FEI Talos F200i S/TEM: imaging in STEM mode
Nicholas G. Rudawski
ngr@ufl.edu
(805) 252-4916
(352) 392-3077
Last updated: 01/14/21

*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 is critical (particularly if trying to achieve atomic resolution); thus, it is imperative that specimens be plasma cleaned (if permissible) prior to analysis.

2. Instrument settings

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

2.2. The “80 kV S/TEM” and “200 kV STEM” FEG registers should be used for STEM operation at 80 and 200 kV, respectively.

2.2.1. NOTE: the FEG register for 200 kV STEM operation is different than the one for 200 kV TEM operation).

3. Before 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 will need to be performed once in STEM mode if atomic-resolution STEM is being performed, but try to get this as close as possible in TEM mode.

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

3.3. Verify the objective and SA apertures are both retracted.
4. Entering STEM mode

4.1. In Microscope Control, select the “STEM” tab and navigate to the “STEM Imaging” control panel; select “STEM” to enter STEM mode (the button will turn yellow); a stationary CBED pattern should be visible on the FluCam.

![STEM模式示意图](image)

4.2. In Microscope Control, set the spot size to be used for STEM imaging (you must do this after entering STEM mode);

4.2.1. Spot size = 1 is recommended for EDS.

4.2.2. If operating at 200 kV and attempting atomic-resolution imaging, spot size = 7 is the minimum recommended value, with spot size = 10 providing the best possible resolution (≈0.14 nm).
5. Alignment

5.1. In Microscope Control, select the “STEM” tab and navigate to the “STEM Detector” control panel; set the indicated “Camera length” ≥ 520 mm (this will be changed later after alignment is complete) and select “Insert detectors” to retract the ADF detector (button will turn gray).

5.2. Insert the 150 μm C2 aperture; then wobble the “Focus” knob (in STEM mode, “Focus” controls the C2 lens) using Focus step = 4 to determine the approximate center position of the Ronchigram (direct CBED disc); roughly center the C2 aperture on this position.
5.3. Alignment should be performed on a region of the specimen that is amorphous or partially amorphous (evaporated C or FIB Pt, for example). If you are not currently positioned over such a region, turn the “Focus” knob counterclockwise to defocus the Ronchigram so an image of the specimen can be observed and use the joystick to move to an appropriate region; once finished, focus the Rochigram to the “blow up” (focus) condition.

5.4. Note the presence of any streaking/elongation of features (rings) in the Rochigram; use the condenser stigmators (available in the “STEM” tab) to make the features round.

5.4.1. In STEM mode, only the condenser stigmators should be adjusted.
5.5. In Microscope Control, navigate to the STEM tab and then to the “Direct Alignments” control panel and then select “Rotation Center (Intensity)”; the Ronchigram will expand/contract as it goes in and out of focus; adjust the “Multifunction” knobs (see diagram below) until the expansion/contraction is straight into/out of the page; select “Done” when finished.

"Multifunction" knob adjustments to achieve straight expansion/contraction of the Rochigram based on the current observed pattern of expansion/contraction.
5.6. Use the “Focus” knob to focus ("blow up") the Rochigram and check for any remaining astigmatism; if needed, correct using the condenser stigmators (a final adjustment to the condenser stigmators will likely need to be performed when observing the STEM image for optimal results).

5.7. Insert and center the 70 µm C2 aperture (in the “Tune” tab described elsewhere) on the Ronchigram; turn the “Focus” knob back and forth through the “blow up” condition to verify the point of expansion/contraction is centered in the aperture (if not, adjust the aperture position accordingly).
5.8. In Microscope Control, select the “STEM” tab and navigate to the “STEM Detector” control panel; set “Camera length” ≤98 mm (for a HAADF-STEM image); then select “Insert detectors” to reinsert the ADF detector (button will turn yellow).

5.9. If the Ronchigram is not centered inside the inner rim of the ADF detector (indicated by arrow), navigate to the “Direct Alignments” control panel and select “Diffraction alignment”; use the “Multifunction” knobs to center the Ronchigram inside the inner rim of the ADF detector and select “Done” when finished.
6. Imaging in STEM mode

6.1. In Velox, select “HAADF” from the toolbar to acquire a live STEM image; adjust the dwell time on the toolbar as needed (4 – 16 µs is usually sufficient).

6.2. To adjust the image brightness/contrast; navigate to the “Detector Settings” control panel and check the box next to “Scope tool” to see the STEM signal (green line) as the beam is scanned. Adjust “Gain” and “Offset” until the STEM signal is just below (above) the top (bottom) red (blue) line. Uncheck “Scope tool” when finished.
6.3. If desired, the STEM image can be rotated to align certain features in the image along preferred directions; simply adjust “Scan Rotation” from the toolbar as needed. Note that the dwell time should be \( 8 \geq \mu s \) and specimen drift should be minimal to ensure the image rotation is as close to as it will appear in the final STEM image.

