FEI Talos F200i S/TEM: imaging in STEM mode
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ANALYSIS OF RADIOACTIVE SPECIMENS IS STRICTLY PROHIBITED

This document assumes the user is already familiar with basic operation of the instrument in TEM mode and use of Microscope Control.

1. Specimen cleanliness when performing STEM imaging is critical (particularly for atomic resolution); thus, it is best that specimens be plasma cleaned prior to analysis (even a few seconds of plasma cleaning is better than nothing).

2. Instrument settings

    2.1. The instrument may be operated in STEM mode at 200 or 80 kV (however, atomic-resolution imaging is only possible at 200 kV on this instrument); configure the instrument accordingly (select high-tension and apply alignments) before starting the session (described elsewhere).

    2.2. The “200kV_nP” (“80kV_nP”) FEG registers should be used for STEM operation at 200 (80) kV.

        2.2.1. NOTE: these are different than the FEG registers for TEM operation at the same voltages and configured to produce the smallest possible STEM probes.

3. Prior to entering STEM mode

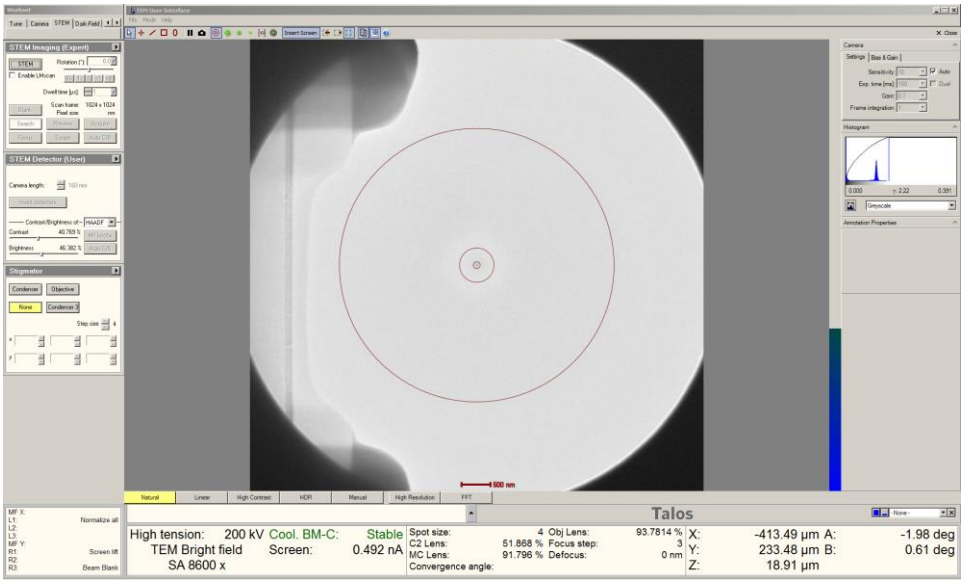
3.1. Find a region of interest and bring it to eucentric height.

3.2. If needed, crystallographically align the area of interest (using diffraction mode) and re-establish eucentric height; finer adjustments to crystallographic alignment may need to be performed once in STEM mode (particularly if attempting atomic-resolution STEM imaging) but try to get this as close as possible in TEM mode.

3.2.1. Remember to re-establish eucentric height if the specimen was tilted.

3.3. Verify the objective and SA apertures are both <u>retracted</u>.

3.4. If possible, move the specimen a few µm to the <u>left of center</u> of the FluCam; this will result in the beam being in vacuum for STEM alignment; the specimen will be moved back under the beam later.

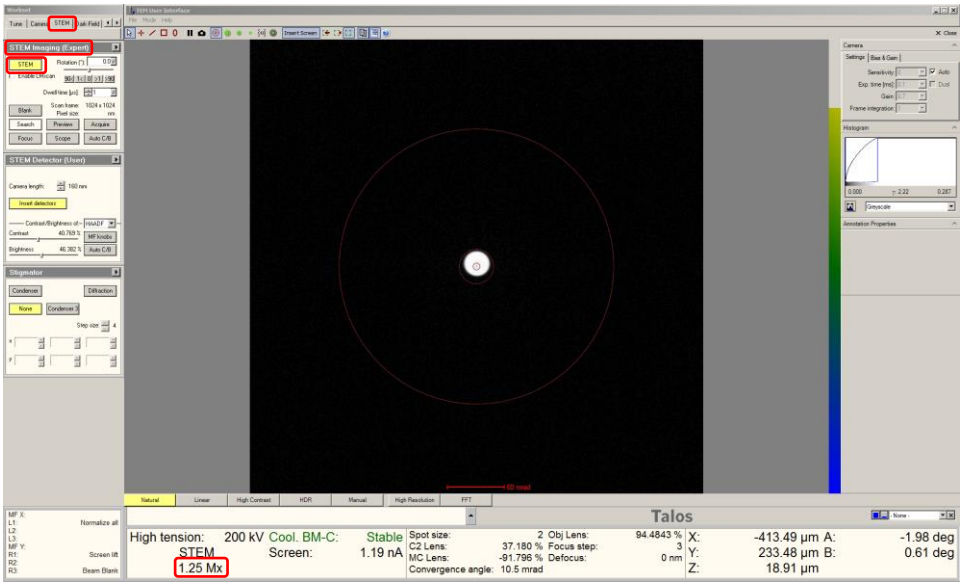


4. Entering STEM mode

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

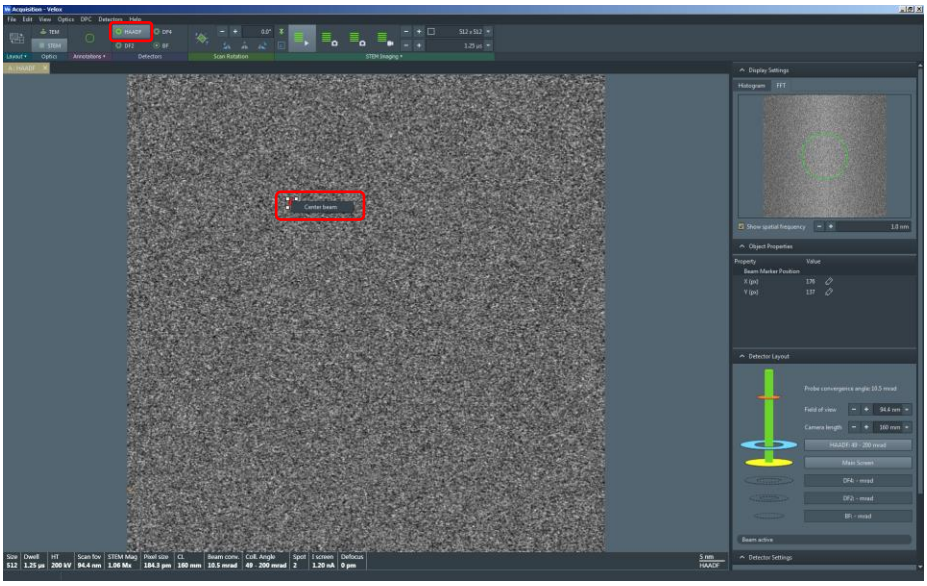
4.1.1. Select “STEM” to enter STEM mode; a stationary CBED pattern (direct disc only) should be visible on the FluCam.

