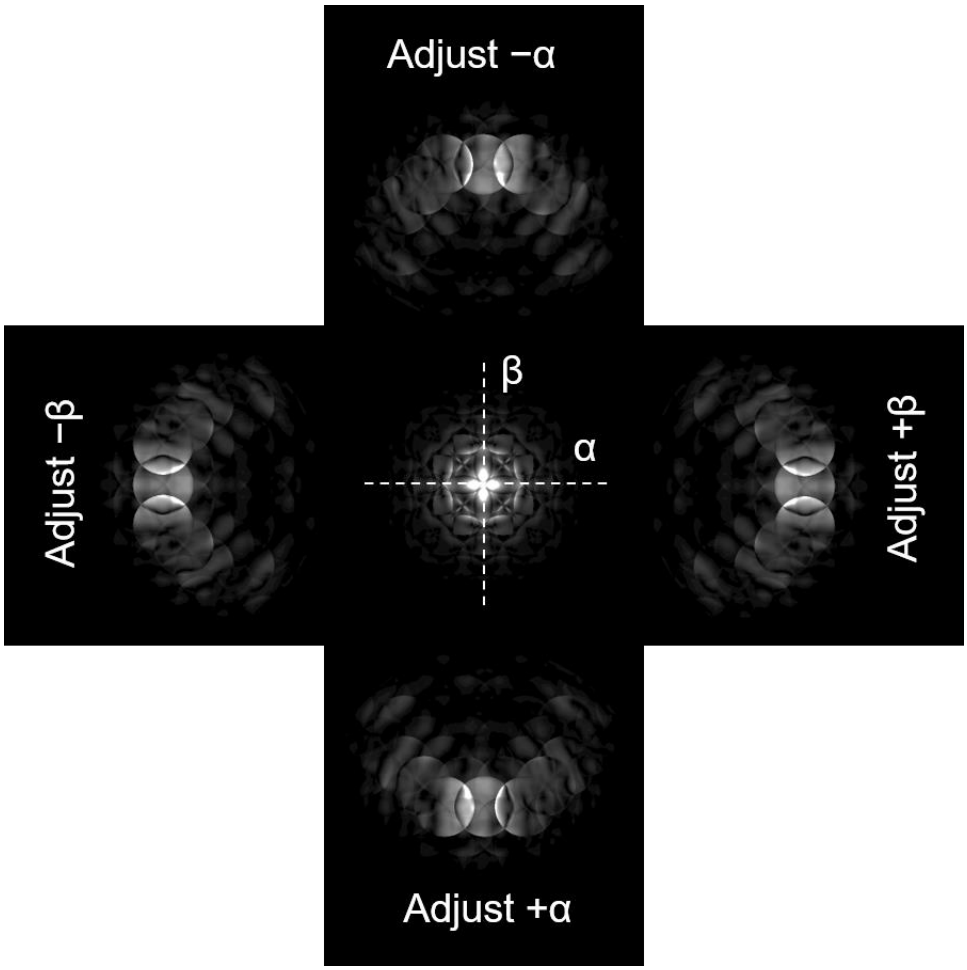
5.4. In Velox, start acquiring a live HAADF-STEM image and adjust the dwell time as needed (not shown; *identical to as described for the Talos*).

5.5. Adjust the detector “Gain” and “Offset” as needed to make optimal use of the detector dynamic range (not shown; *identical to as described for the Talos*).

5.6. Adjust the STEM image rotation as needed (not shown; *identical to as described for the Talos*).

5.7. If needed, tilt the specimen to align along a major crystallographic zone axis (not shown; *identical to as described for the Talos*; see zone axis tilt map below).

5.7.1. Remember to refocus the image after tilting by adjusting the “Z axis” buttons and/or “Piezo Move Z”.



6. Current adjustments (as needed)

6.1. The probe current can be quickly altered by adjusting the monochromator focus without changing any of the other optics thus eliminating the need for extensive instrument realignment.

6.2. In Velox, start acquiring a live HAADF-STEM image (not shown; *identical to as described for the Talos*).

6.3. Freeze the scan (but *do not* blank) the beam; position the beam over vacuum (if not possible; position over area that will *weakly* scatter).

