FEI Themis Z S/TEM: STEM-EELS
Nicholas G. Rudawski
ngr@ufl.edu
(805) 252-4916
(352) 392-3077
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ANALYSIS OF RADIOACTIVE SPECIMENS IS <u>STRICTLY</u> PROHIBITED

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

1. Instrument voltage and specimen considerations

    1.1.   It is recommended to perform STEM-EELS at the default voltage setting of 200 kV; If you need to perform STEM-EELS at a different voltage (60 or 200 kV), please contact staff for assistance setting up the instrument and GIF.

        1.1.1.   The examples shown here were performed with the instrument operating at 200 kV.

    1.2.   Specimen thickness is crucial for STEM-EELS to be effective. In general, the sample thickness should only be a few 10s of nm and at worst should not exceed 100 nm.

        1.2.1.   If the sample thickness exceeds 100 nm, it is probably better to perform STEM-EDS instead of STEM-EELS.

2. GIF tuning

    2.1. _Before switching to STEM mode, the GIF must be tuned while in TEM mode_ to ensure the GIF apertures are all properly centered; _full TEM mode alignment is not necessary for GIF tuning_; move the stage so the beam is in vacuum.

        2.1.1. If this is not possible, the beam should be over a region of the specimen/grid that _scatters weakly_.

    2.2. Set the indicated magnification = 44000×, expand the beam to the size shown in the image below (approximately _twice the diameter of the second circle marking_) and center the beam on the FluCam.
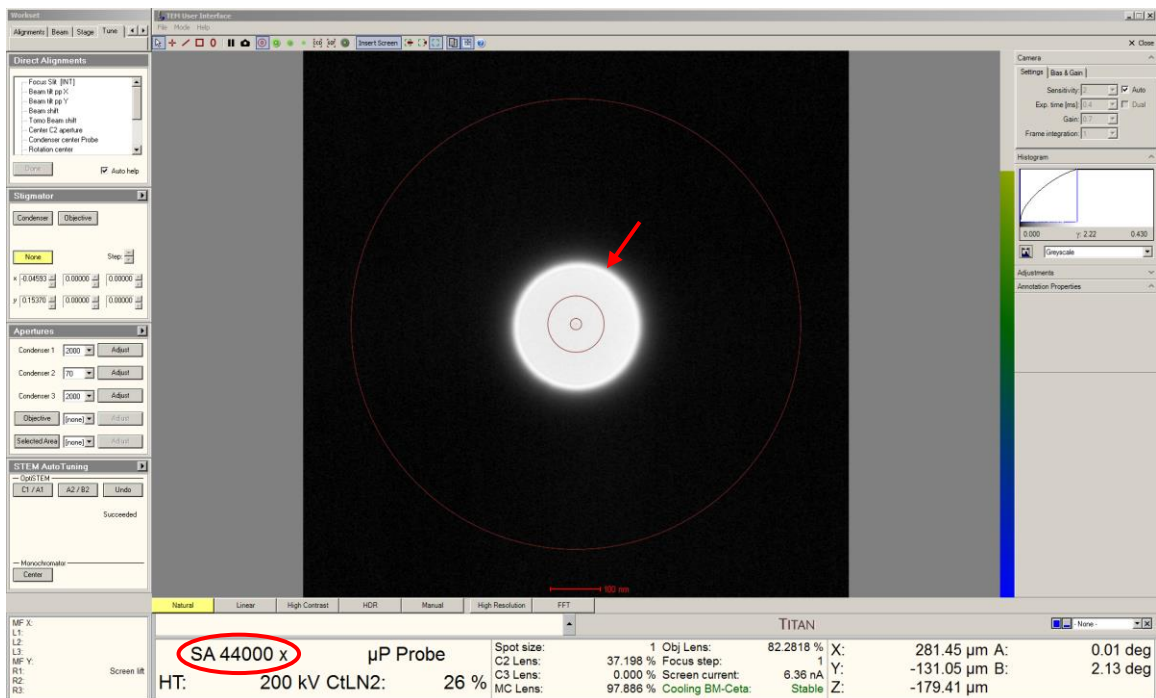
   2.3. Verify the Ceta camera and all microscope STEM detectors are _retracted_.

    2.4. Retract the viewing screen so the beam can enter the GIF.

        2.4.1. NOTE: the viewing screen is a physical barrier for entry of the beam to the GIF. _If at any point there is concern about the beam potentially damaging the GIF, simply re-insert the viewing screen_.

2.5. In DigitalMicrograph, navigate to the "Microscope System" (left) side panel and select "EFTEM".

2.5.1. If prompted to switch to "EFTEM" mode; select "OK".

2.6. Navigate to the "Tune GIF" (left) side panel; select "Center ZLP" and then "Tune GIF"; pop up windows should appear indicating if the tuning was successful (or not).
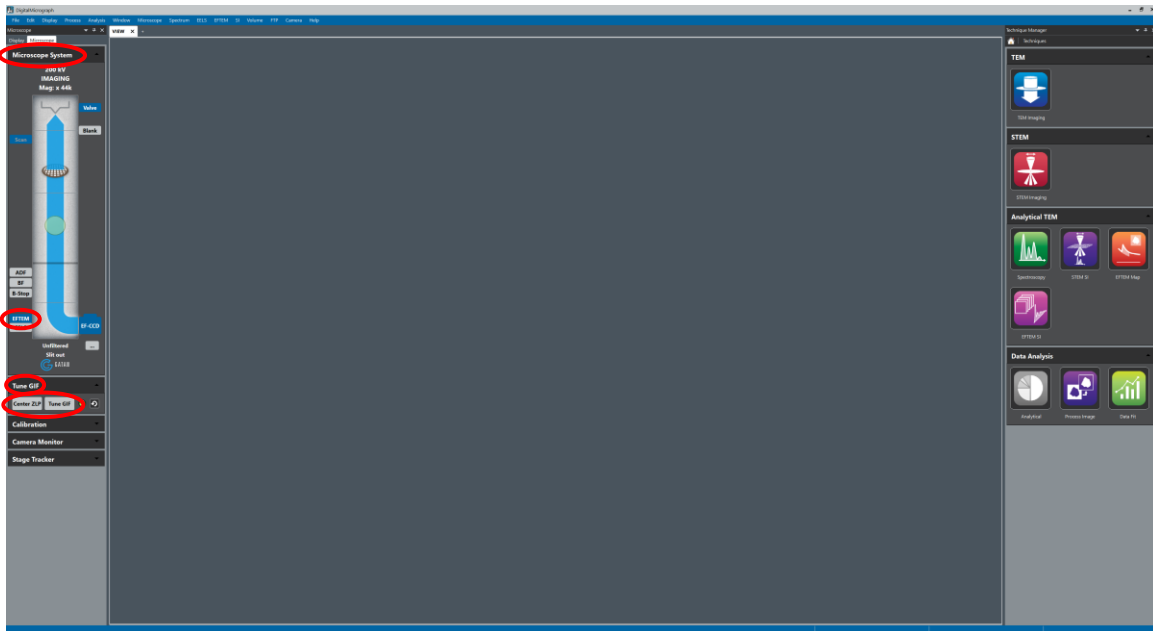

2.7. Re-insert the viewing screen after GIF tuning is complete; no additional GIF tuning should be needed during the session _unless the microscope voltage is changed_.

3. Instrument alignment/settings for STEM-EELS

    3.1. Perform the same alignment in STEM mode using a HAADF-STEM image before switching to EFSTEM mode noting the following:

        3.1.1. _Set the probe current to ~400 pA_; this value can always be increased/decreased later as need, but usually serves as a good starting value.

        3.1.2. Verify the indicated camera length = 115 mm; this ensures a HAADF-STEM image will be collected in DigitalMicrograph after switching to EFSTEM mode.

    3.2. Verify the Ceta camera and all microscope STEM detectors are _retracted_; otherwise, _GIF operation and HAADF-STEM imaging in DigitalMicrograph will not work properly_.

4. Setting up EFSTEM mode

   4.1. In Microscope Control, select the "EFTEM" tab, navigate to the "Filter" control panel, and select "EFTEM".

   4.2. Activate the "High Resolution" display mode for the FluCam.
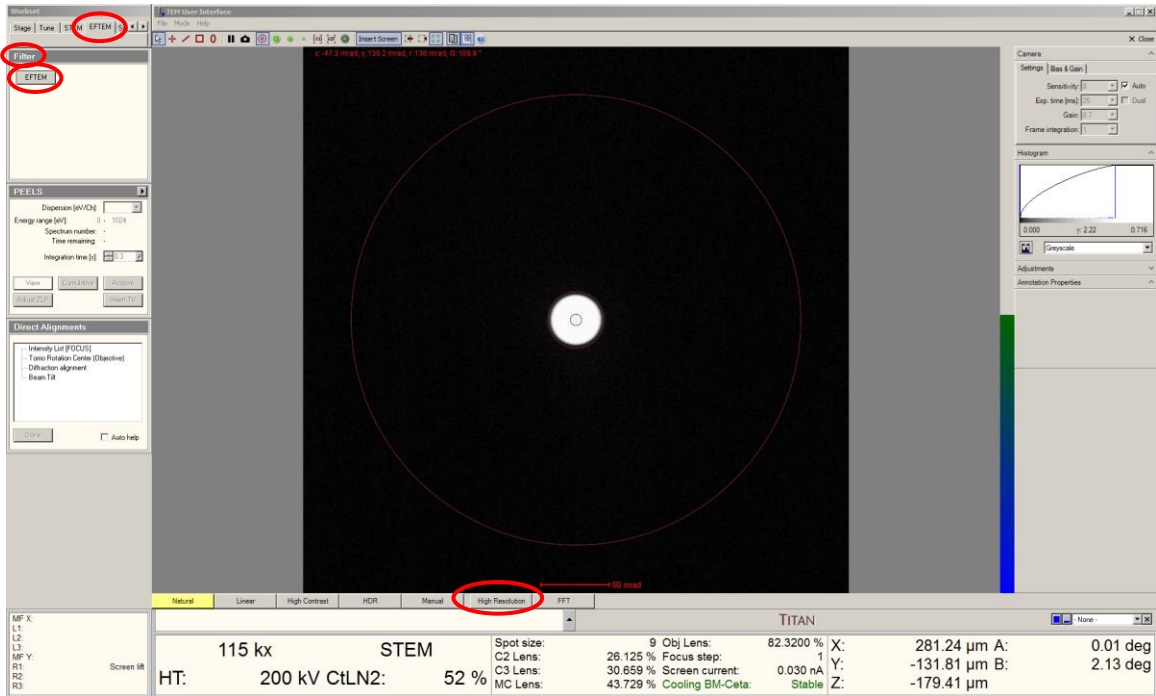
4.3. Activate the "GIF" entrance aperture markings from the FluCam viewer tool bar.

