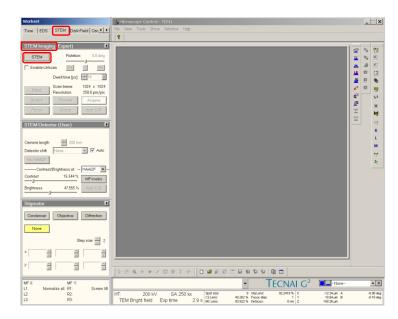
FEI Tecnai F20 S/TEM: STEM imaging Nicholas G. Rudawski ngr@ufl.edu (805) 252-4916 (352) 392-3077 Last updated: 05/01/24

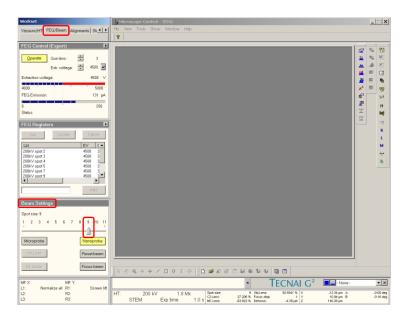
This document assumes the user is already familiar with basic operation of the instrument in TEM mode and use of the Microscope Control interface.

- 1. Specimen cleaning
 - 1.1. Plasma cleaning of the grid and holder prior to insertion into the column should be performed (if possible); specimen cleanliness is <u>critical</u> to obtaining high-quality STEM images.
 - 1.2. If the grid cannot be plasma cleaned, <u>beam showering</u> is recommended instead (please contact RSC staff for assistance).
- 2. Before entering STEM mode
 - 2.1. Find a region of interest, crystallographically orient it (if needed), and bring it to eucentric height.
 - 2.2. Select and apply a FEG register; "200kV spot 2" or "200kV spot 3" are recommended for performing STEM-EDS; "200kV spot 9" is recommended for performing high-resolution STEM imaging (shown here).
 - 2.3. Verify the objective and SA apertures are both retracted.
 - 2.4. If possible, adjust the stage so the <u>field of view is completely in vacuum</u> (this will facilitate easier alignment). Otherwise, position the stage so the field of view contains weakly-scattering material (e.g., empty C support film).

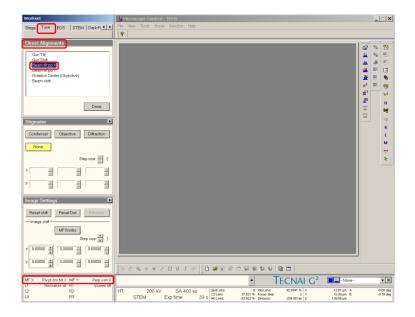
- 3. Entering STEM mode
 - 3.1. In Microscope Control, select the "STEM" tab and then the "STEM Imaging" control panel; select "STEM" to enter STEM mode; a stationary CBED pattern should be visible on the viewing screen.



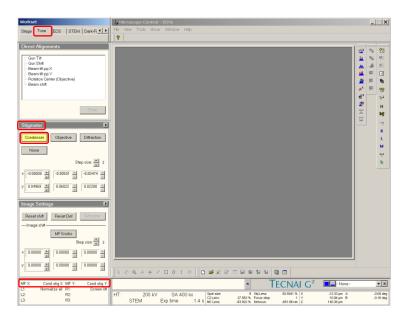
3.2. In Microscope Control, select the "FEG/Beam" tab and then the "Beam Settings" control panel; set "Spot size" to the value indicated by the FEG register that was applied previously.



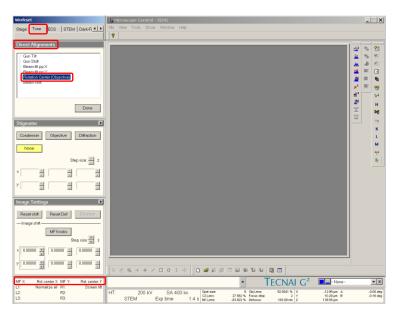
- 4. Alignment
 - 4.1. Select "Diffraction" (R control pad) to view an image of the probe; set the indicated magnification = SA 400k× (highest possible).
 - 4.2. Push "Eucentric focus" (R control pad).
 - 4.3. Insert and center the #1 C2 aperture using the same procedure described for TEM mode operation.
 - 4.4. Use the "Intensity" knob (L control pad) to focus the beam until a sharp, caustic spot surrounded by a diffuse halo is observed (use the binoculars and the focusing screen to assist with this).
 - 4.5. In Microscope Control, select the "Tune" tab and then the "Direct Alignments" control panel; select "Beam tilt pp X" and use the "Multifunction" knobs to adjust the "wings" until the bright tips touch. Next, select "Beam tilt pp Y" and repeat.



- 4.6. Remain in the "Tune" tab and select the "Stigmator" control panel; select "Condenser" and use the "Multifunction" knobs to make the beam circular.
 - 4.6.1. This is only an initial correction of condenser astigmatism; final correction of condenser astigmatism must be done using the live STEM image.