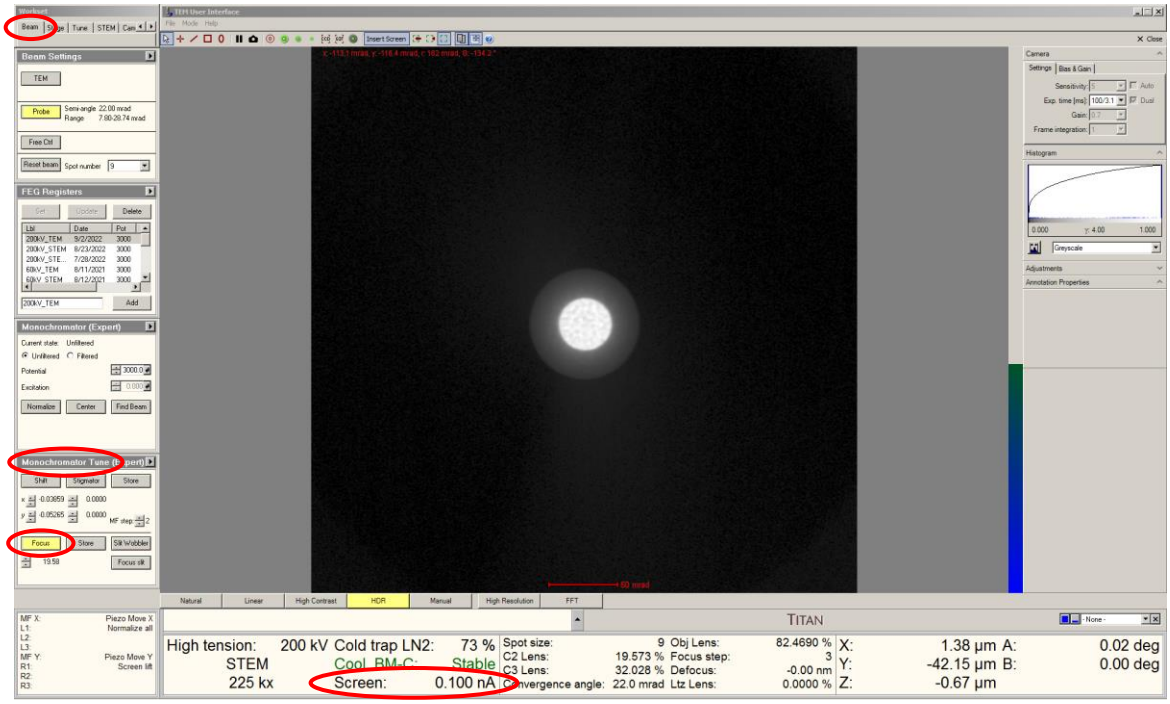
6.3.1. Note the measured screen current in the bottom Microscope Control info panel.

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

6.4.1. Select “Focus”.

6.4.2. Adjust the “Intensity” knob to set the desired probe current.

6.4.3. Deselect “Focus” when finished.



6.4.4. If the probe current is changed, the detector “Gain” and “Offset” will also need to be adjusted.

7. STEM AutoTuning (as needed)

    7.1. Specimen drift and contamination must be minimal for STEM AutoTuning to work properly.

    7.2. STEM AutoTuning tends to work best on single crystal samples aligned to a major crystallographic zone axis, but can also work on crystalline particulate samples, too.

    7.3. Set the indicated magnification such that at least 3 unique pairs of spots are present in the FFT (should be > 1M×).

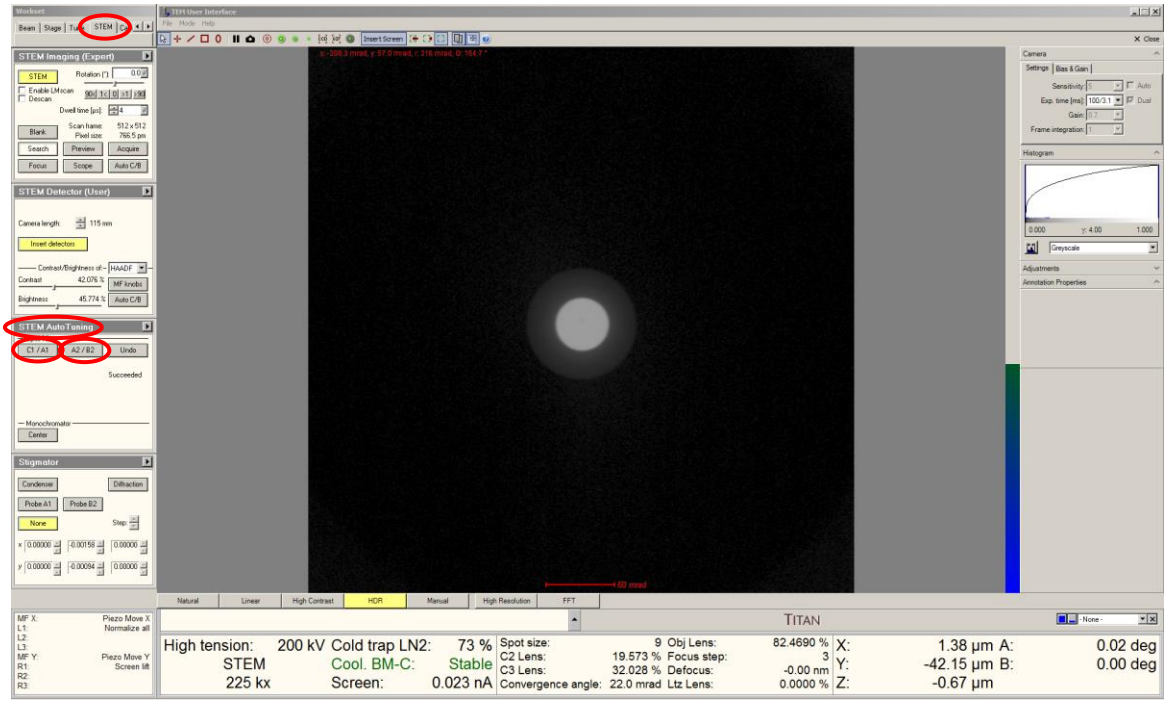
    7.4. Focus the image and adjust detector gain/offset to make optimal use of the detector dynamic range (not shown; *identical to as described for the Talos*).