4.4. Roughly center the direct disk inside the green GIF entrance aperture circle marking (indicated by arrow) using the "Diffraction Alignment" direct alignment.

    4.4.1. More precise alignment will be performed subsequently.
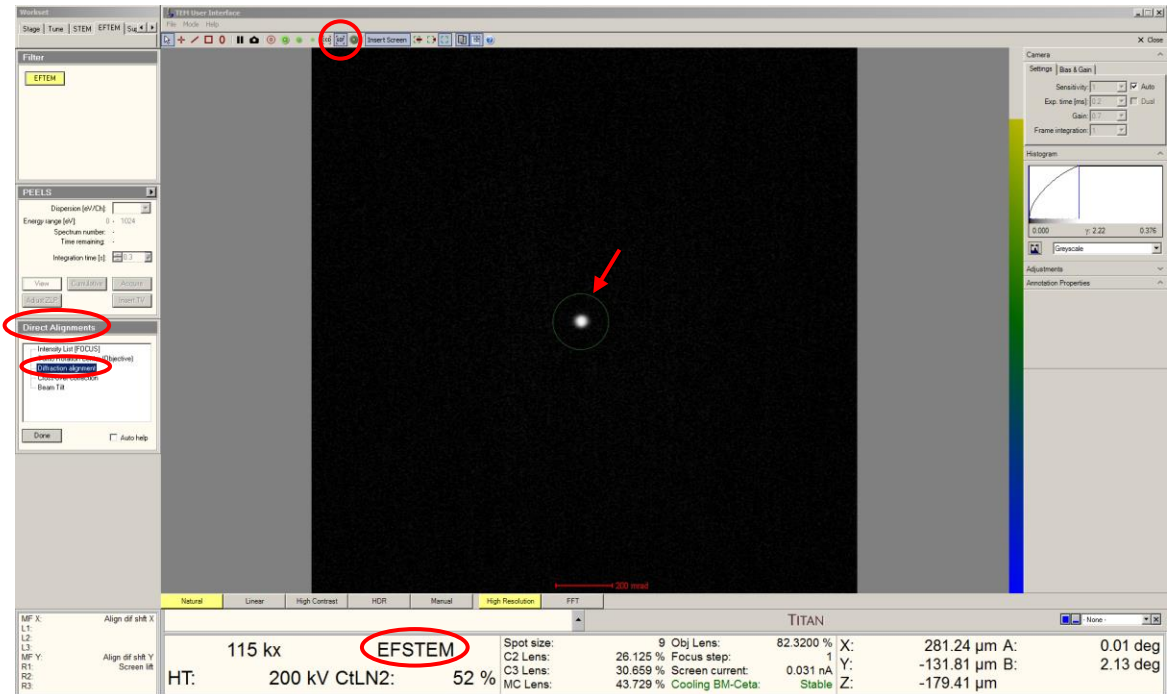
4.5. Retract the viewing screen so the beam can enter the GIF.

5. Precise CBED alignment

   5.1. In DigitalMicrograph, navigate to the "Technique Manager" (right) side panel and select the "Home" icon; then select "STEM SI" from the (right) side panel.
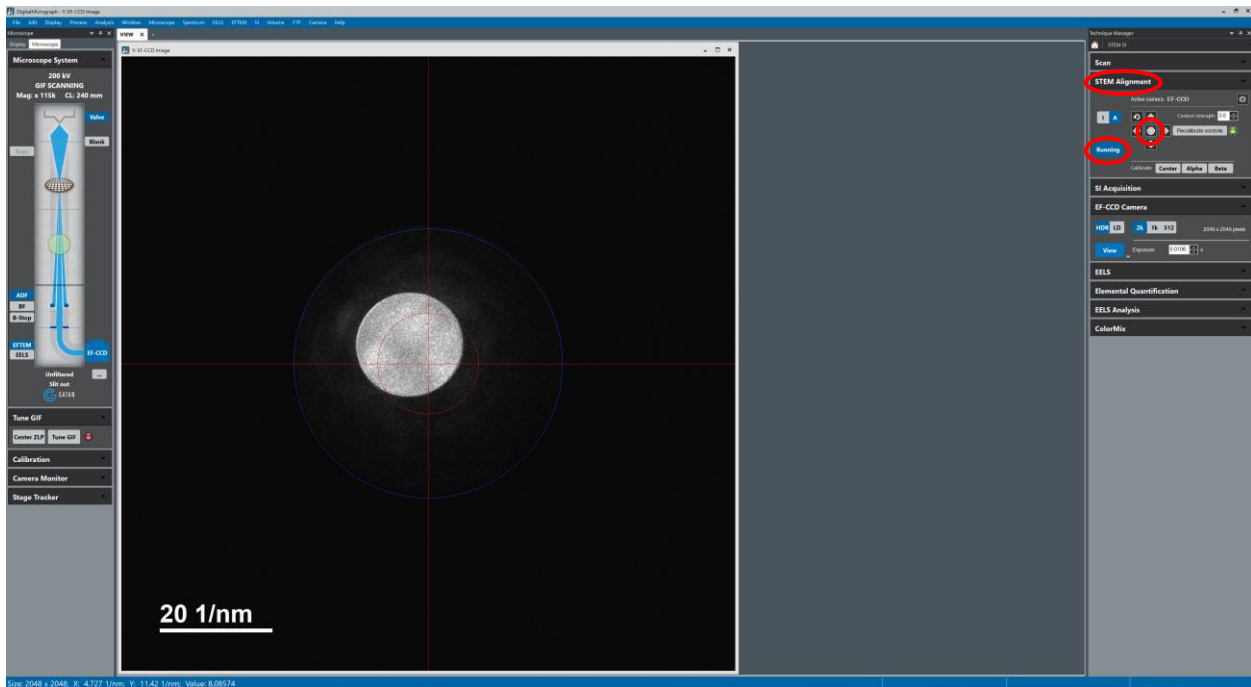
      5.1.1. If prompted to switch to "EELS" mode; select "NO"; the system needs to remain in "EFTEM" mode for CBED alignment.
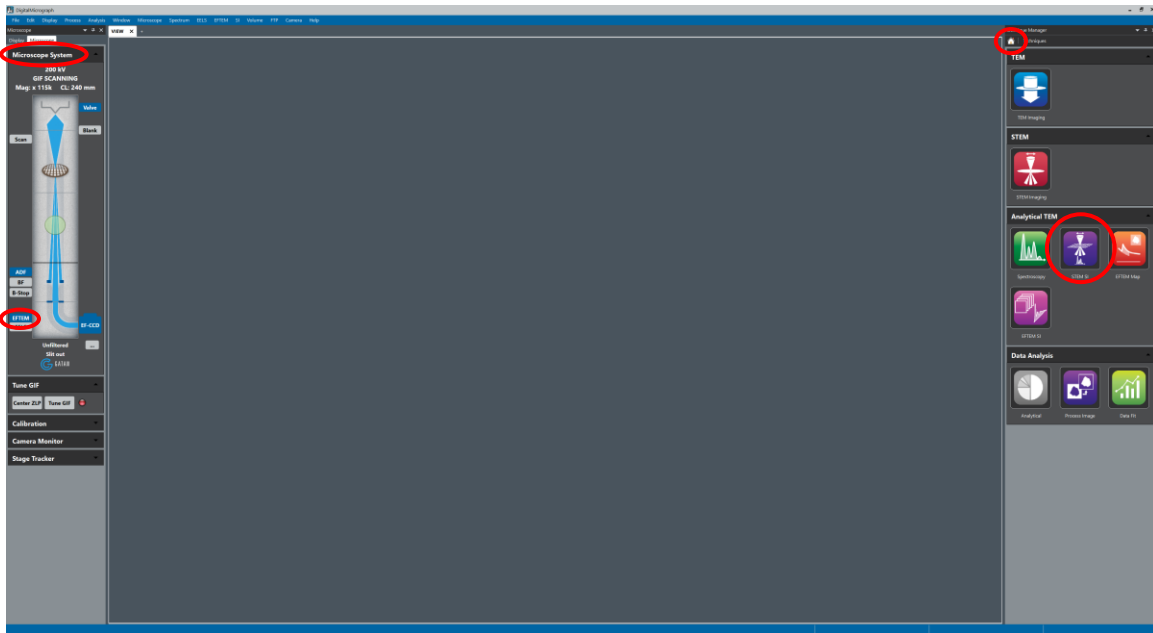
5.2. Navigate to the "STEM Alignment" (right) side panel and select "Start".
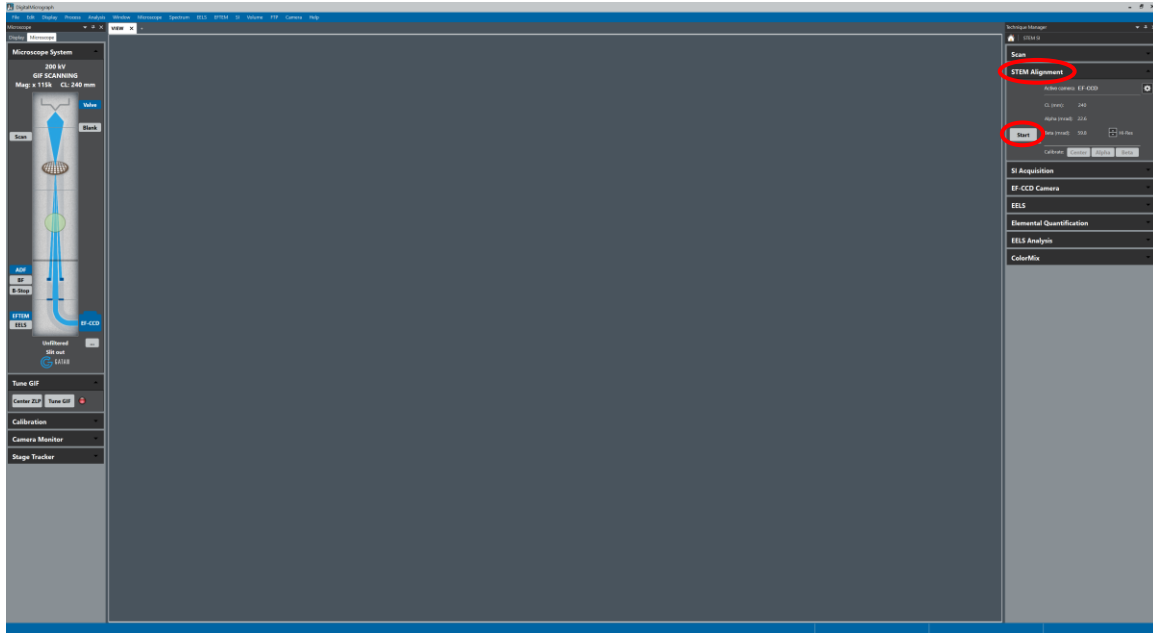
5.3. Remain in the "STEM Alignment" (right) side panel; select "Auto center disc" to center the CBED pattern in the image. When finished, select "Running".