4.1.2. Set the indicated STEM magnification = 1.25 M×.

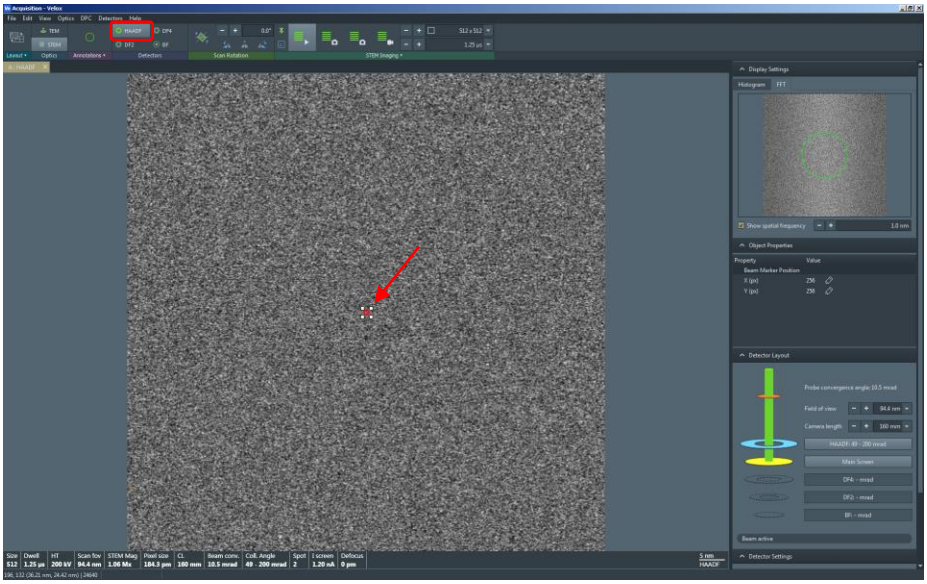


4.2. In Velox Acquisition, select “HAADF” from the toolbar to start live STEM imaging.

4.2.1. Right click on the beam position marker and select “Center beam”.



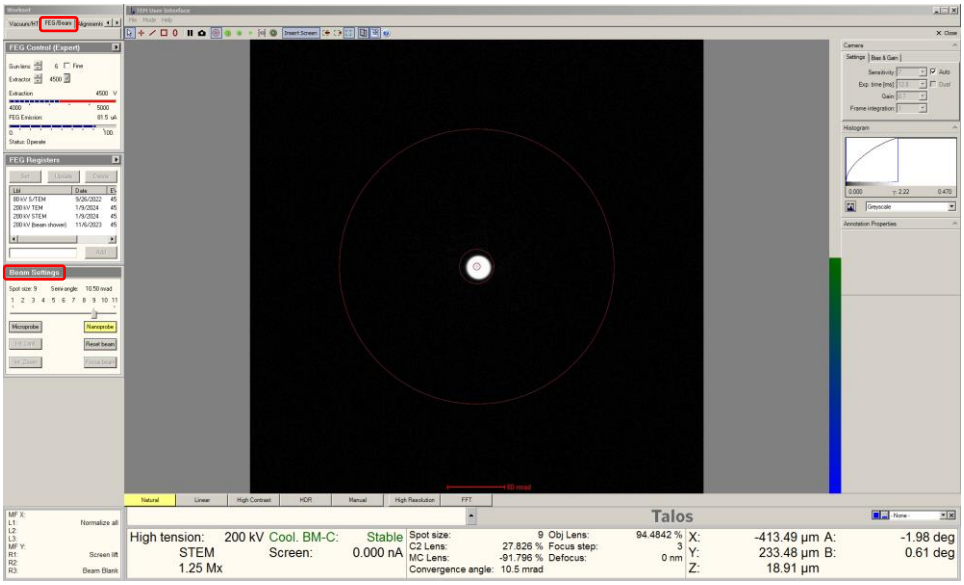
4.3. After verifying the beam position marker is centered in the STEM image, select “HAADF” from the toolbar again to stop the live STEM image and retract the HAADF detector.



4.4. Select the spot size to be used for STEM imaging (not shown; identical to TEM mode operation).

4.4.1. Spot size = 9 is recommended for atomic-resolution imaging; with the 70 µm C2 aperture, this will produce ~15 pA of probe current.

4.4.2. Spot size = 2, 3, or 4 is recommended for performing EDS; with the 70 µm C2 aperture, this will generate ~1.03, 0.67, or 0.47 nA of probe current, respectively.



5. C2 aperture alignment

5.1. Press the “Diffraction” button on the right-hand control panel; this will result in an image of the probe appearing on the FluCam; the microscope will now be in “SA” mode.

5.1.1. Reduce the magnification until the probe is visible.

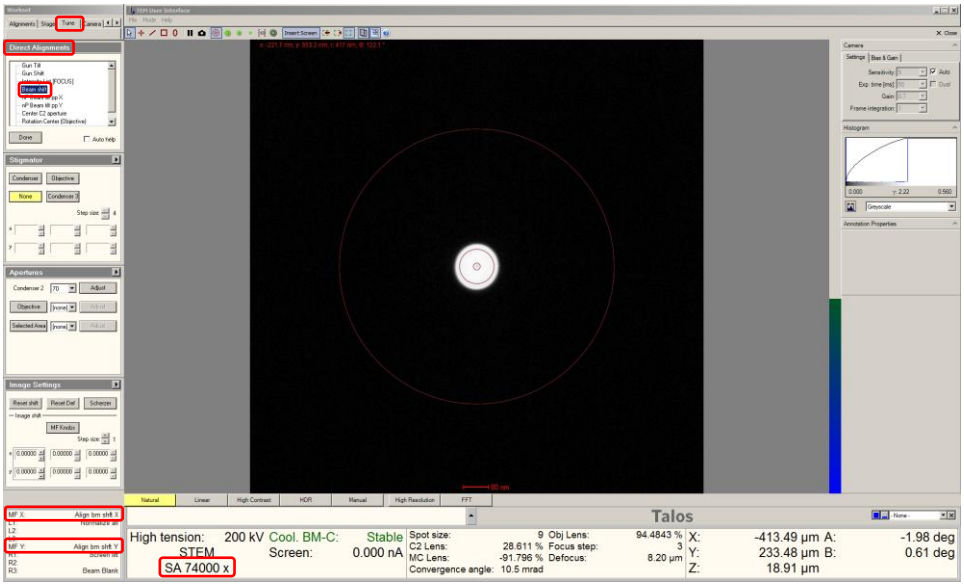
5.2. Select the “Tune” tab and navigate to the “Direct Alignments” control panel.

5.2.1. Select “Beam shift” and use the “Multifunction” knobs to shift the probe to the center of the FluCam.

5.2.2. Set the indicated magnification = 74k×.

5.2.3. Use the “Intensity” knob to expand the probe clockwise from crossover until it is slightly larger than the 4 mm circle marking.

5.2.4. Precisely center the (expanded) probe on the FluCam using the “Multifunction” knobs.

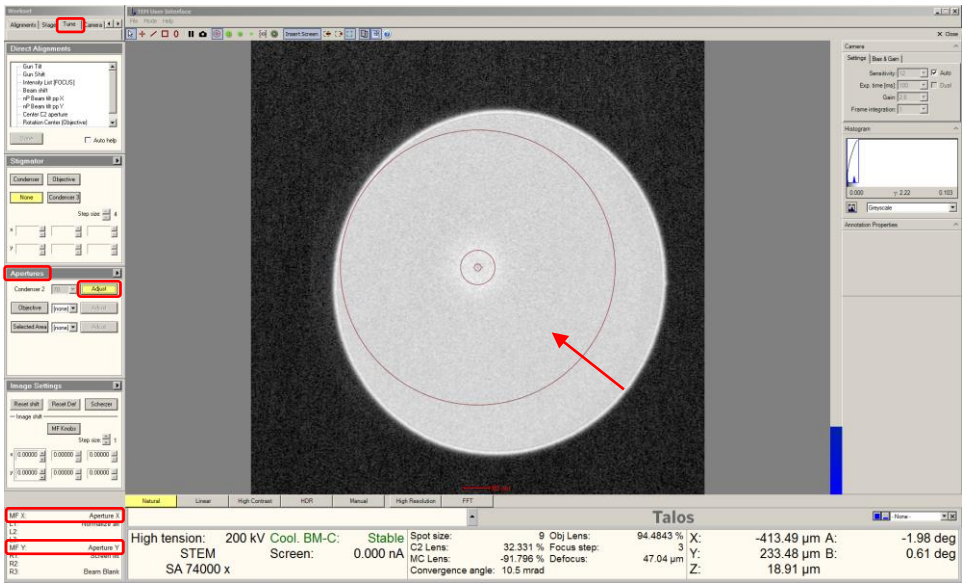


5.3. Remain in the “Tune” tab and navigate to the “Apertures” control panel.

5.3.1. Verify the 70 µm “Condenser 2” aperture is inserted, then activate “Adjust”.

5.3.2. Turn the “Intensity” knob clockwise to expand the probe so it is slightly larger than the 40 mm circle marking.

5.3.3. Use the “Multifunction” knobs to recenter the (expanded) probe on the FluCam via shifting of the C2 aperture.



5.4. Repeat step 5.2 and then 5.3 iteratively until the probe expands while remaining centered on the FluCam (3 iterations is usually sufficient).

5.4.1. When finished, navigate to the “Apertures” control panel; next to “Condenser 2” deactivate “Adjust”.

6. Setting the C2 lens

6.1. Remain in the “Tune” tab and navigate to the “Direct Alignments” control panel.

6.1.1. Select “Intensity List [FOCUS]” to couple the “Focus” knob to the C2 lens.

6.1.2. Use the “Focus” knob to coarsely focus the probe.

6.2. Set the indicated magnification = 630k×

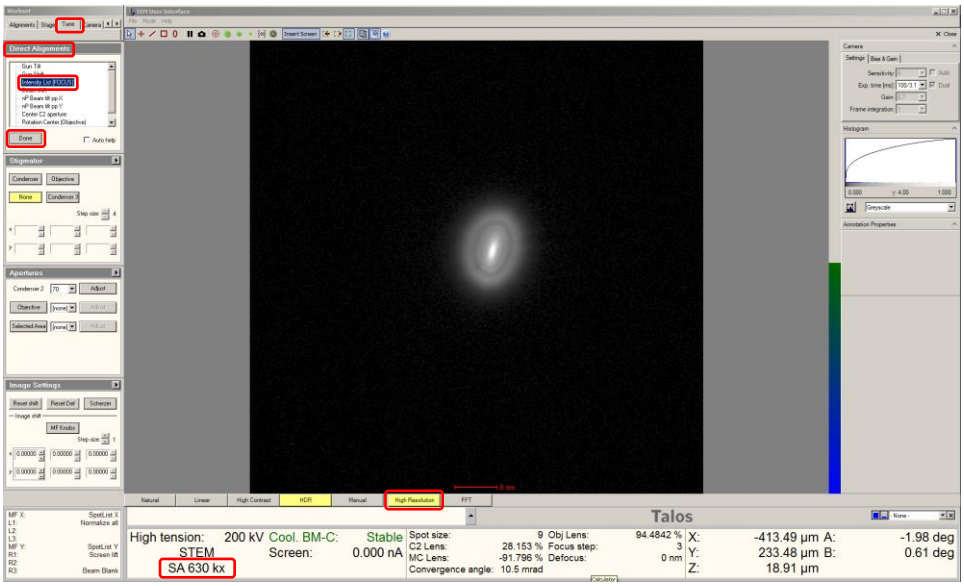
6.2.1. If needed, select “Beam Shift” and use the “Multifunction” knobs to approximately center the probe on the FluCam (it need not be precise).