6.4. Use the joystick to center a region of the specimen with very fine features (e.g. FIB Pt grains) in the STEM image, then use the “Magnification” knob to set the indicated magnification to \( \sim 1M \times \). In Velox, select “Reduced Area” from the toolbar to open a reduced area scan window (focusing box) in the live STEM image. Use the “Focus” knob to focus the image as best as possible and note the presence of any streaking (astigmatism).
6.5. In Microscope Control, return to the “STEM” tab, navigate to the “Stigmator” control panel and select “Condenser”; use the “Multifunction” knobs to make the image as sharp as possible and then select “Done”. Use the “Focus” knob to refocus the image. Navigate back to Velox and select “Reduced Area” from the toolbar to resume full frame imaging.

6.6. Use the joystick to navigate to a region of interest; then set the indicated magnification higher than will be used for final image acquisition and focus the image, then set the indicated magnification as needed. In Velox, select “Acquire” from the toolbar to acquire the final STEM image; adjust the dwell time as needed (16 – 64 µs is usually sufficient). If the dwell time is adjusted while acquiring an image, the new dwell time will not take effect until subsequent acquisitions.
7. Series acquisition (drift-corrected imaging)

7.1. Series acquisition is used to sequentially acquire several STEM images (typically, 20 – 30) with a fixed dwell time (typically, only 1 – 2 µs); the series of images can then be used to create a drift-corrected image using drift-correct frame integration (DCFI). Select “STEM Imaging” from the toolbar, then the “Series” tab and then set the parameters for Series acquisition.

7.2. Select “Series” acquisition from the toolbar to start acquiring the series (progress shown at the lower left corner of the window).
7.3. To generate the drift-corrected image, switch to the “Processing” part of Velox and make sure the series just acquired is selected. Select “Processing” from the pull-down menu and then “DCFI”.

7.3.1. If the Series does not contain atomic-level resolution, select the “DCFI” option.

7.3.2. The “DCFI – Optimized for periodic images” option is generally only used for atomic-resolution images of single crystals (see subsequent section on considerations for atomic-resolution imaging).

7.4. Next to the panel containing the original image series, a new panel will be generated with the drift-corrected image generated by DCFI.

7.4.1. The signal to noise ratio in the drift-corrected image generated by \( N_f \) frames each with dwell time = \( t_d \) will be approximately equivalent to a single frame image acquired with dwell time = \( t_d \times N_f \) (e.g. 30 frames with 1 \( \mu \)s dwell time compared to 1 frame with 30 \( \mu \)s dwell time).
8. Blanking the beam (if needed)

8.1. If you need to leave the instrument for an extended period, but want to leave the instrument in STEM mode, it is best practice to blank the beam to minimize specimen irradiation. In Velox, select “Beam Blank” from the toolbar to blank the beam (the live image will continue, but will just be noise).

![Blanked Beam Image](image1.png)

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

![Unblanked Beam Image](image2.png)
9. Additional considerations for atomic-resolution STEM imaging

9.1. The highest quality atomic-resolution STEM images will be 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, you must look at the CBED pattern on the FluCam. To complicate matters, you must also achieve this alignment at your desired ROI (e.g. at a film/substrate interface).

9.1.1. If you properly aligned your ROI while still in TEM mode (making sure it was at eucentric height afterwards), then tilting more than a few mrad when in STEM mode to fine tune the crystallographic alignment should not be necessary.

9.2. While acquiring a live STEM image in Velox, select “View” from the toolbar to freeze the live image; then click and drag on the beam position marker on the frozen STEM image (automatically inserted) and position it at the desired ROI; the CBED pattern observed on the FluCam will correspond to the beam position marker location.

9.2.1. The indicated magnification does not need to be (and should not be) very high while tilting to perform crystallographic alignment; 50 k× – 200 k× will be sufficient for doing this.
9.3. **Use the following iterative process to align the sample along the zone axis:**

9.3.1. Move the beam position marker to your area of interest and observe the CBED pattern on the FluCam.

9.3.2. Make an appropriate change in either $\alpha$ or $\beta$ tilt (see below for an indication of how to tilt to converge on the zone axis based on $L$ and the appearance of the CBED pattern).