20 1/nm

6. Changing scan inputs

    6.1.   Locate the gray scan input box on the workstation desktop; select the "EELS SCAN" button; this ensures the STEM image can be collected in DigitalMicrograph.
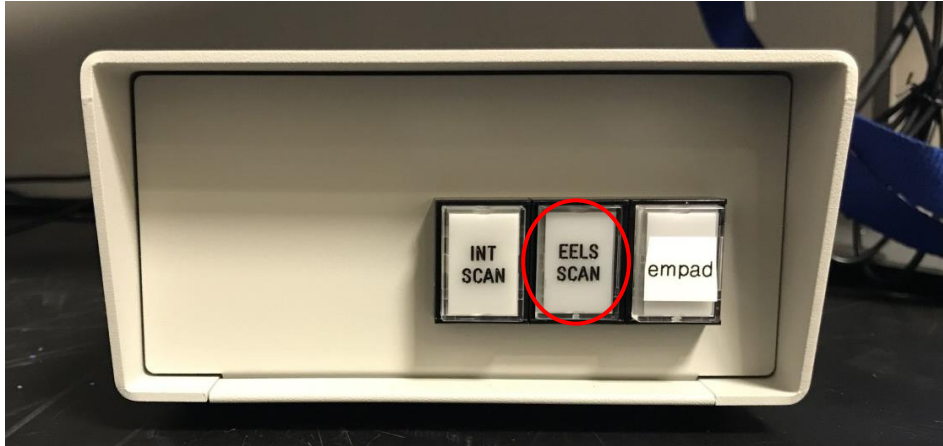
7. STEM imaging with the GIF.

    7.1. Navigate to the "Scan" (right) side panel; verify "Preview" mode is selected and select "View to start acquiring a live HAADF-STEM image; adjust the indicated magnification and "Pixel Time" (dwell time) as needed.

        7.1.1. In general, the level of detail in a spectrum image will be like that observed in the STEM image, so set the indicated magnification accordingly.

    7.2. Navigate to the "Microscope System" (left) side panel; right click on "ADF" and select "Auto Gain" to automatically adjust image contract/brightness (perform as needed).
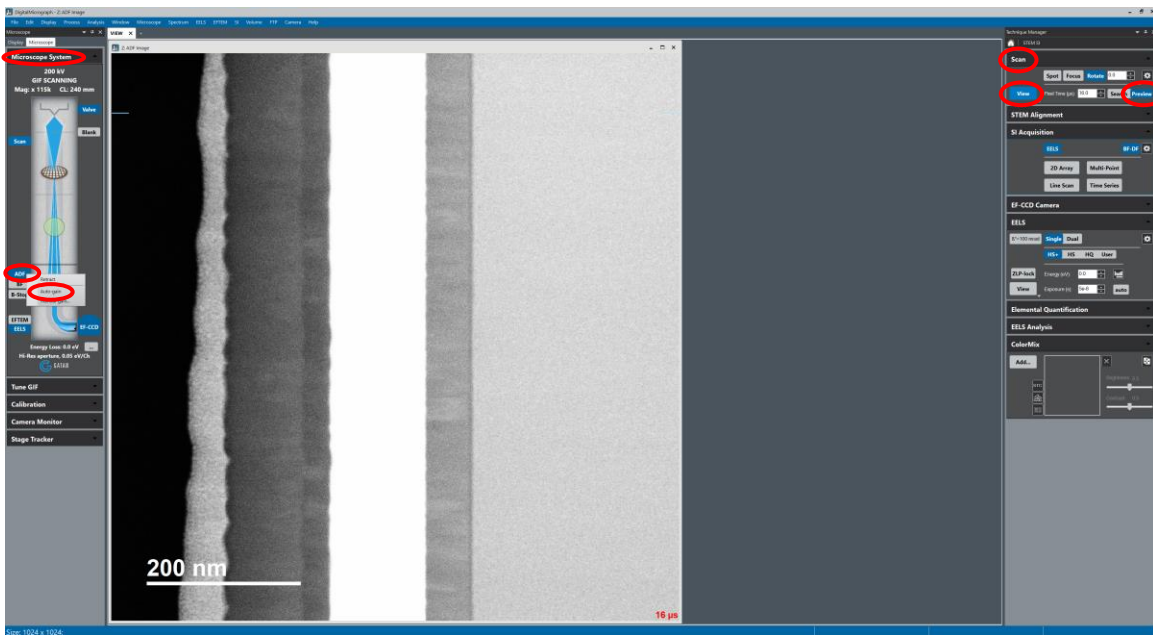
7.3. Right-click on the live STEM image; select the feature align tool and follow the instructions to rotate the STEM image so the feature(s) of interest is/are aligned as desired (perform as needed).
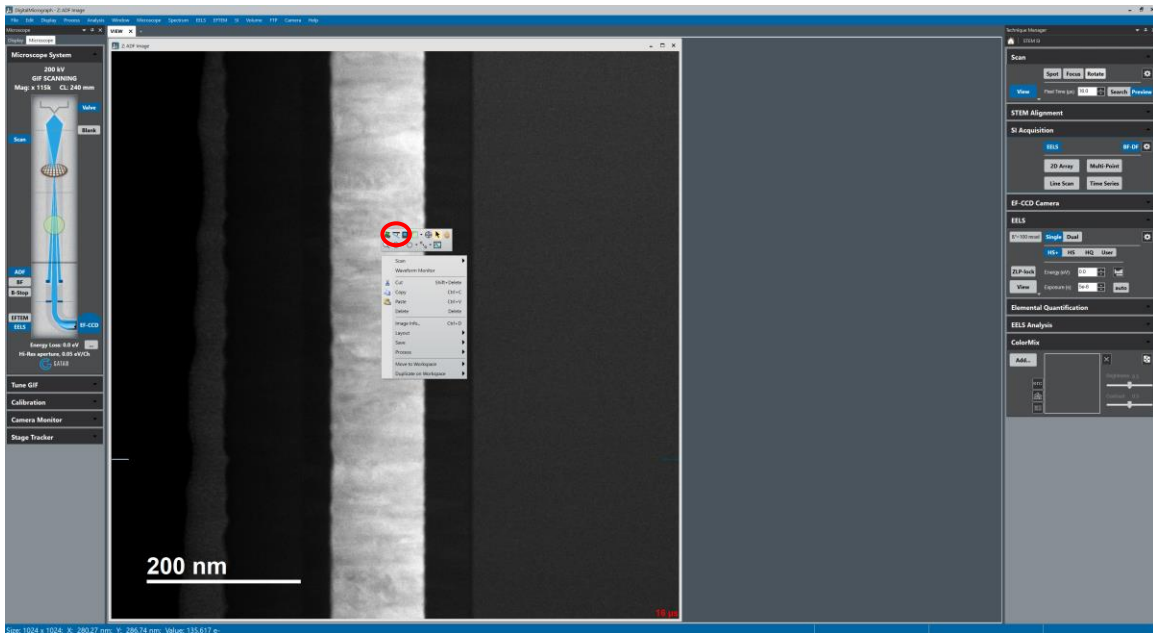

7.4. Remain in the "Scan" (right) side panel; select "Focus" to place a reduced area scanning box in the live image; use this to focus and correct astigmatism as needed.
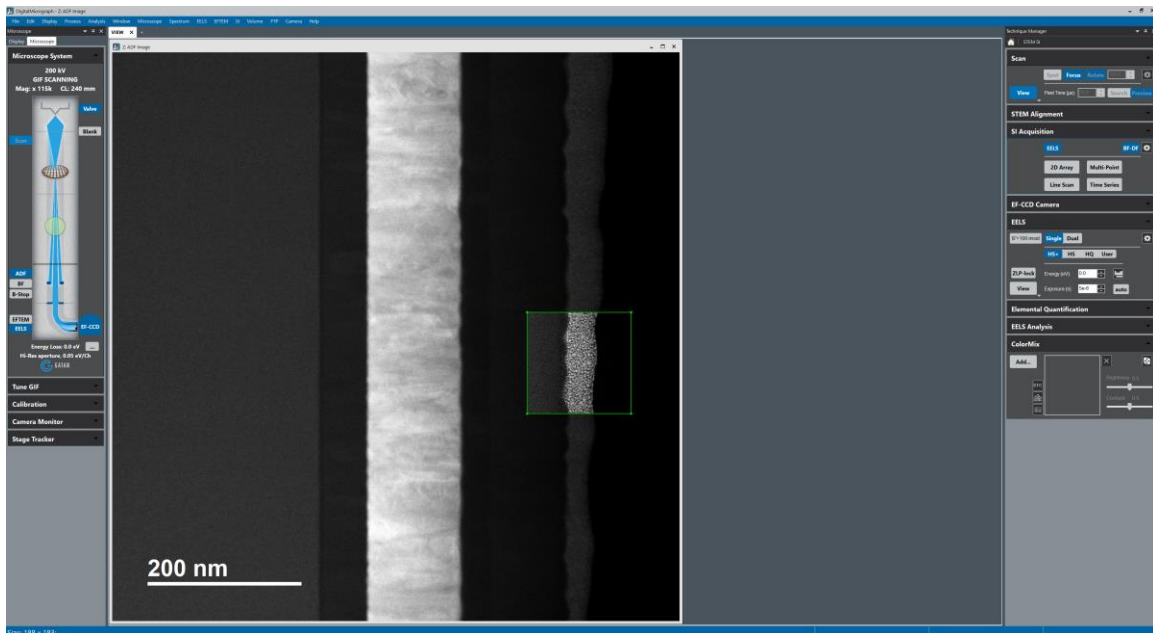
7.5. Remain in the "Scan" (right) side panel; select "Spot" to add the beam "Park" position marker in the image; left click and drag on the marker to position the beam over vacuum; the beam will "park" there *when scanning is stopped*.