6.2.2. Select “Done” when finished to store this as the probe position.

6.2.3. Select “Intensity List [FOCUS]” to couple the “Focus” knob to the C2 lens again.

6.2.4. Use the “Focus” knob to finely focus the probe; when done properly, this should appear as a small bright (caustic) spot surrounded by a diffuse halo.

6.2.5. Select “Done” when finished to store this setting for the C2 lens.



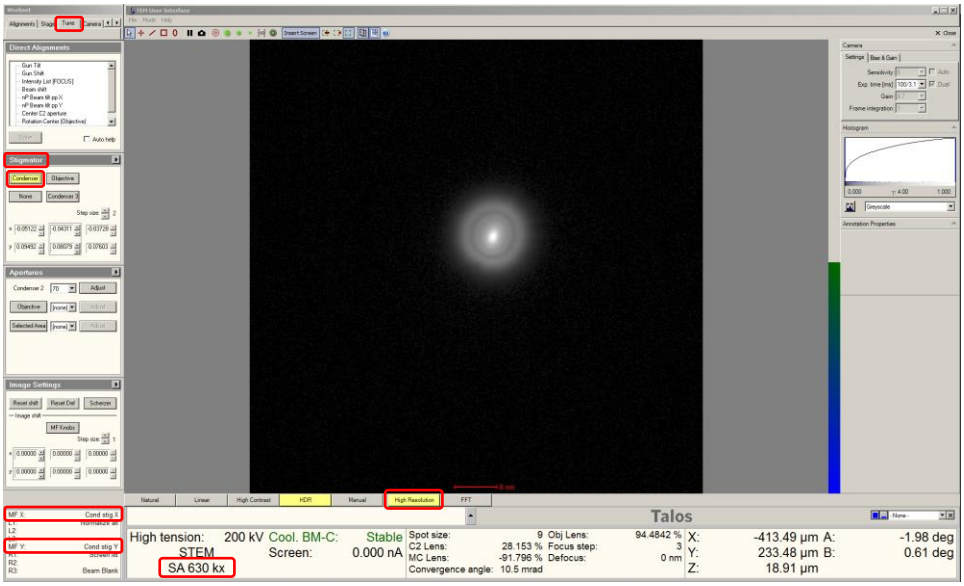
6.3. IMPORTANT: after storing the setting for the C2 lens, <u>do not perform any further adjustments</u> to the probe using the “Intensity” knob.

7. Coarse probe astigmatism correction

7.1. Remain in the “Tune” tab and navigate to the “Stigmator” control panel.

7.1.1. Select “Condenser” and use the “Multifunction” knobs to make the probe as circular as possible.

7.1.2. NOTE: finer probe astigmatism correction will be performed during STEM imaging.



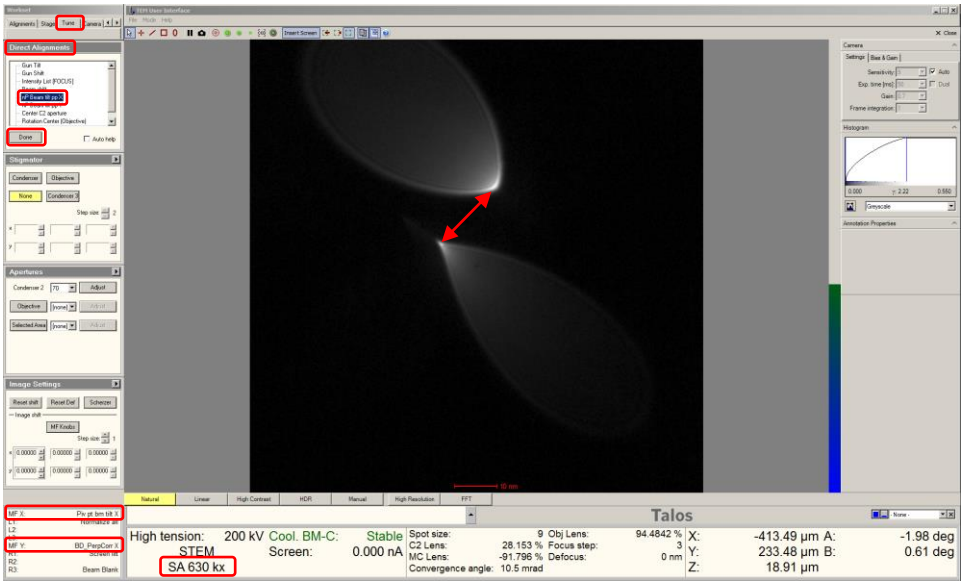
8. Beam tilt pivot points

8.1. Remain in the “Tune” tab and navigate to the ““Direct Alignments” control panel.

8.1.1. Select “nP Beam tilt ppx”; the probe will separate out into two “wings”.

8.1.2. Use the “Multifunction” knobs to align the “wings” so the bright tips touch together and create what looks like a figure 8.

8.1.3. Select “Done” when finished.



8.1.4. In the “Direct Alignments” control panel, select “nP Beam tilt ppy” and repeat.

9. Rotation centering

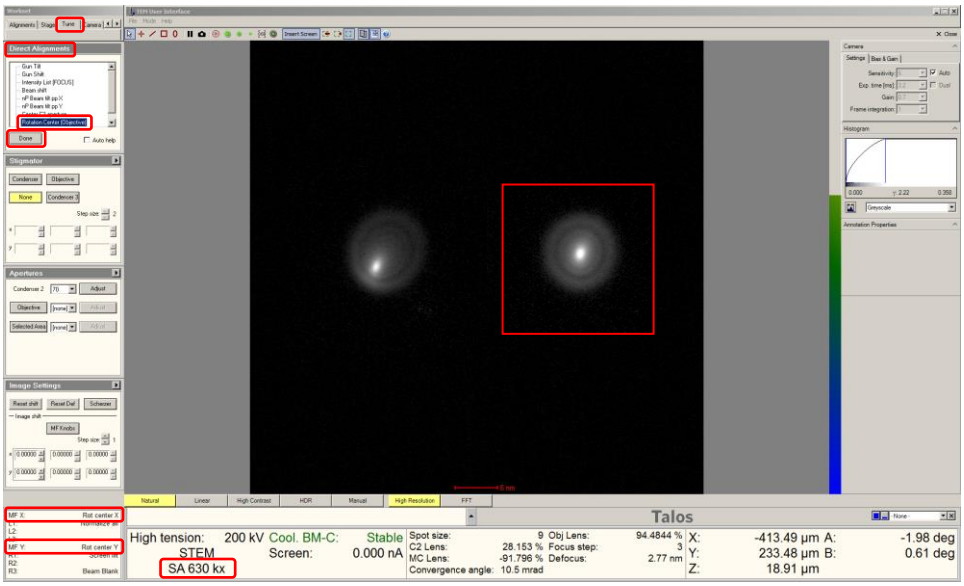
9.1. Remain in the “Tune” tab and navigate to the “Direct Alignments” control panel.

9.1.1. Select “Rotation Center (Objective)”, the probe will start expanding and contracting.

9.1.2. Turn the outer “Focus” knob counterclockwise until the expansion and contraction stops.

9.1.3. Use the “Multifunction” knobs to precisely center the caustic spot inside the diffuse halo.

9.1.4. Select “Done” when finished.



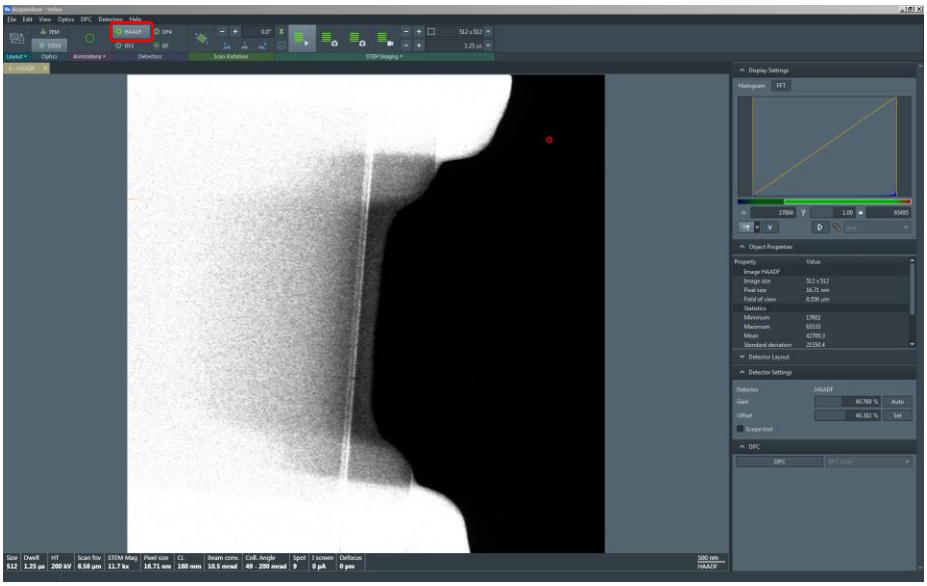
9.2. If the probe appears noticeably astigmatic after correcting the rotation center, coarsely correct it once more using the condenser stigmators as described previously (not shown).

9.3. Select “Diffraction” on the right-hand control panel to switch back to showing a stationary CBED pattern (direct disc only) on the FluCam (not shown).