- 4.7. Remain in the "Tune" tab and the "Direct Alignments" control panel and select "Rotation Center"; if the beam shifts from the field of view, use the trackball to recenter it.
- 4.8. Turn the ring of the "Focus" knob counterclockwise to stop the pulsing of the beam; if needed, use the "Intensity" knob to adjust the beam so a sharp, caustic spot surrounded by a diffuse halo is observed.
- 4.9. Use the "Multifunction" knobs to precisely center the sharp, caustic spot inside the diffuse halo (use the binoculars and focusing screen to assist with this).

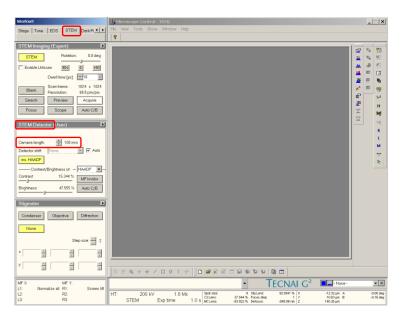


- 4.10. DO NOT perform any further adjustments to the "Intensity" knob (C2 lens).
- 4.11. Press "Diffraction" (R control pad) to observe a stationary CBED pattern again.

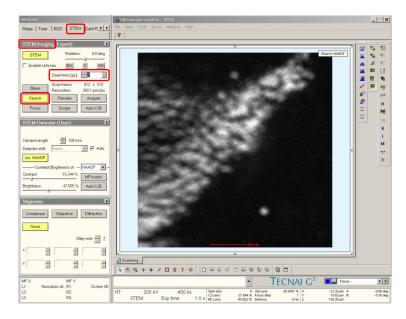
4.12. In Microscope Control, select the "STEM" tab and then the "STEM Imaging" control panel; select the flap-out arrow and then the "Focus" tab and then finally "Reset Df".

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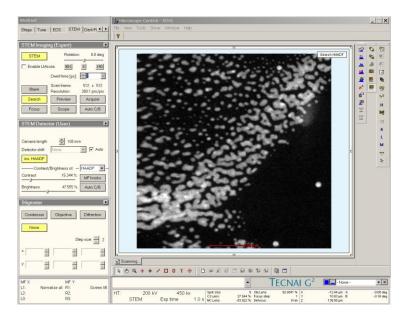
4.13. Remain in the "STEM" tab and select the "STEM Detector" control panel; adjust the spin controls to set "Camera length" = 100 mm (for a HAADF-STEM image).



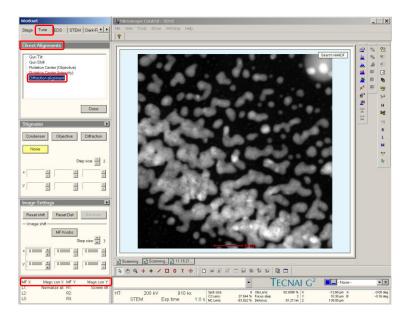
- 5. Live imaging and fine tuning
 - 5.1. Remain in the "STEM" tab and select the "STEM Imaging" control panel; select "Search" to start acquiring a live STEM image in TIA.
 - 5.1.1. Adjust the "Dwell time" spin controls to adjust the dwell time as needed.



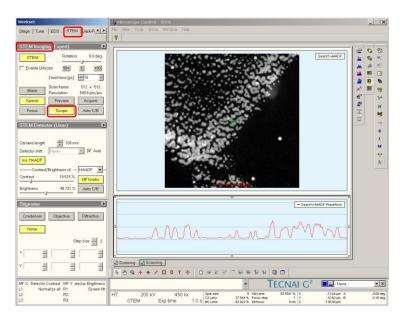
5.2. Adjust the "Z axis" buttons (R control pad) to focus the live image as best possible; any further focusing may be performed using the "Focus" knob.



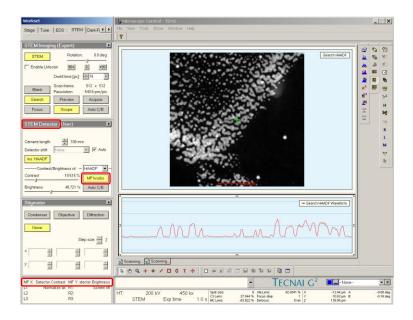
5.3. Select the "Tune" tab and the "Direct Alignments" control panel; select "Diffraction alignment" and then use the "Multifunction" knobs to center the direct disk of the CBED pattern inside the STEM detector rim.



5.4. Return to the "STEM" tab and select the "STEM Imaging" control panel; select "Scope" to generate a new pane below the live STEM image.



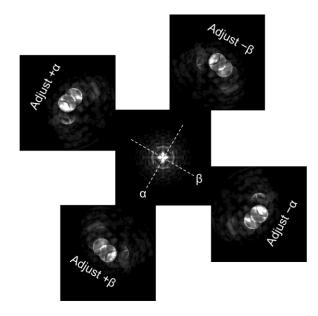
5.5. Select the "STEM Detector" control panel and then "MF knobs"; use the "Multifunction" knobs to adjust the detector signal so it takes up approximately the middle 50% of the dynamic range.