7.5.1. If possible, it is always best to *park the beam over vacuum*; otherwise, *position it at a location not of interest for analysis* or that is otherwise expendable.
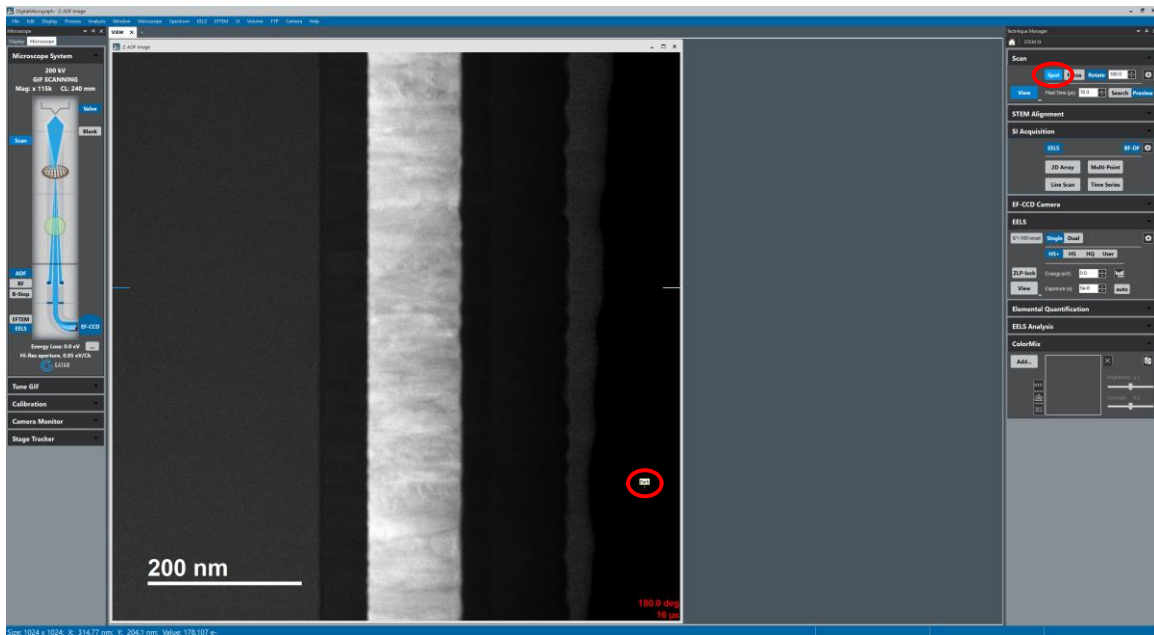
8. GIF focusing

    8.1.   Navigate to the "Microscope System" (left) side panel and select "EELS".

    8.2.   Navigate to the "Scan" (right) side panel and select "View" to stop live STEM imaging; then *park the beam over vacuum*.

        8.2.1.   This is critical for GIF focusing to work correctly; if a vacuum region is not present in the image, place the beam over a region that will *weakly scatter*.

    8.3.   Remain in the "Microscope System" (left) side panel; select "…" to open the "Filter State" dialogue box; select "Filter State" and then "Hi Res"; select an appropriate dispersion (usually 0.750 or 1.500 eV/Ch for element maps).

        8.3.1.   Higher values of dispersion increase the energy range that can be observed in a spectrum at the cost of reduce spectral detail; this is where knowing the core-loss edges of the elements of interest can be useful, to determine the energy range needed to detect all core-loss edges of interest.
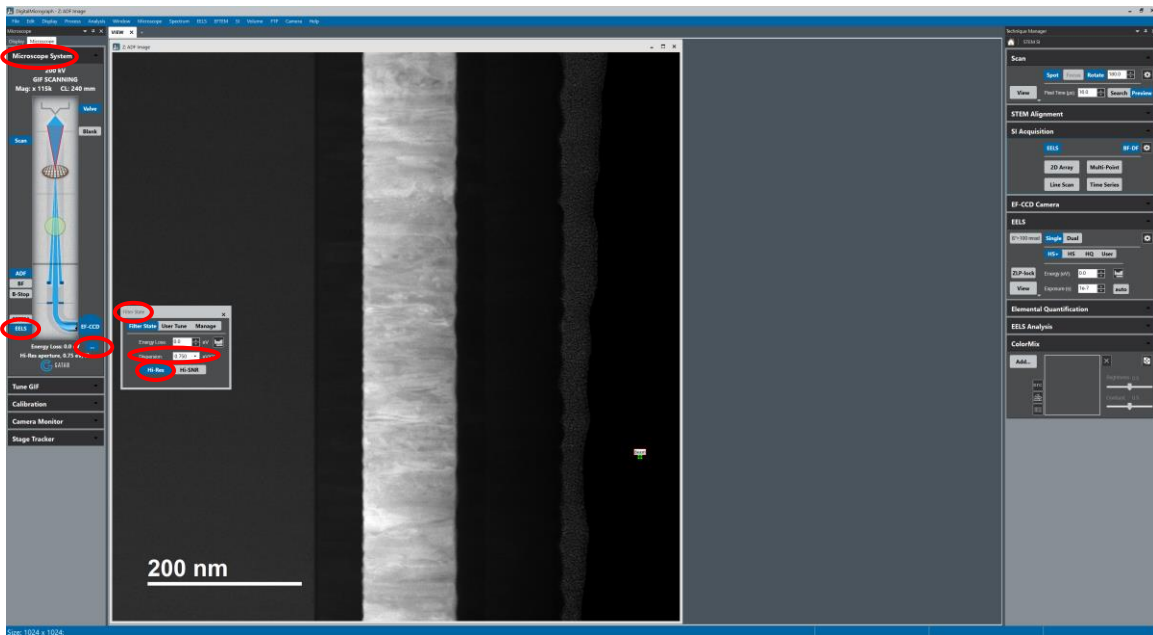
8.4. Navigate to the "Tune GIF" (left) side panel, select "Find ZLP", and then "AutoFocus"; "AutoFocus" should only need to be performed once during the session *unless the dispersion is changed*.
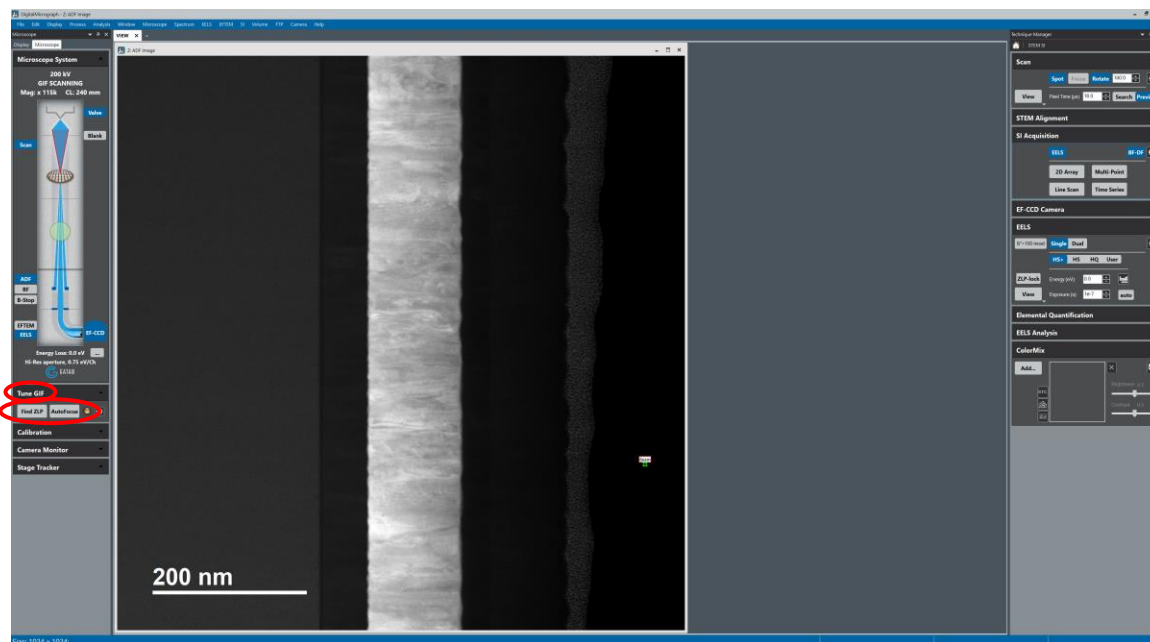
9. Determine spectrum imaging exposure time