10. Setting camera length; diffraction alignment

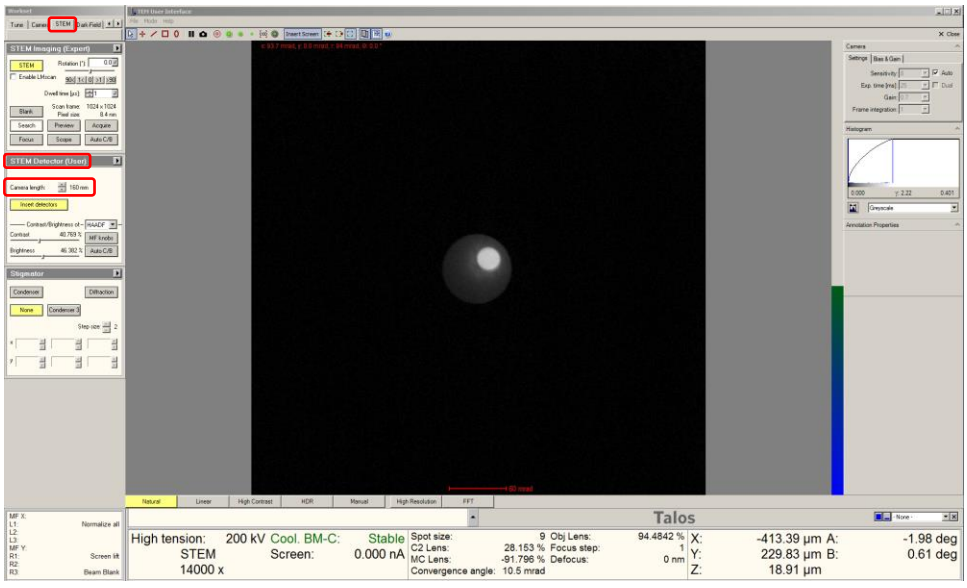
10.1. In the Velox Acquisition, select “HAADF” from the toolbar to start live STEM imaging (512×512 resolution); decrease the indicated magnification until the specimen is in the field of view.

10.2. Use the Joystick to move the specimen into the middle of the STEM image.



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

10.3.1. If not already set accordingly, set “Camera length” = 160 mm for HAADF-STEM imaging.

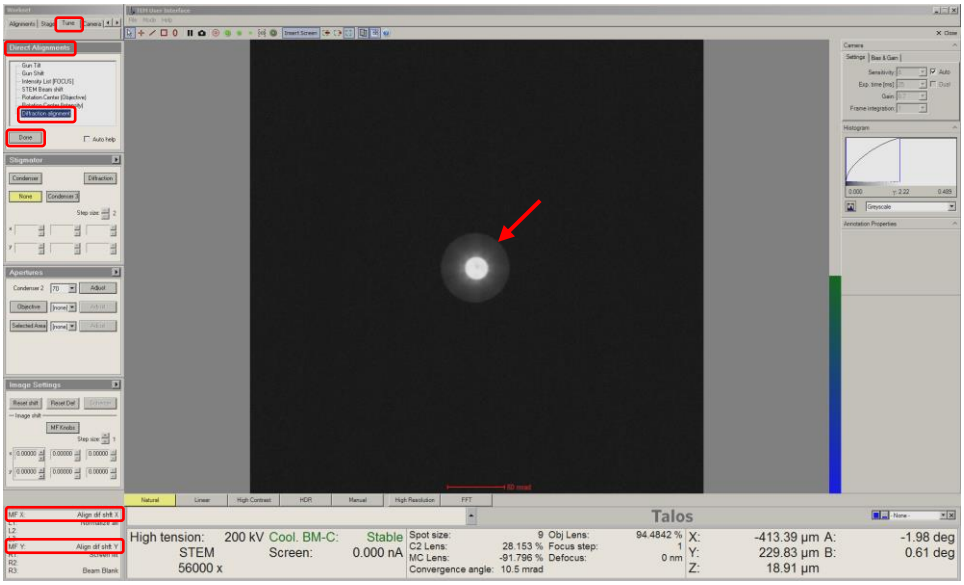


10.4. Select the “Tune” tab and navigate to the “Direct Alignments” control panel.

10.4.1. Select “Diffraction alignment”

10.4.2. Use the “Multifunction” knobs to center the direct disc of the CBED pattern inside the inner rim of the HAADF detector (indicated by arrow).

10.4.3. Select “Done” when finished.



11. STEM imaging in Velox

11.1. Return to Velox Acquisition and adjust the dwell time on the toolbar for live imaging as needed.

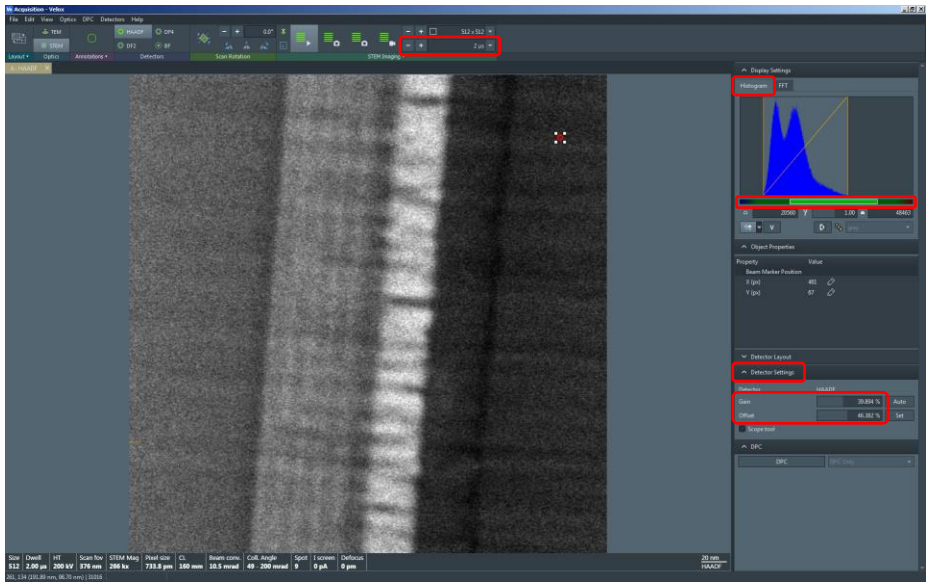
11.1.1. Shorter dwell times (1 – 2 µs) tend to work better for navigating around the specimen.

11.1.2. Longer dwell times (≥10 µs) tend to work better for focusing, fine astigmatism correction, and detector gain/offset adjustments.

11.2. To properly set the detector gain and offset; navigate to the “Display Settings” side panel and select “Histogram” to see the image histogram.

11.2.1. Navigate to the “Detector Settings” side panel and adjust “Gain” and “Offset” until the range marker under the image histogram takes up approximately the middle of the dynamic range.

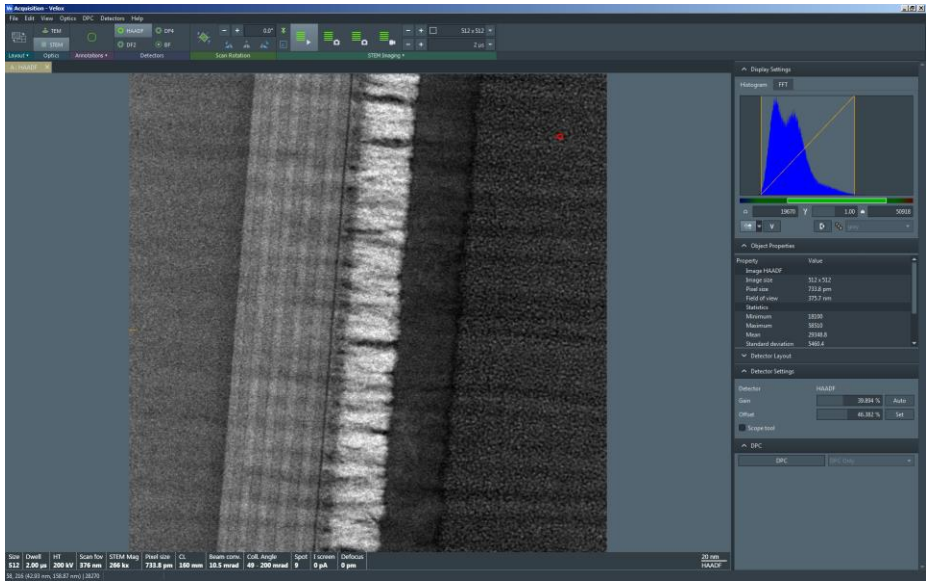
11.2.2. NOTE: optimal gain and offset settings will depend on focal state and what is in the field of view; the gain and offset must therefore be adjusted as needed.



11.3. Use the “Z axis” buttons on the right-hand control panel to focus the STEM image as best as possible.

11.3.1. Additional focusing of the STEM image may be performed with the “Focus” knob.

11.3.2. Once again, <u>do not perform any further adjustments</u> to the “Intensity” knob.

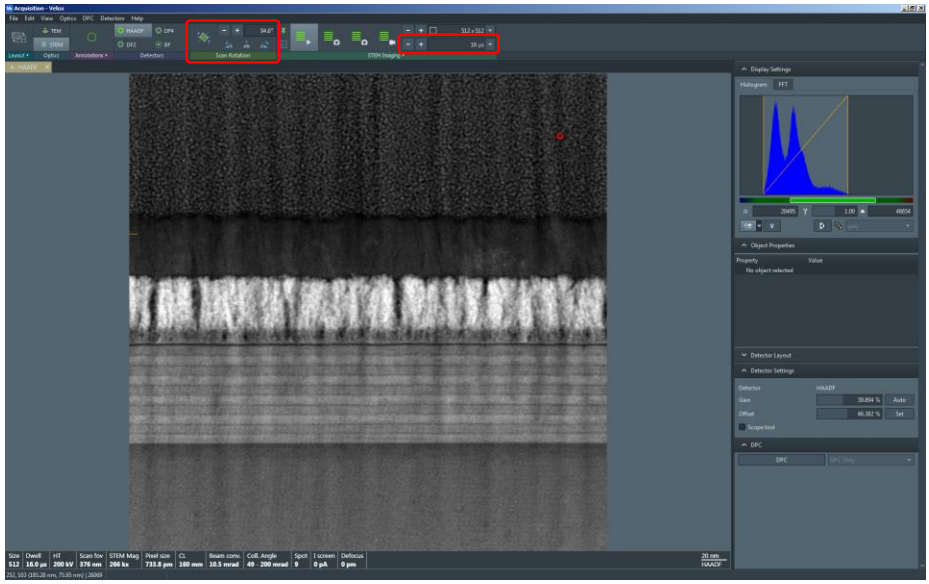


11.4. From the toolbar, adjust “Scan Rotation” to rotate the STEM image as needed.

11.4.1. Note the following to ensure the scan rotation in the final acquired STEM image will be the same as observed in the live STEM image.