    7.5. Freeze the scan (but *do not* blank).

7.6. Select the “STEM” tab and navigate to the “STEM AutoTuning” control panel.

7.6.1. Select “C1/A1” to correct focus (C1) and 2-fold astigmatism (A1); this should take < 30 s.

7.6.2. Select “A2/B2” to correct C1, A1, 3-fold astigmatism (A2), and axial coma (B2); this may take up to 5 min.



7.7. C1 and A1 typically exhibit stability over a few minutes (or until the sample is moved) and thus C1/A1 correction may be performed as needed.

7.7.1. Remember, the focus should be first optimized by adjusting Z to ensure any deviations from the default focus value are minimal.

7.8. A2 and B2 typically exhibit stability over a few hours. Should A2/B2 correction succeed, this should not need correcting again during the remainder of the session.

7.8.1. If A2/B2 correction fails, the system will revert to the initial settings and must be used as is.

7.8.2. If B2 was properly corrected earlier, <90 pm resolution should still be possible even without successful A2/B2 STEM AutoTuning.

8. ABF imaging (if needed)

8.1. Full alignment using a HAADF image should be performed as described previously *before* attempting to set up an ABF image.

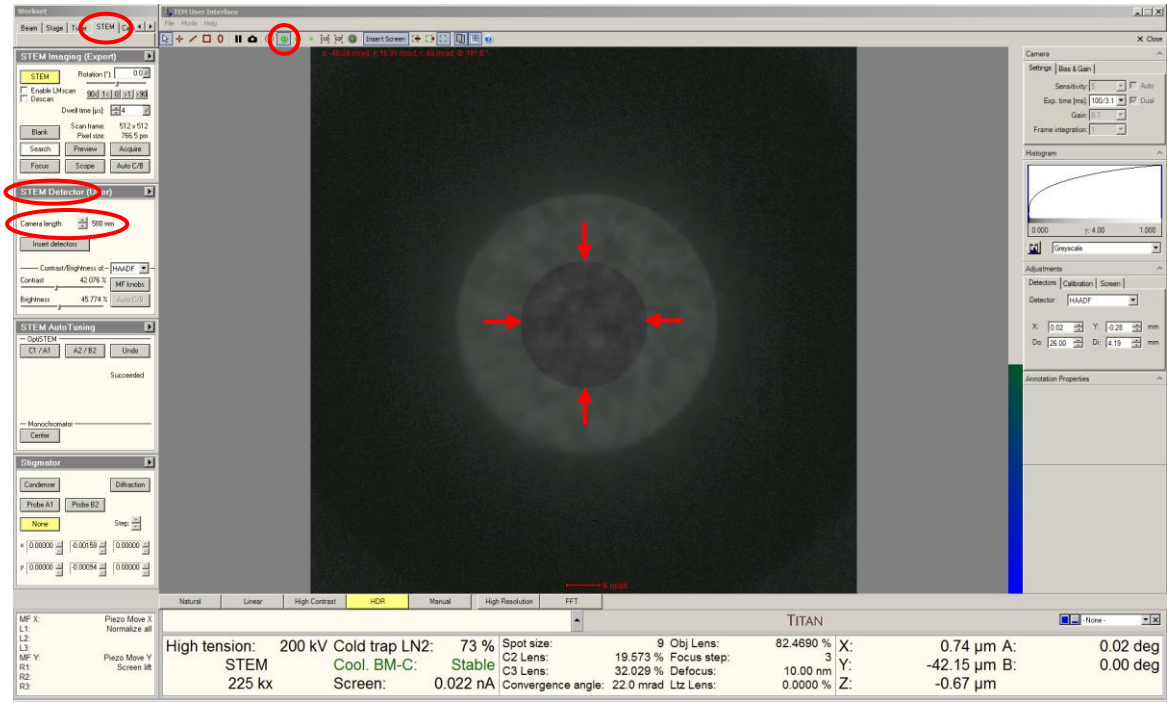
8.2. Zone axis alignment, if needed, needs to be performed using a HAADF imaging condition *before* attempting to set up an ABF image.