  9.1. Navigate to the "Scan" (right) side panel; if necessary, set "Pixel Time" = 16 and acquire a live STEM image.
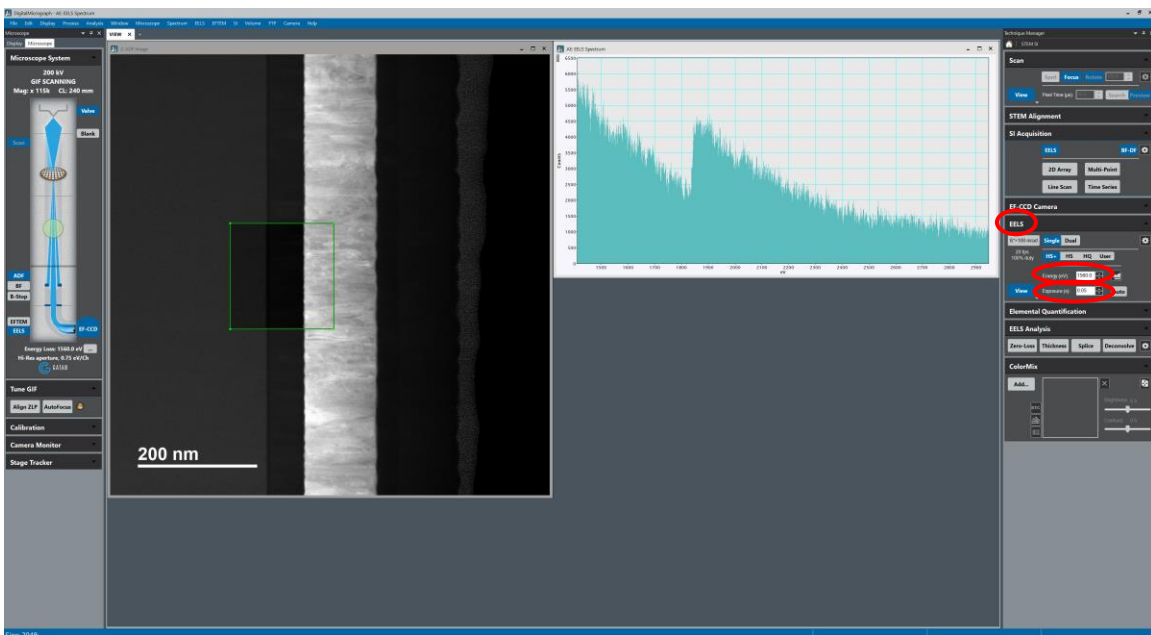
  9.2. Navigate to the "Scan" (right) side panel and select "Focus"; adjust/position the green box in the STEM image approximately where the analysis is to be performed (avoid placing vacuum in the analysis area, if possible).
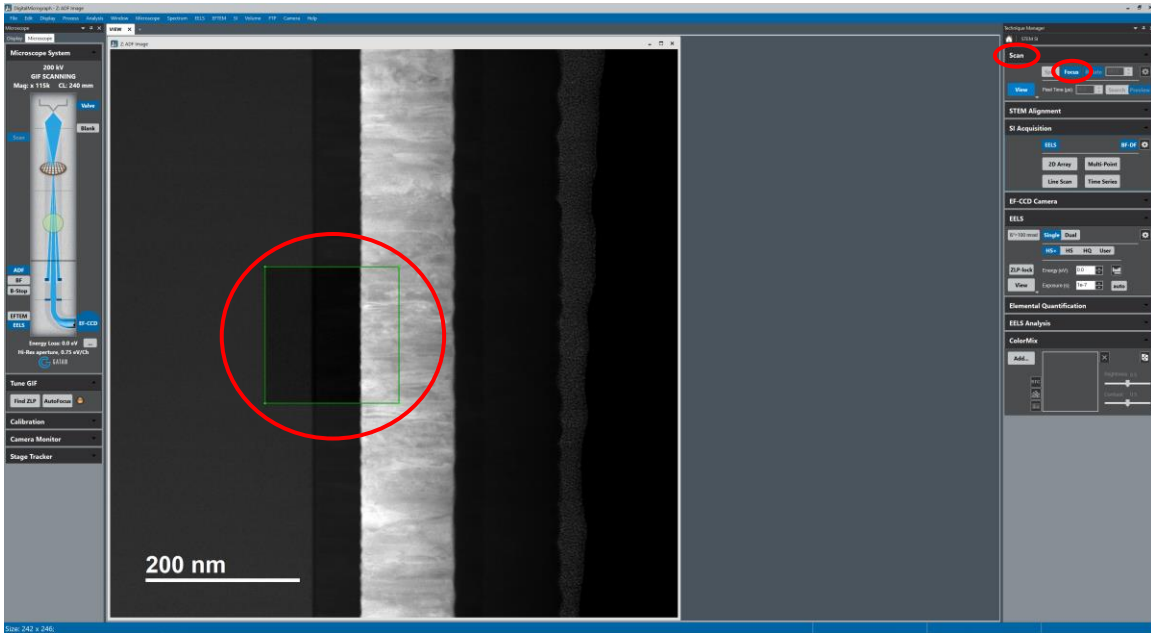
9.3.   Navigate to the "EELS" (right) side panel; <u>set "Exposure" time to 1.0×10⁻⁷ s</u> and select "View" to view an EELS spectrum from the area being scanned.
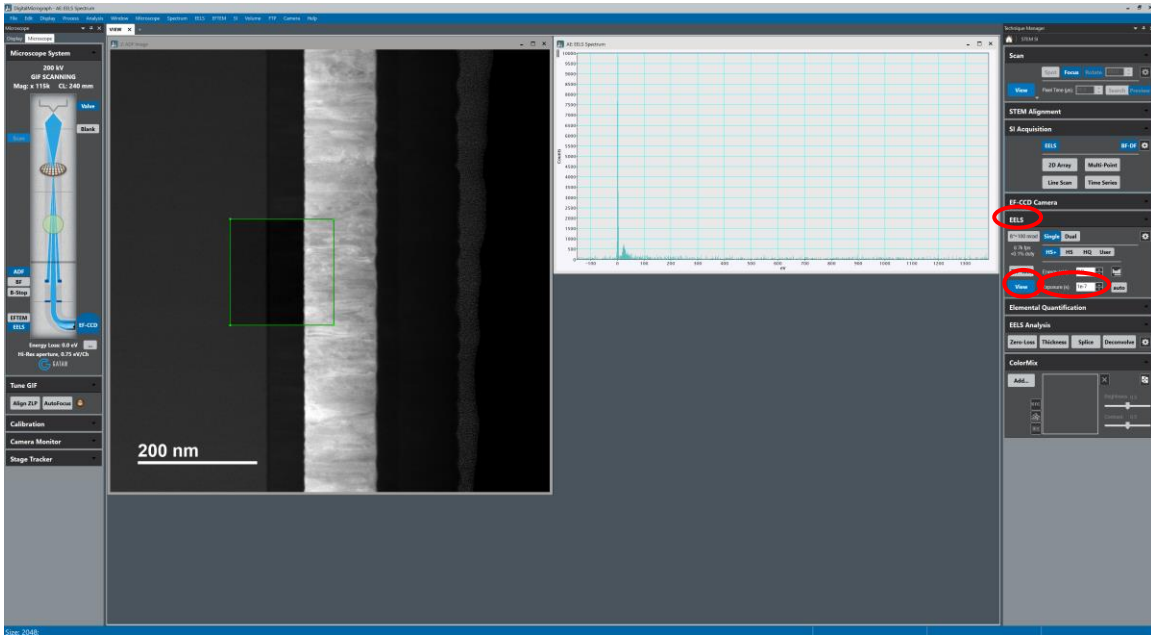

9.4.   Navigate to the "EELS" (right) side panel; adjust the "Energy" offset to shift the ZLP off the spectrum and to move the first core-loss edge of interest to the left side (channel zero) of the spectrum; *increase the "Exposure" time until all desired core-loss edges are visible* (DO NOT let the spectrum turn red) and *note this "Exposure" time*.

9.5.   Navigate to the "EELS" (right) side panel and select "View" to stop acquiring the spectrum ("View" will turn gray").

9.6.   Navigate to the "Scan" (right) side panel and select "Focus" to resume full-frame scanning; position and focus the image as needed and select "View" to stop scanning ("View" will turn gray after frame completes).

9.6.1.   When the scan is completed; be sure to move the beam position marker off the specimen (if possible) or away from the area of interest.
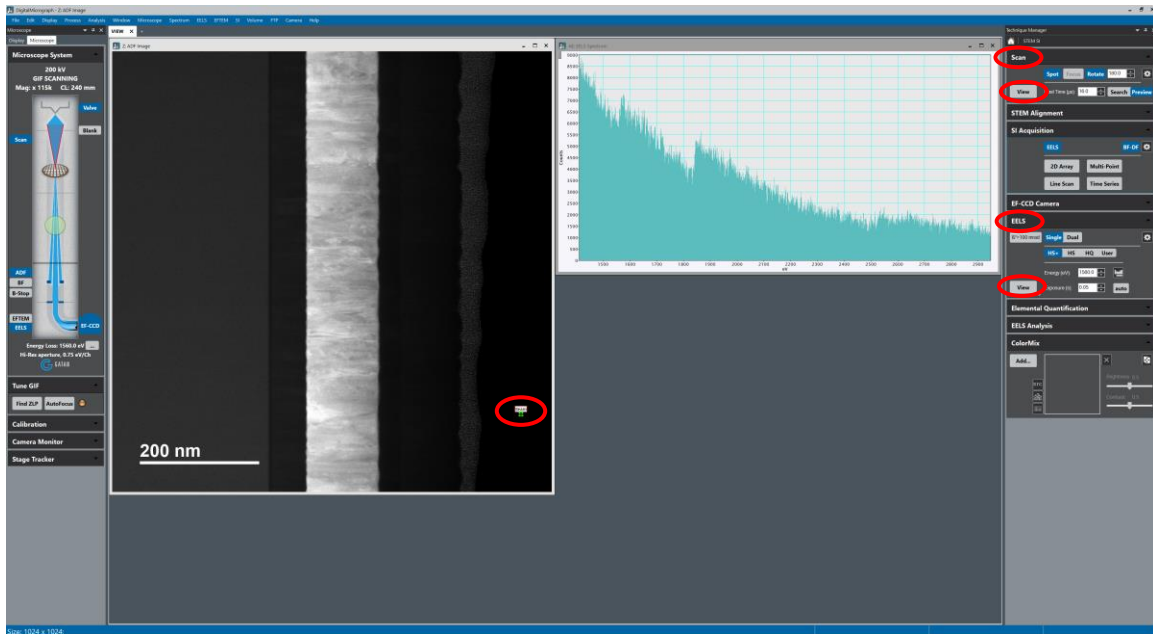
10. Spectrum imaging (2D array)