11.4.2. Specimen drift should be minimal.

11.4.3. Set the dwell time ≥ 10 µs in the live image.



11.5. To fine tune the focus and correct any remnant astigmatism, use the joystick to center a region of the specimen with very fine features (e.g. protective Pt grains) in the STEM image.

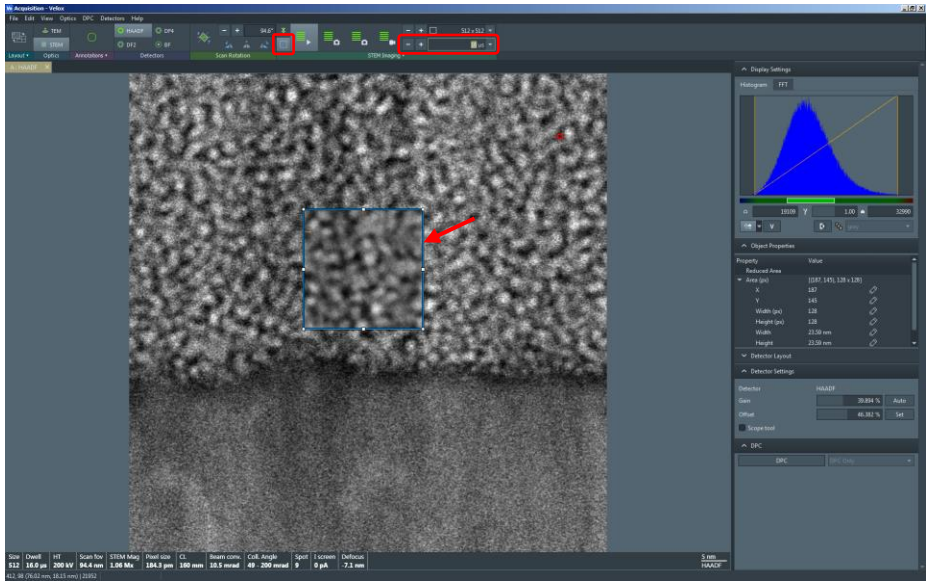
11.5.1. Use the “Magnification” knob to set the indicated magnification in Microscope Control to ~1M×.

11.5.2. If the image is noticeably out of focus after moving the stage, first refocus using the “Z axis” buttons.

11.5.3. Set the dwell time ≥10 µs.

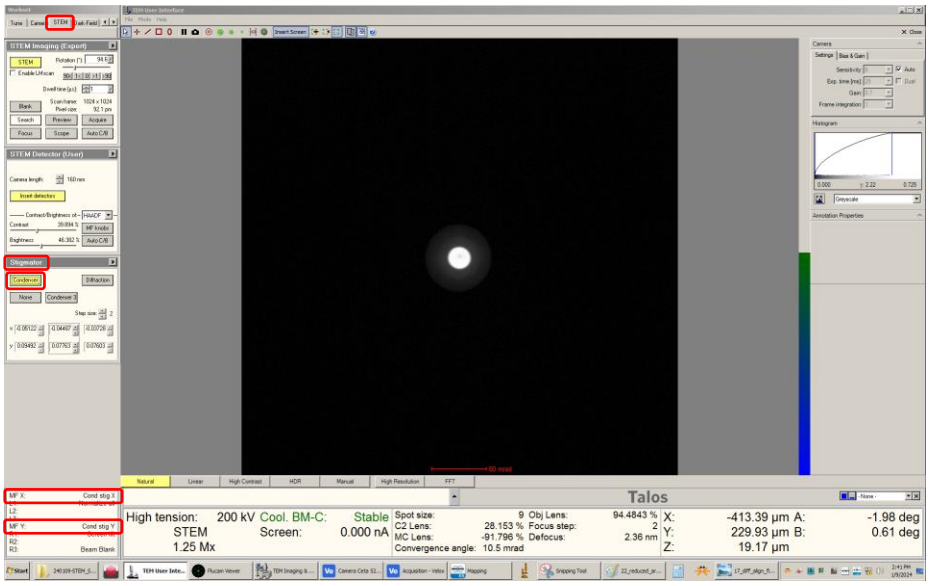
11.5.4. Select “Reduced Area” from the toolbar to open a reduced area scan window (focusing box) in the live STEM image (indicated by arrow); adjust/position the box as needed.

11.5.5. Use the “Focus” knob to (finely) focus the image as best as possible and note the presence of any streaking (astigmatism).



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

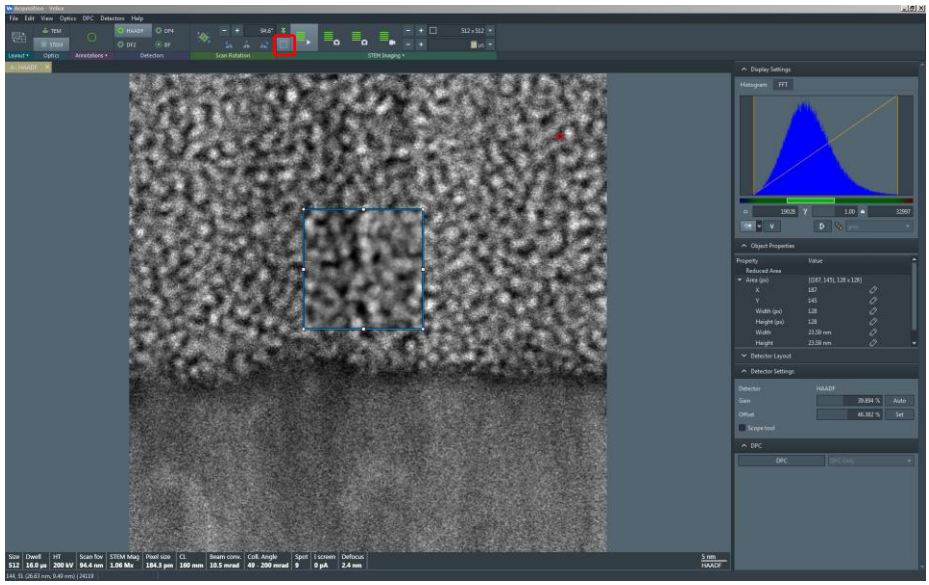
11.5.7. Select “Condenser”.



11.5.8. Use the “Multifunction” knobs to make the STEM image as sharp as possible.

11.5.9. Use the “Focus” knob to refocus the image after the additional astigmatism correction.

11.5.10. Navigate back to Velox Acquisition and select “Reduced Area” from the toolbar to resume full frame imaging as needed.



11.6. When ready to acquire the final image, first use the joystick to center the region of interest in the live image.

11.6.1. Use the “Magnification” knob to set the indicated magnification higher than will be used for final image acquisition (not shown).

11.6.2. If the live image is noticeably out of focus, refocus using the “Z axis” buttons (not shown).

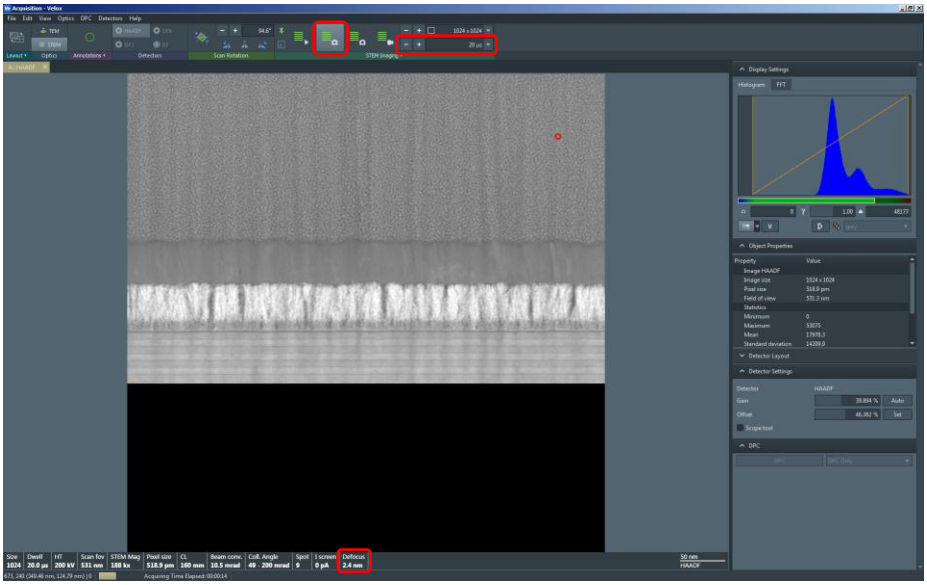
11.6.3. Use the “Focus” knob (along with the “Reduced Area” box, if needed) to finely focus the image (not shown).