![Diagram of tilting angles and CBED patterns]

Tilting to the zone axis: when $L < 98$ mm (left) and when $L \geq 98$ mm (right)

9.3.3. Return to Velox and select “View” to resume the live STEM image in Velox; if the image is clearly out of focus after tilting, **refocus by adjusting the Z axis of the stage**.

9.3.4. Re-center the region of interest in the STEM image and then freeze live STEM image again.

9.3.5. Move the beam position marker back over to the area of interest and look at the CBED pattern on the FluCam again (you should be closer to the zone axis than you were before).

9.3.6. Repeat this process until alignment with the zone axis for your region of interest has been achieved as indicated by the CBED pattern (which may take many iterations). With skill, you should be able to achieve zone axis alignment with $<1$ mrad precision.

9.3.7. Once zone axis alignment is complete, resume live STEM imaging.
9.4. While live imaging, center the region of interest in the image and set the indicated STEM magnification in Microscope Control to $7.2 \times$ (this can be adjusted later, but is best for fine tuning of an atomic-resolution image).

9.5. Stop the scanning and observe the CBED pattern on the FluCam; adjust “Focus” (focus step = 2) until the probe is focused on the sample (this corresponds to the “blow up” condition where CBED pattern does not exhibit any features resembling lattice fringes).

9.6. Start live imaging again; set focus step = 1 and adjust “Focus” until the sharpest atomic resolution possible is evident in the image (this can only be done effectively with focus step = 1).
9.7. If imaging a single crystal, the scanning pattern of the STEM image may be rotated to align major crystallographic directions with the vertical and horizontal image directions to facilitate easier removal any remaining astigmatism. In Velox, set dwell time ~ 30 µs for the live image and adjust “Scan Rotation” from the toolbar until the STEM image is oriented as needed.
9.8. In Microscope Control, activate the condenser stigmators and adjust the “Multifunction” knobs to remove any remnant astigmatism from the probe (use the finest possible setting); astigmatism will show up as preferential streaking that reorients by ~90° as the focal condition goes from under- to over-focus; if no such streaking is evident as the focal condition is changed, then the astigmatism has been properly corrected.

Astigmatic atomic-resolution STEM images: under- (left) and over-focused (right)

Non-astigmatic atomic-resolution STEM images: under- (left) and over-focused (right)
9.9. Finely focus the live image, set the magnification as needed (lowest practical indicated magnification for atomic-resolution STEM is 1.25 M×), adjust image contrast/brightness (if needed), and acquire the final STEM image.

9.9.1. A “Dwell time” of 32 – 64 µs is usually sufficient.

9.10. Due to the long acquisition times needed for an atomic-resolution STEM image with sufficient signal to noise, a stable specimen is necessary to obtain images with minimal drift; it may take 0.5 – 1.0 h (or longer) after loading of a specimen for it stabilize sufficiently such that a single-frame atomic-resolution STEM image with minimal drift can be produced.

9.10.1. Another option to produce a drift-free image is to acquire an image series and perform DCFI as described subsequently.
9.11. Collecting an image series and performing DCFI is particularly effective when performing atomic-resolution imaging. In Velox, Select “STEM Imaging” from the toolbar, select the “Series” tab and adjust the parameters accordingly; adjust the live image (focus, contrast/brightness, etc.) and select “Series” from the toolbar (as described previously) to acquire the series.

9.11.1. DCFI is effective for atomic-resolution imaging, but only if specimen drift is not excessive; if the drift is extremely high, your best option is still to wait until the drift has stabilized before proceeding (again, this may take ≥0.5 h).

9.11.2. 30 frames with dwell time = 1 µs are good parameters to start with for atomic-resolution DCFI.
9.12. Switch to the “Processing” part of Velox and perform DCFI as described previously.

9.12.1. If the imaged area is a single crystal, the “DCFI – Optimized for periodic images” option is most appropriate.

9.12.2. If the imaged area is not a single crystal (e.g. a group of nanoparticles), the regular “DCFI” option is most appropriate.
10. Finishing in STEM mode

10.1. In Velox, verify “Beam Blank” from the toolbar is not activated (beam is *not* blanked) and a live image is actively being acquired and set “Scan Rotation” from the toolbar back to 0° (if needed). Finally, select “TEM” from the toolbar to return to TEM mode (live STEM image will stop and STEM detector will automatically retract).

10.2. Re-insert the appropriate C2 aperture for TEM mode (if necessary) and re-center (as described elsewhere).

10.2.1. NOTE: C2 aperture alignment will be different between TEM and STEM modes.

10.3. If TEM imaging is to be performed, the full TEM alignment procedure should be carried out.

10.4. If no TEM imaging is to be performed, simply finish the session as you would normally (close column valves, reset stage, remove holder, etc).