10.1. Navigate to the "SI Acquisition" (right) side panel and select "2D Array".
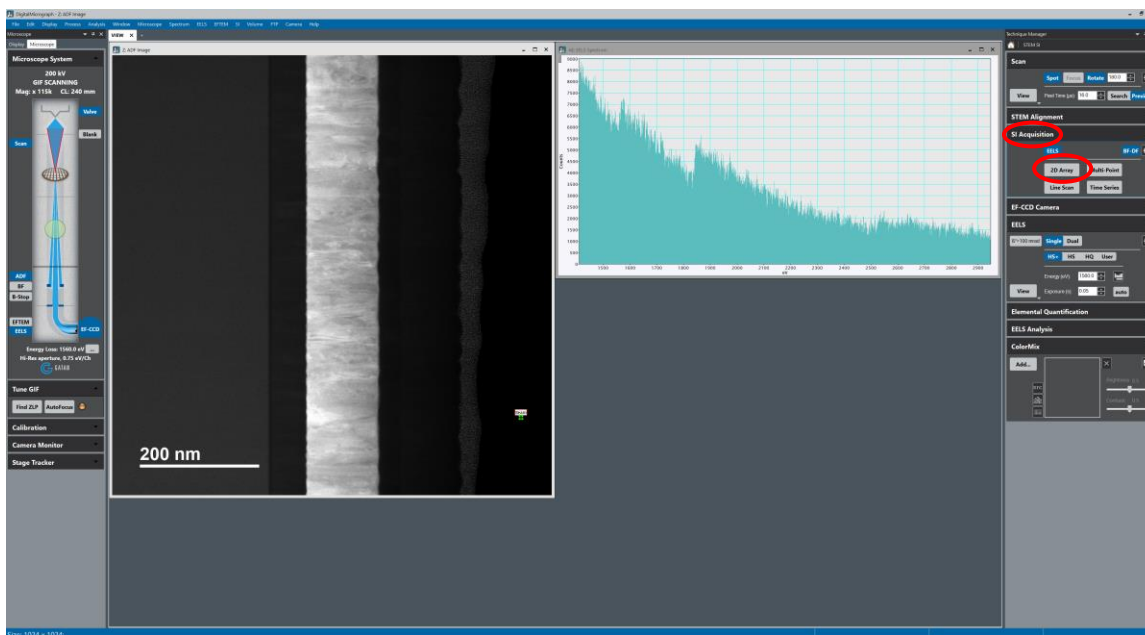
10.2. Position/size the green "Spectrum Image" box on the STEM image as needed; Reman in the "SI Acquisition" (right) side panel; set the "Pixel Time" to the exposure time determined in 7.4 and set the "Step" (pixel) size to be used for the spectrum image.

10.2.1. In general, it is best to set the "Step" size to the pixel size for the image; right click on the image and select "Image info" to check the image pixel size.
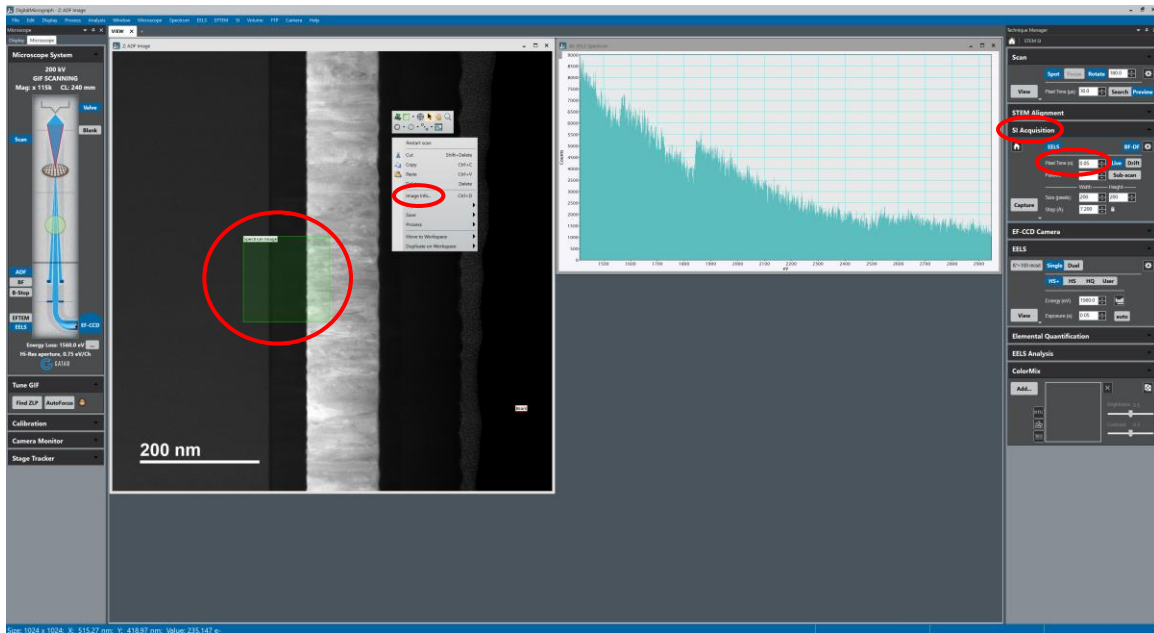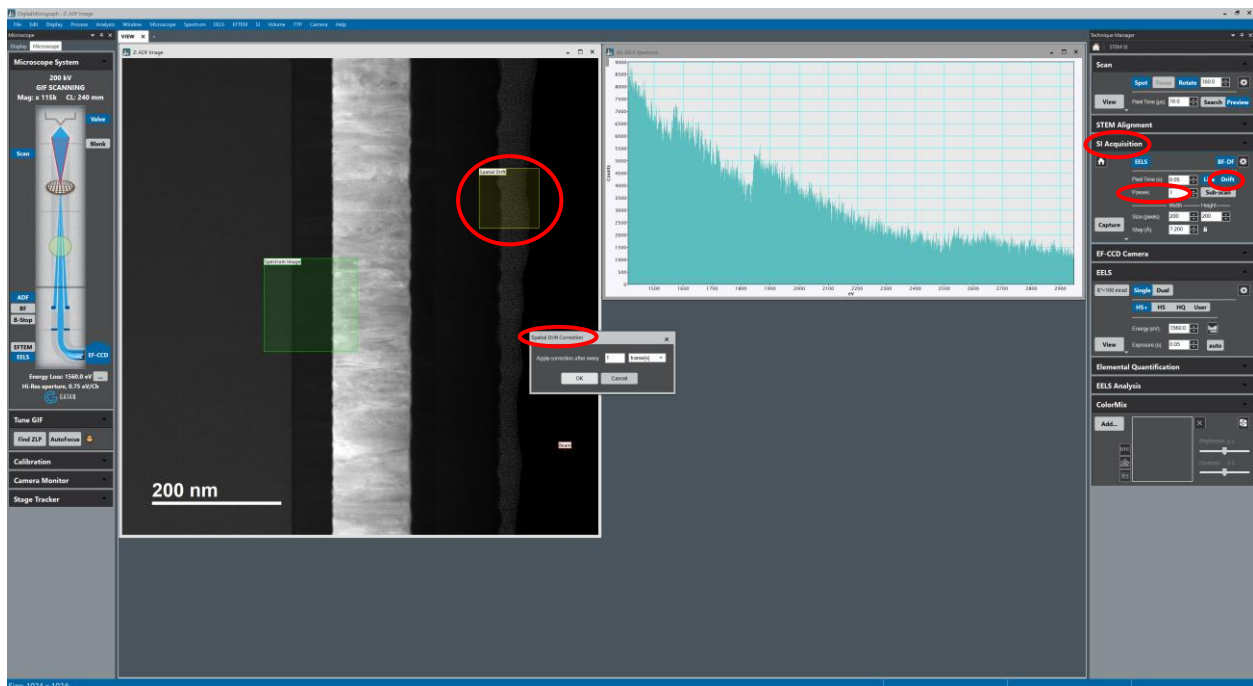
10.3. Remain in the "SI Acquisition" (right) side panel and set the number of "Passes" as needed.

   10.3.1. Multiple passes are general only used if the time for a single frame is <30 s.

10.4. Remain in the "SI" Acquisition" (right) side panel and select "Drift"; position the yellow "Spatial Drift" box over an area suitable for drift correction (a region with distinct features).

    10.4.1. If a single pass is being collected, *perform drift correction after every single (1) row*.

    10.4.2. If multiple passes are being collected, *perform drift correction after every single (1) frame*.

10.5. Right-click on the STEM image and select "Restart Scan" to scan the beam one more time; reposition the boxes as needed; remain in the "SI Acquisition" (right) side panel and select "Capture" to start acquisition.
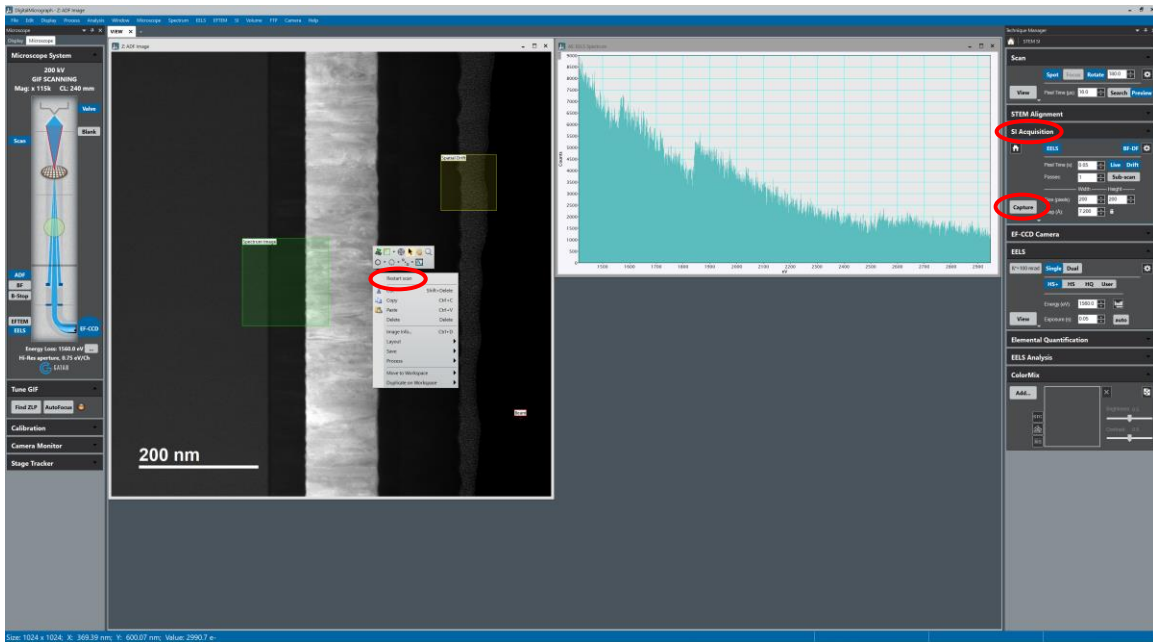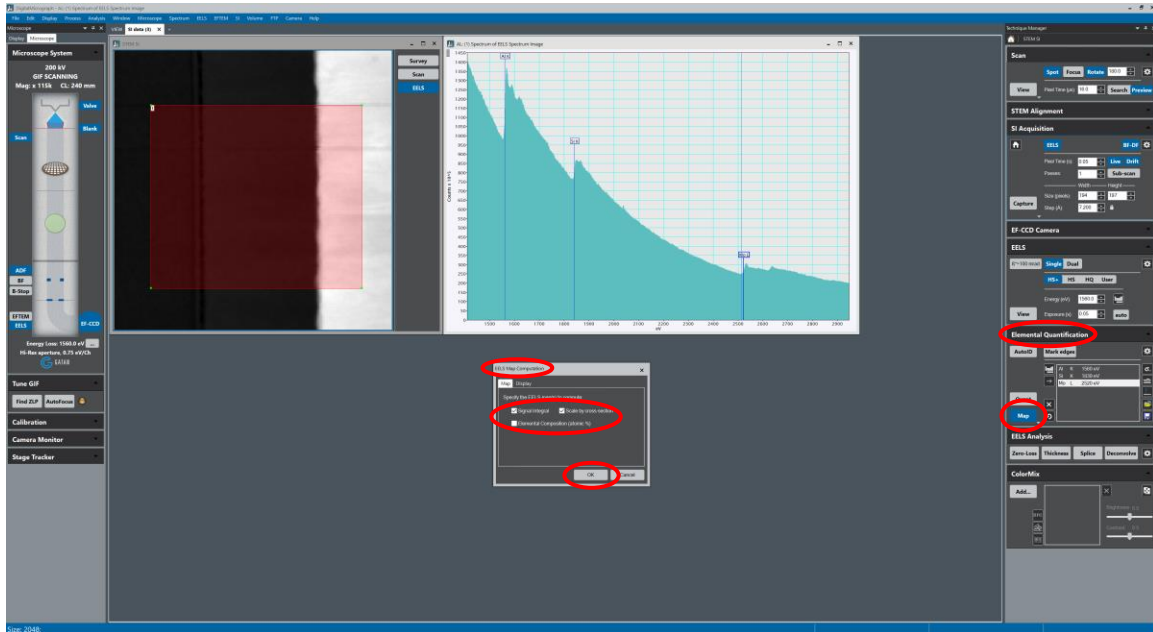