11.6.4. NOTE: if the alignment was done properly and image properly focused (first by adjusting the “Z axis” buttons and then by finely adjusting the “Focus” knob), “Defocus” should be within ±100 nm.

11.6.5. Use the “Magnification” knob to set indicated magnification as needed for the final image (not shown).

11.6.6. In Velox Acquisition, select “Acquire” from the toolbar to acquire the final STEM image (1024×1024 resolution); the first (second) option has dwell time = 20 (50) µs.

11.6.7. DO NOT adjust the default “Acquire” settings without consulting with RSC staff first.

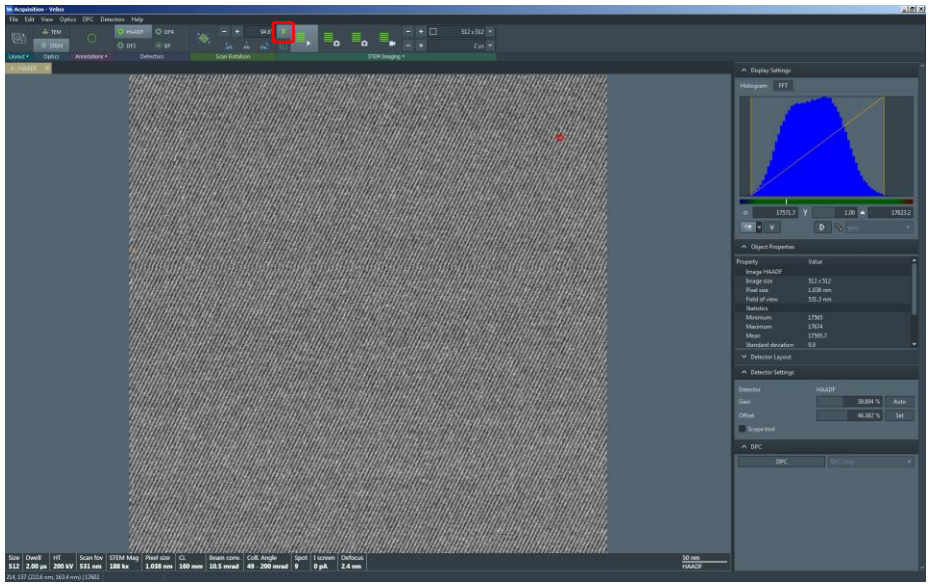


12. Blanking the beam (as needed)

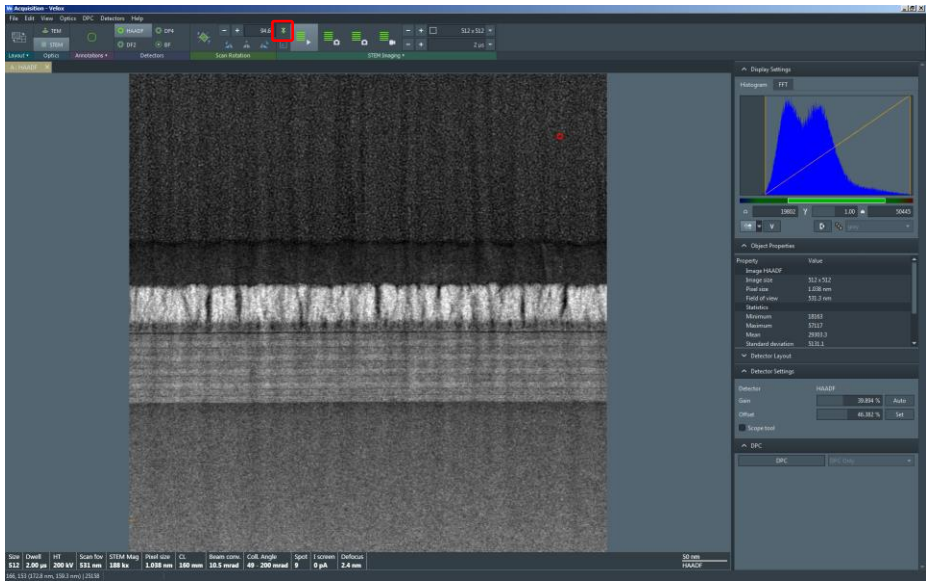
12.1. If needing to leave the instrument for only a few minutes, it is best practice to blank the beam to minimize specimen irradiation.

12.1.1. If needing to leave the instrument for more than a few minutes, it is best practice to close the column valves (not shown).

12.2. In Velox Acquisition, select “Beam Blank” from the toolbar to blank the beam (the live image will continue, but will just be noise).



12.3. When ready to un-blank the beam, simply select “Beam Blank” from the toolbar and the live image will reappear in Velox.



13. Additional considerations for atomic-resolution STEM imaging

13.1. The highest quality atomic-resolution STEM images are obtained when the sample is as closely aligned along a major crystallographic zone axis as possible. Generally, performing this alignment is only feasible in single-crystal samples or very large grains in polycrystalline samples. To check the crystallographic alignment, observe the CBED pattern on the FluCam. To complicate matters, this alignment must be achieved at the desired ROI (e.g. at a film/substrate interface).

13.1.1. If the ROI was crystallographically aligned previously in TEM mode, then only a small amount of tilting (few mrad) in STEM mode may be necessary.

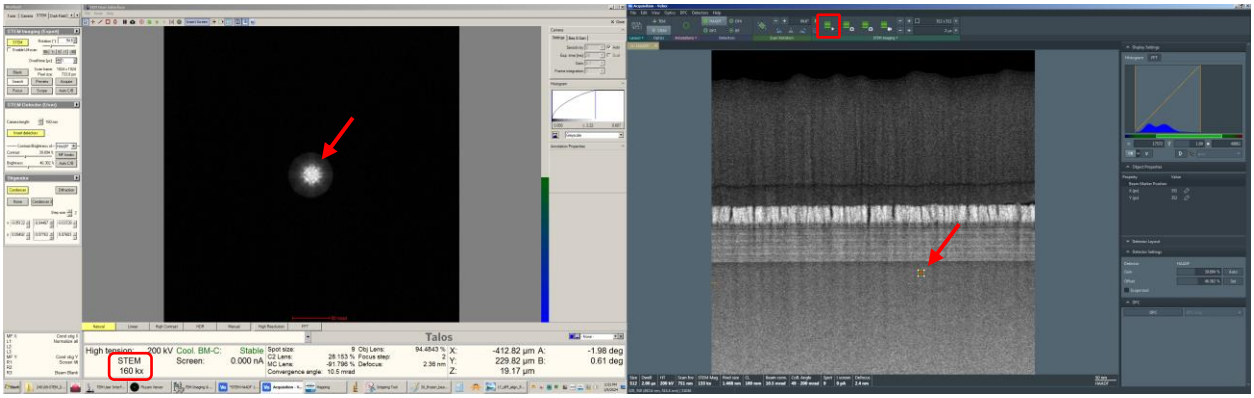
13.2. Prior to performing any tilting, verify the STEM image is focused; if not, refocus using the “Z axis” buttons.

13.3. While acquiring a live STEM image in Velox Acquisition, select “View” from the toolbar to freeze the live image.

13.3.1. Click and drag on the beam position marker on the frozen STEM image (automatically inserted) and position it at the desired ROI.

13.3.2. The CBED pattern observed on the FluCam will correspond to the beam position marker location.

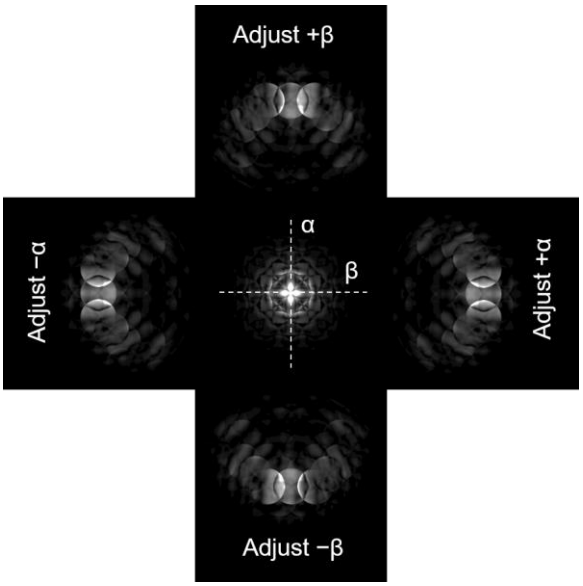
13.3.3. The indicated STEM magnification need not be very high to perform crystallographic alignment; 50 k× – 200 k× will be sufficient for doing this.



13.4. Use the following iterative process to align the sample along the zone axis:

13.4.1. Move the beam position marker to the ROI and observe the CBED pattern on the FluCam.

13.4.2. Make an appropriate change in either the α or β tilt based on the following zone axis tilt map and the observed CBED pattern.



STEM mode zone axis tilt map when camera length L ≥ 98 mm

13.4.3. Return to Velox and select “View” to resume the live STEM image; use the joystick to re-center the ROI in the STEM image.

13.4.4. <u>If necessary, refocus using the “Z axis” buttons</u> and then freeze the live STEM image again.