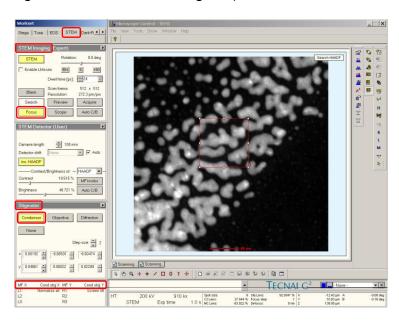
- 5.6. Remain in the "STEM" tab and select the "STEM Imaging" control panel; adjust the "Rotation" slide control to rotate the image as desired.

 - 5.6.1. NOTE: a <u>clockwise</u> rotation corresponds to a <u>negative</u> rotation angle.

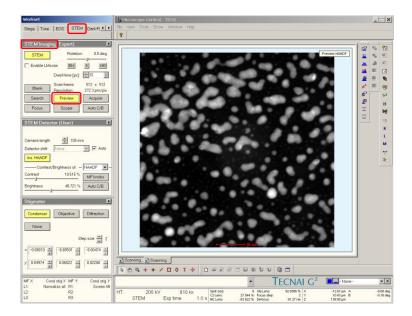
- 5.7. If needed, fine tuning of the crystallographic alignment of the sample may also be performed. Observe the CBED pattern as the probe scans across the region of interest; use the following zone axis tilt map (assuming L = 100 mm) to align to the zone axis.
 - 5.7.1. The image will likely go out of focus after tilting the stage; be sure to refocus the image as best as possible using the "Z axis" buttons (R control pad).



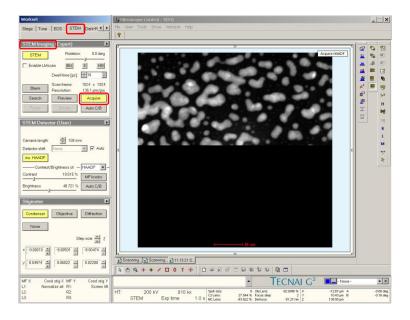
- 5.8. Remain in the "STEM" tab and select the "STEM Imaging" control panel; select "Focus" to generate a focusing box on the live image.
 - 5.8.1. Click and drag on the box to position/resize it as needed.
 - 5.8.2. If needed, adjust the "Dwell time".
 - 5.8.3. Use the "Focus" knob to finely focus the image.
 - 5.8.4. Select the "Stigmator" control panel and select "Condenser"; use the "Multifunction" knobs to finely stigmate the image (FIB-deposited Pt grains work well for doing this).



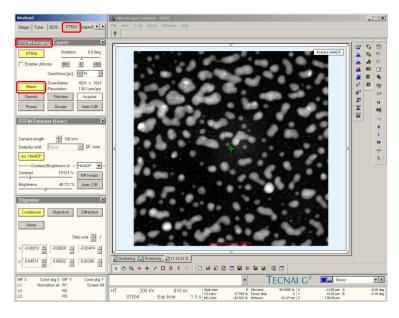
- 6. Acquiring the final STEM image
 - 6.1. Adjust the "Magnification" knob to set the field of view as needed; use the "Focus" knob to focus the image as precisely as possible.
 - 6.2. In the "STEM" tab, select the "STEM Imaging" control panel and then "Preview"; a new panel will be generated in TIA with the "Preview" image.



6.3. If the "Preview" image is acceptable, select "Acquire" to acquire the final image in a new panel in TIA.

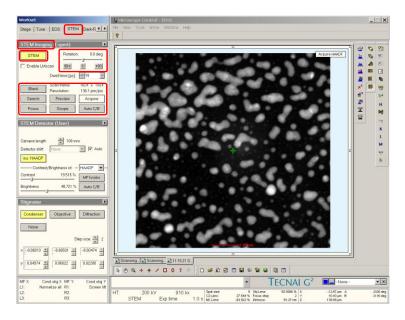


- 7. Blanking the beam (if needed)
 - 7.1. If needing to stop STEM imaging for an extended period, but wanting to leave the instrument in STEM mode, the scanning should be stopped, and the beam blanked.
 - 7.2. In the "STEM Imaging" control panel, make sure "Search", "Preview", "Acquire", or "Focus" are not activated; if one of these buttons is yellow, simply select it and this will turn the scanning off.
 - 7.3. In the "STEM imaging" control panel, select "Blank" to blank the beam.



7.4. When ready to resume imaging, select "Blank" to un-blank the beam.

- 8. Finishing in STEM mode
 - 8.1. In Microscope Control, select the "STEM" tab and then the "STEM Imaging" control panel. Verify "Search", "Preview", "Acquire", and "Focus" are not activated; if one of these buttons is activated, deactivate it.
 - 8.2. Additionally, verify "Blank" is not activated.
 - 8.3. If "Rotation" was adjusted, reset it to 0°.
 - 8.4. Select "STEM" to exit STEM mode.



- 8.5. Re-insert and recenter the #2 C2 aperture.
- 8.6. If TEM imaging is to be performed, the full TEM alignment procedure should be carried out.
- 8.7. If no TEM imaging is to be performed, simply finish the session as per usual (close column valves, reset stage, remove holder, etc).