10.6. Navigate to the newly generated "SI data" workspace; in the "STEM SI" window, select "EELS" in the upper right corner; right-click on the image and select "SI Picker".
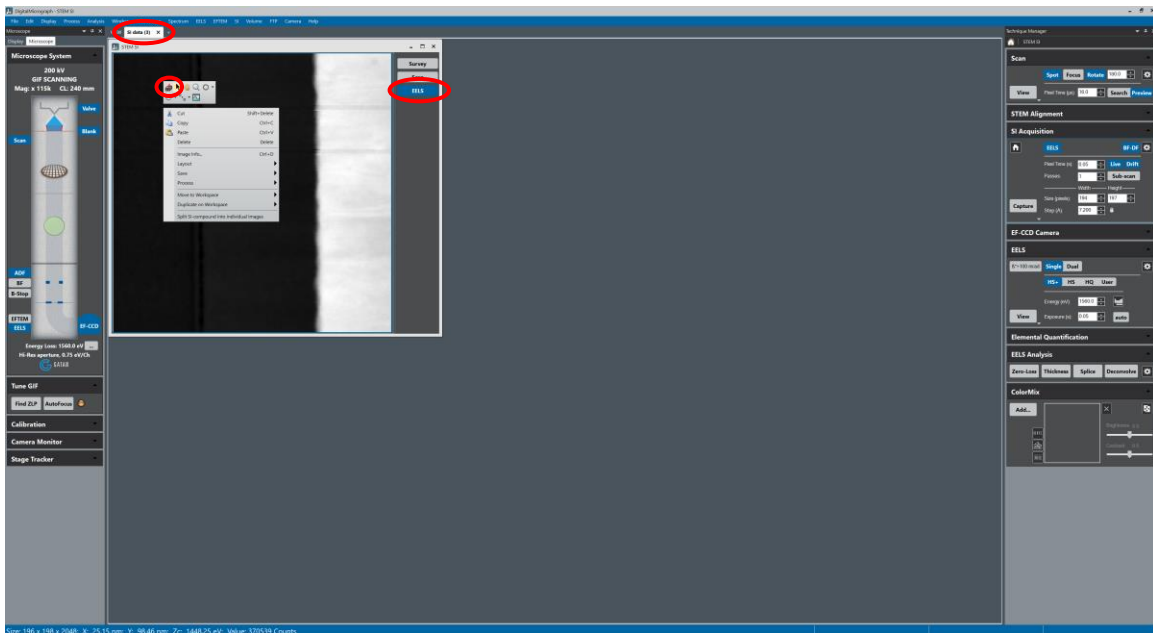
10.7. Left-click and drag to position/size the red box as needed; the extracted EELS sum spectrum will be displayed; right click on a core-loss edge, select "Add to Quant", and then select the element of interest; repeat this for all other elements of interest.
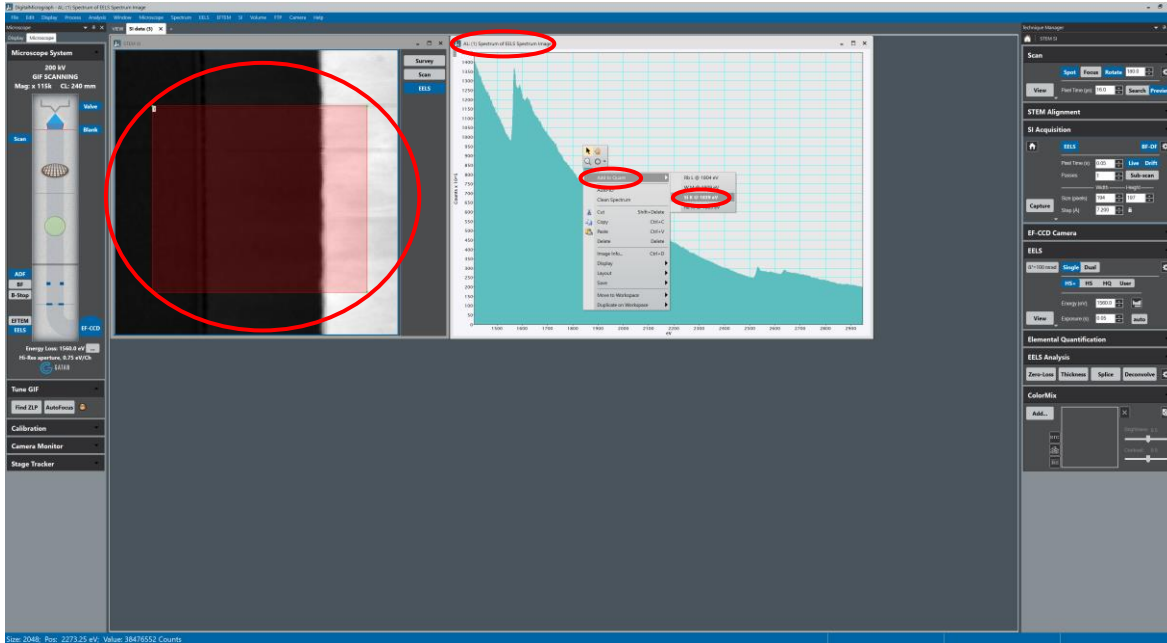
10.8. Navigate to the "Elemental Quantification" (right) side panel and select "Map"; in the "EELS Map Computation" dialogue box, check "Signal Integral" and "Scale by cross-section" to produce scaled counts maps or "Elemental Composition (atomic %)" to produce atomic % maps and select "OK".
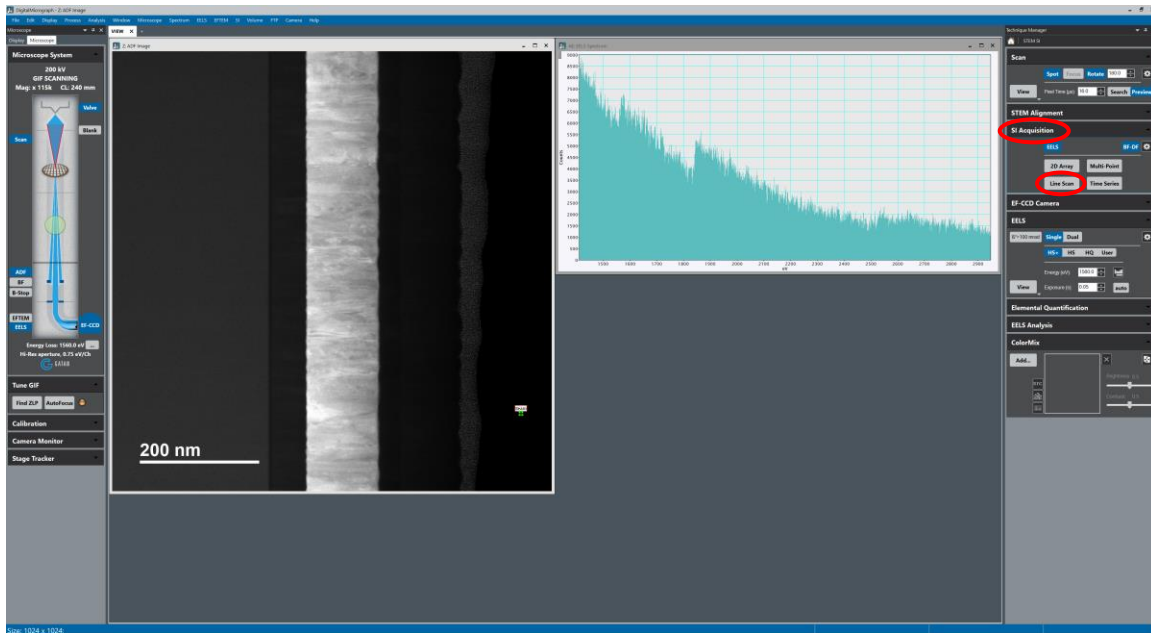