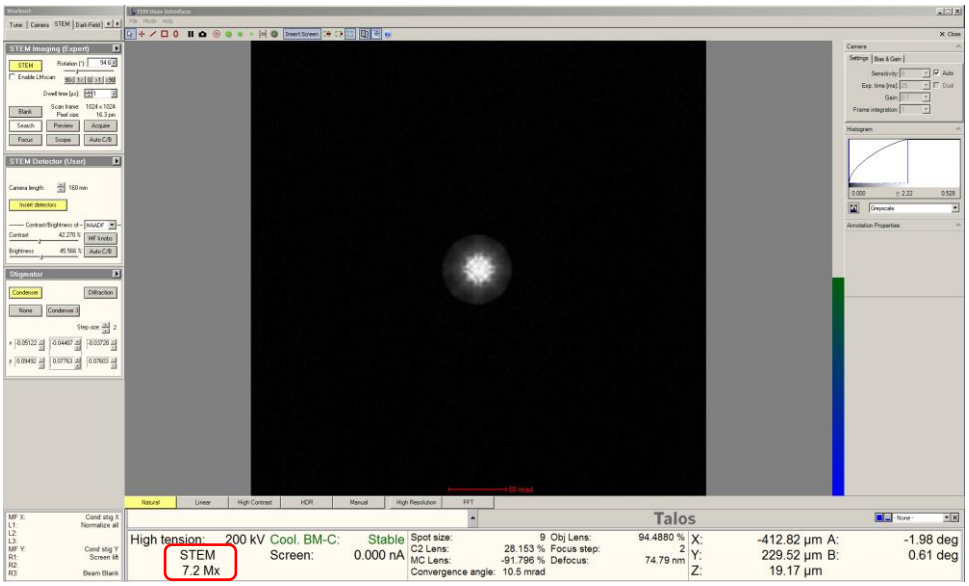
13.4.5. Move the beam position marker back over to the ROI and observe the CBED pattern on the FluCam again (it should be closer to being aligned to the zone axis than before).

13.4.6. Repeat until alignment with the zone axis for the ROI is achieved as indicated by the CBED pattern (this may take many iterations). With skill and patience, zone axis alignment with <1 mrad precision will be possible.

13.4.7. Once zone axis alignment is complete, resume live STEM imaging; again, <u>refocus using the “Z axis” buttons</u>.

13.5. While live imaging, center the region of interest in the image.

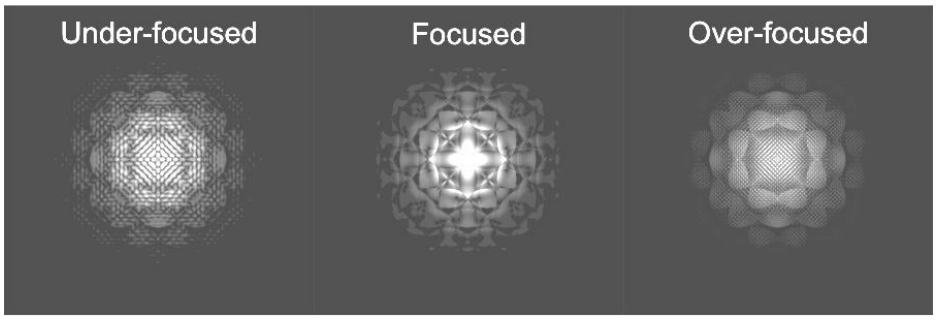
13.5.1. Set indicated STEM magnification in Microscope Control to 7.2 M× (appropriate for fine tuning of an atomic-resolution image).



13.6. Freeze the live image and observe the CBED pattern on the FluCam (not shown).

13.6.1. Adjust the “Focus” knob (focus step = 2) to obtain the “blow up” condition.

13.6.2. When the probe is properly focused, the CBED pattern will not exhibit any features resembling lattice fringes (see below).

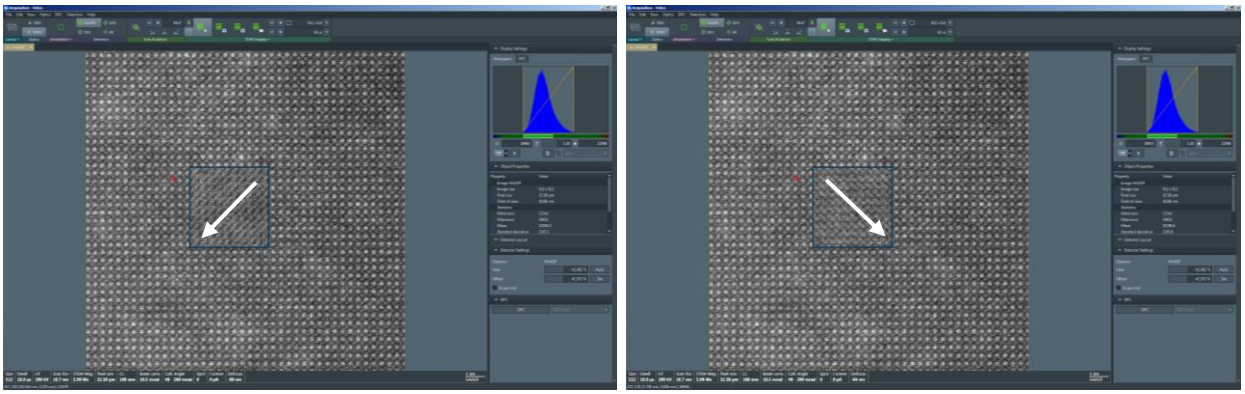


13.7. Restart scanning to resume the live STEM image; atomic-level detail should be evident in the image.

13.8. In Microscope Control, activate the condenser stigmators; set the sensitivity to the finest possible setting.

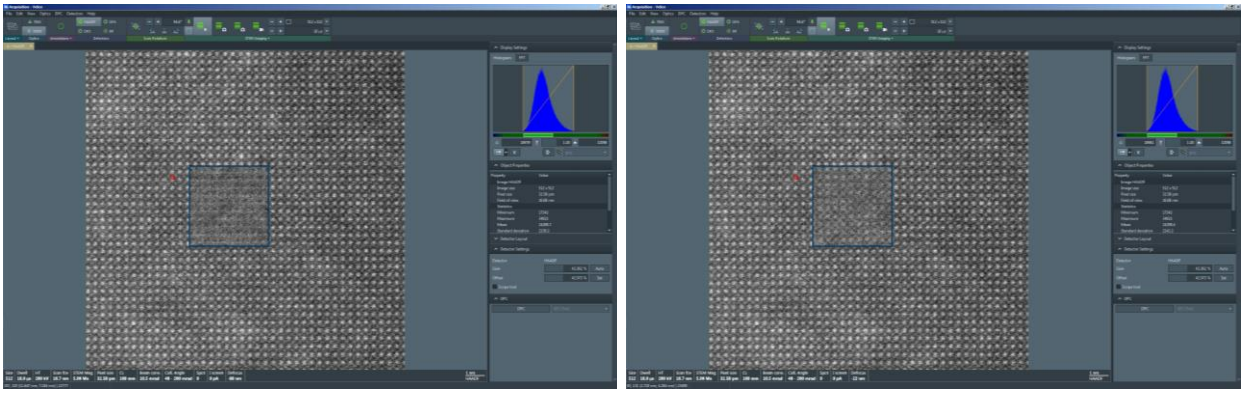
13.8.1. In Velox Acquisition, select “Reduced Area” and use the “Focus” knob (focus step = 1) to finely focus the image as best as possible; use the “Multifunction” knobs to obtain the sharpest possible image.

13.8.2. Astigmatism presents as directional streaking that reorients by ~90° as the focal condition changes from under- to over-focus.



Astigmatic atomic-resolution STEM images (reduced area): under- (left) and over-focused (right); directional streaking indicated by white arrows.

13.8.3. If no such streaking is evident as the focal condition is changed, then the astigmatism is properly corrected.



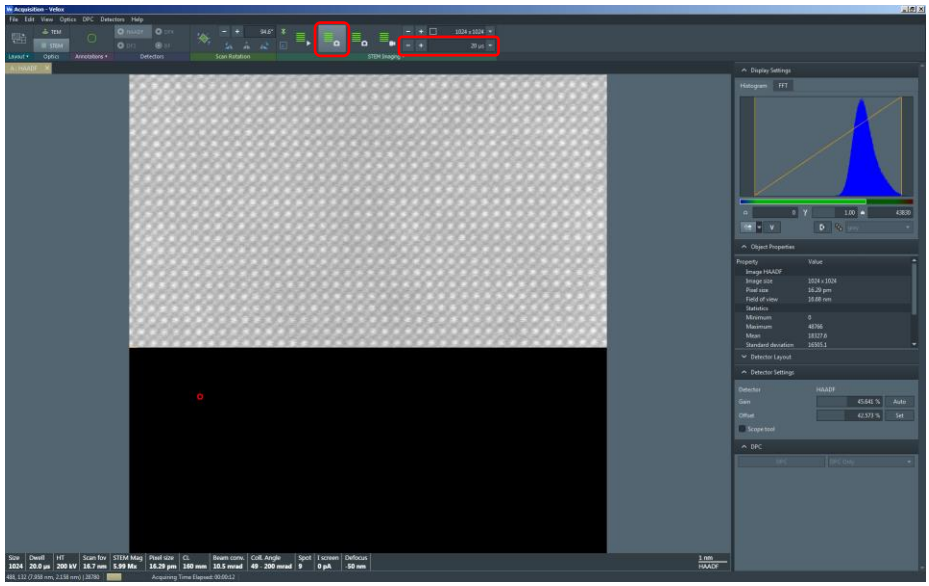
Non-astigmatic atomic-resolution STEM images (reduced area): under- (left) and over-focused (right).