8.3. Freeze the live STEM image and position the beam over an open area or an area that should weakly scatter the beam; do not blank the beam (not shown; *identical to as described for the Talos*).

8.4. Set the indicated camera length = 580 mm and retract the HAADF detector (not shown; *identical to as described for the Talos*).

8.5. Activate the HAADF detector indicator markings from the FluCam toolbar.

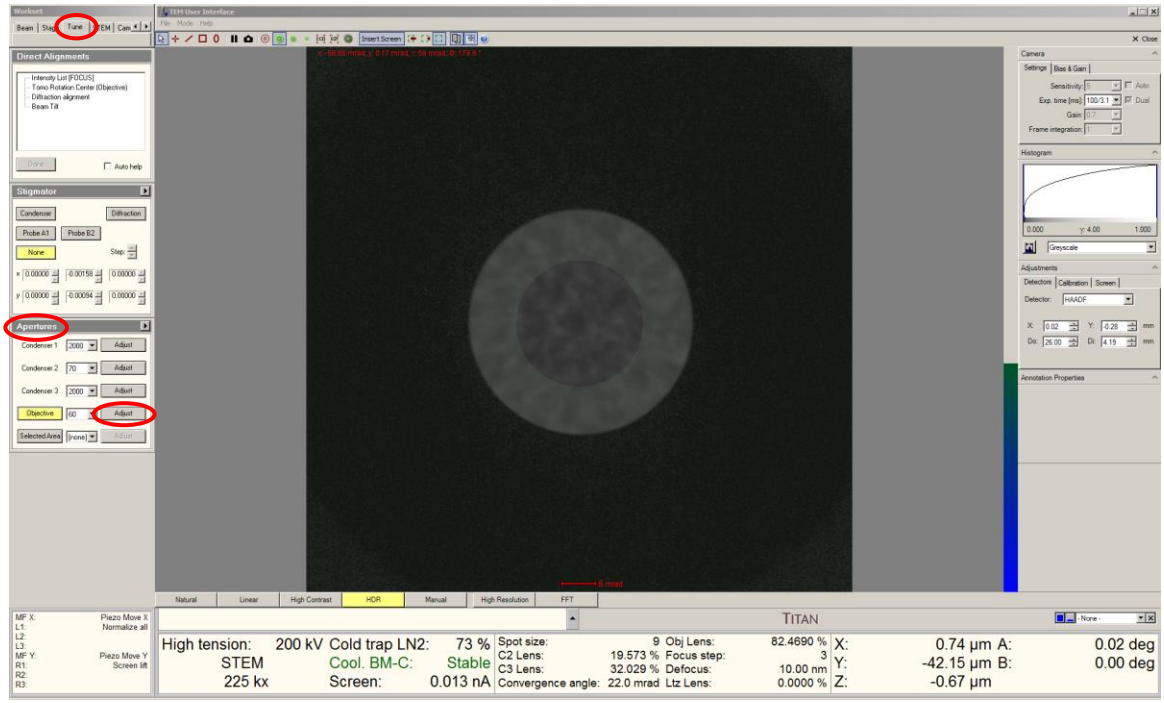
8.6. Use the “Diffraction Alignment” direct alignment to *precisely* center the direct disk relative to the position of the inner HAADF detector rim (indicated by arrows).



8.7. Select and insert the 60 µm *objective* aperture and *precisely* center it on the FluCam (only a slightly cutoff direct disk should be left).

8.7.1. This assumes the semi-angle of convergence = 22 mrad; depending of this value, a smaller or larger objective aperture may be necessary.

8.7.2. This step is critical; if the objective aperture is not *precisely* centered on the direct disk, ABF imaging will not work properly.



8.8. Re-insert the HAADF detector and resume STEM imaging in Velox as per usual.

8.9. Due to increased signal on the HAADF detector when ABF imaging, readjust the detector “Gain” and “Offset” accordingly.

8.10. Finely focus the image, adjust the condenser stigmators, and acquire STEM images as per usual.

8.10.1. While ABF imaging, C1/A1 STEM AutoTuning may not work properly and A2/B2 STEM AutoTuning should be avoided.

9. Finishing in STEM mode

9.1. In Velox, verify the beam is not being blanked and the scan rotation is returned to 0° (if needed) and then return to TEM mode (not shown; *identical to as described for the Talos*).

9.2. If ABF imaging was performed, retract the objective aperture (as described elsewhere).

9.3. If TEM imaging is to be performed, the full TEM alignment procedure should be performed.

9.4. If no TEM imaging is to be performed, simply finish the session as per usual (close column valves, reset stage, reset piezo controller, remove holder, etc).