13.9. Finely focus the live image and set the indicated magnification as needed.

13.9.1. NOTE: the lowest indicated magnification for effective atomic-resolution STEM is ~1.25 M×.

13.9.2. Check the detector dynamic range and adjust the “gain” and “offset” accordingly; this will usually be necessary for atomic-resolution imaging.

13.9.3. Acquire the final image.



13.10. NOTE: a very stable specimen is necessary to obtain high-quality, drift-free atomic-resolution STEM images.

13.10.1. It may take 1 – 2 h (or longer) after loading a specimen for it stabilize sufficiently such that a single-frame atomic-resolution STEM image with minimal drift can be produced.

13.10.2. Another option to address this is to acquire an image series and perform series acquisition with drift-corrected frame integration as described subsequently.

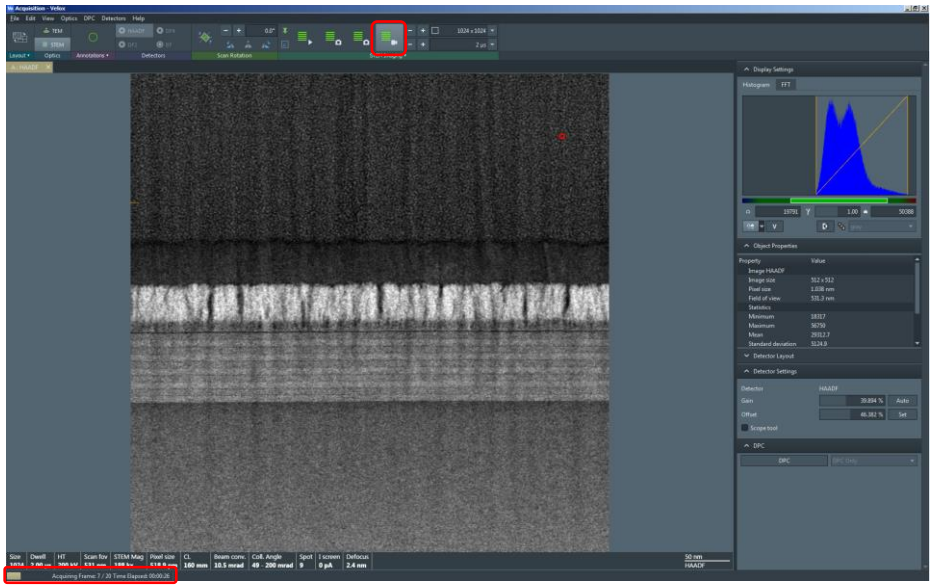
14. Series acquisition

14.1. Series acquisition may be used to sequentially acquire many STEM images with a short dwell time; the series of images can then be integrated and aligned to create a drift-corrected, frame-integrated (DCFI) image series.

14.2. The default settings for series acquisition will be sufficient for most situations and should not need to be adjusted.

14.2.1. Please DO NOT adjust the series acquisition settings without consulting with RSC staff first.

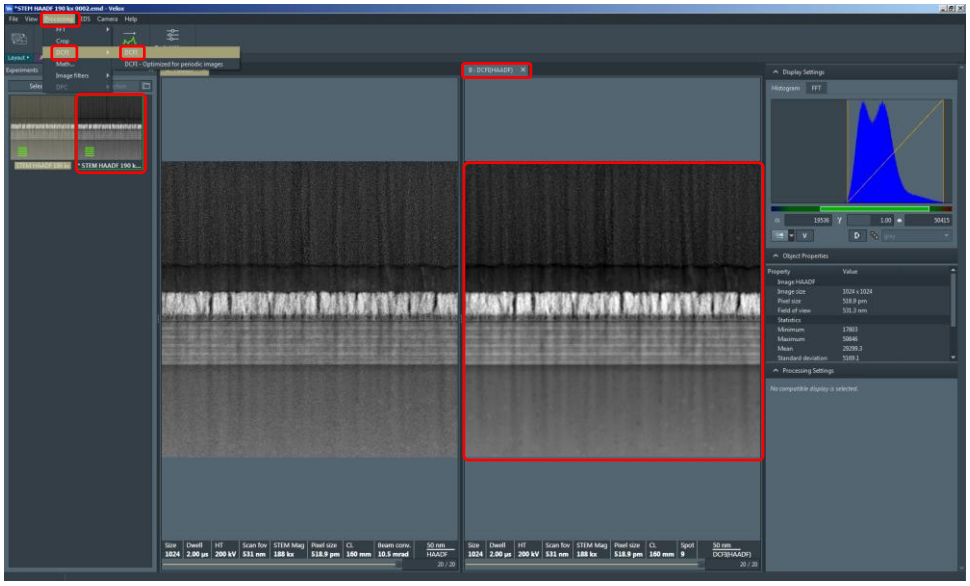
14.3. Set up the live image just as if preparing for a single frame acquisition; then select “Series” from the toolbar to start acquiring the series.



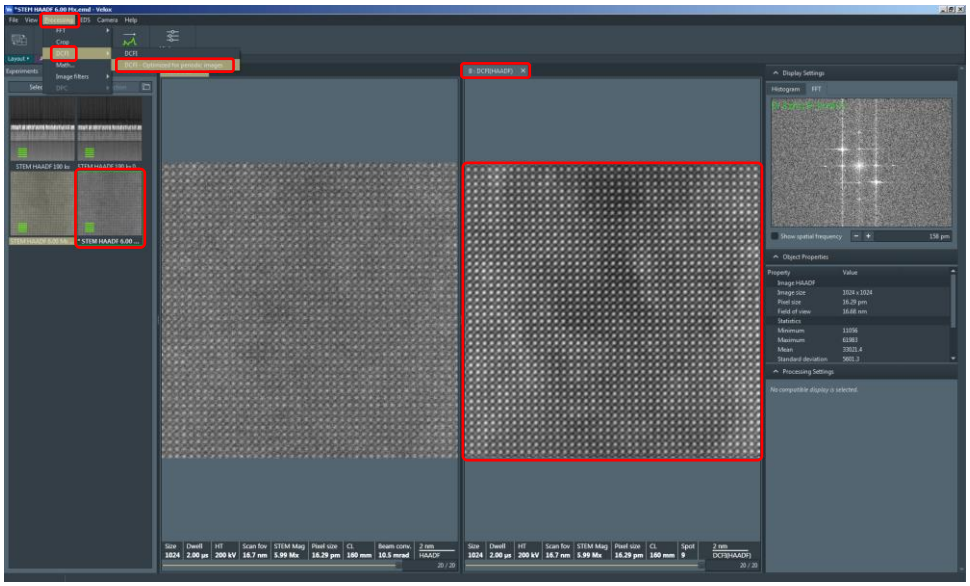
14.4. To generate the DCFI image series, switch to Velox Processing and make sure the series just acquired is selected and open.

14.4.1. Select “Processing” from the pull-down menu and then “DCFI”.

14.4.2. If the series <u>does not</u> contain atomic-level detail, select “DCFI”.



14.4.3. If the series <u>does</u> contain atomic-level detail, select “DCFI – Optimized for periodic images”.



14.4.4. To the right of the panel containing the image series, a new panel will be generated with the DCFI image series.

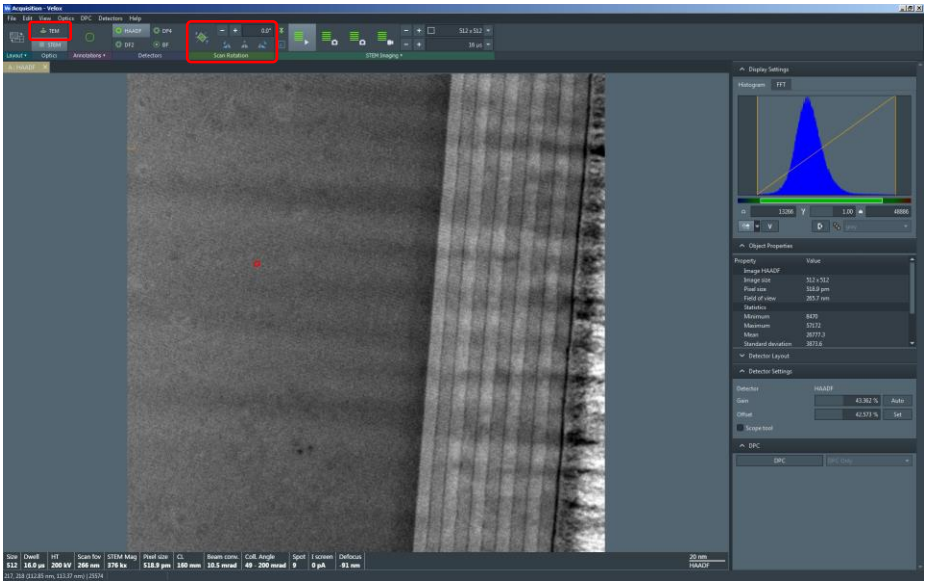
15. Finishing in STEM mode

15.1. Return to Velox Acquisition.

15.1.1. Verify “Beam Blank” from the toolbar is not activated (the beam is not blanked).

15.1.2. Set “Scan Rotation” = 0° in the toolbar (if needed).

15.1.3. Select “TEM” from the toolbar to return to TEM mode (the live STEM image will stop and the HAADF detector will automatically retract).



15.2. If needing to perform TEM imaging, full TEM alignment should be performed; alignment is sensitive to the operating mode of the instrument.

15.2.1. Remember to use the “200kV_uP” FEG register for TEM imaging.

15.3. If no additional TEM imaging is needed, simply finish the session as per usual (close column valves, reset holder, remove holder, etc).