10.9. Navigate to the newly generated "EELS Map" workspace; to generate a composite map, navigate to the "ColorMix" (right) side panel and select "Add"; in the "Select Color Mix Components" dialogue box, select the maps of interest and select "OK" to generate the composite map.
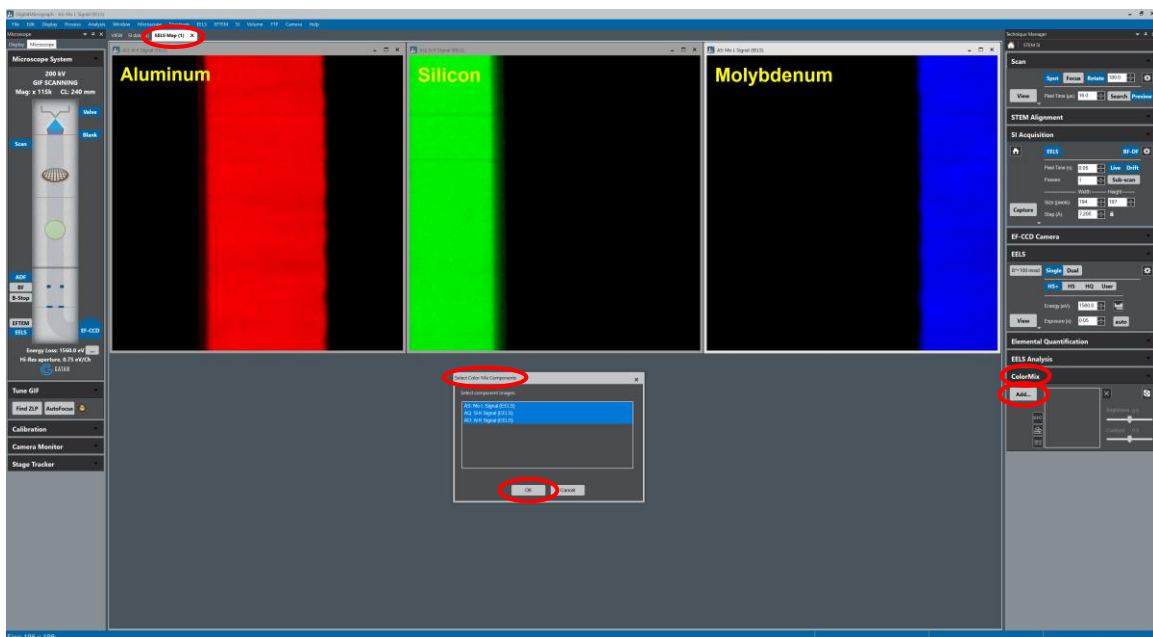
11. Spectrum Imaging (Line Scan)

   11.1. Navigate to the "SI Acquisition" (right) side panel and select "Line Scan".


   11.2. Setting the parameters for a line scan (Pixel time, Step, Passes, Drift correction, etc.) is essentially identical to as described for a 2D array.

      11.2.1. If using drift correction for a line scan, be sure to *apply correction after every single (1) frame* (a "frame" in this instance is now taken to be *one pass along the line*).
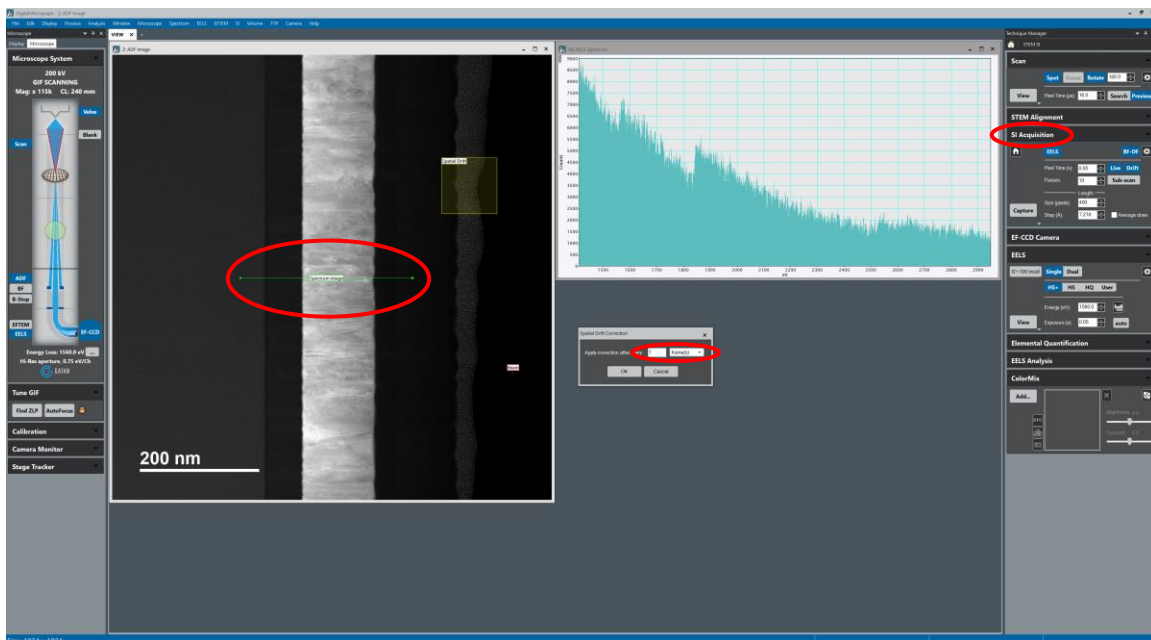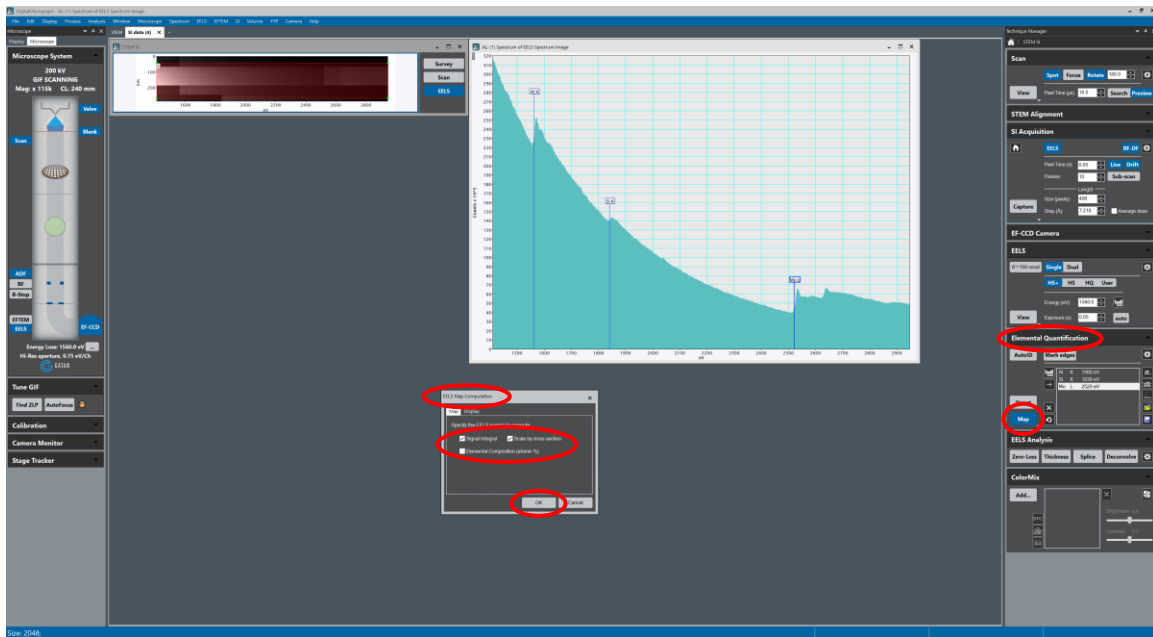
11.3. A newly generated "SI data" workspace will be created; the process of identifying and selecting core-loss edges for the line scan is essentially identical to as described for a 2D array.
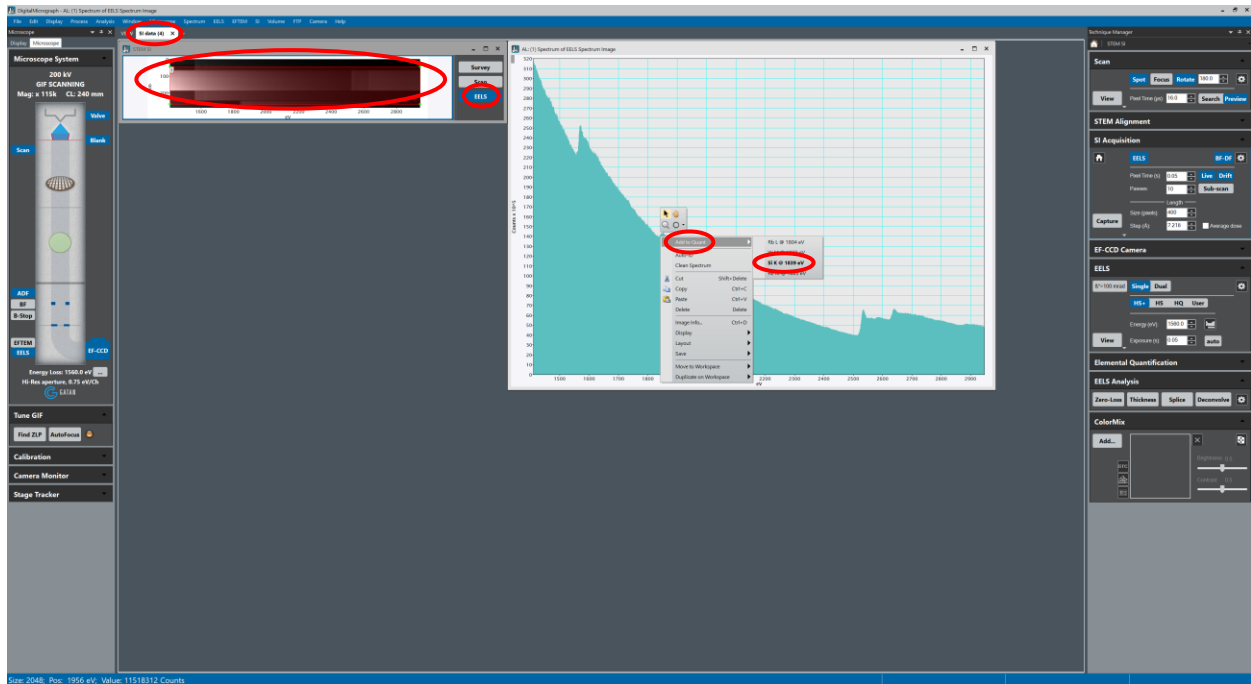
11.4. After adding all core-loss edges of interest, generate the line scans (either scaled counts or atomic %) similarly to as described for a 2D array.

**11.5.** A new "EELS Map" workspace with the composite line scan will be generated.
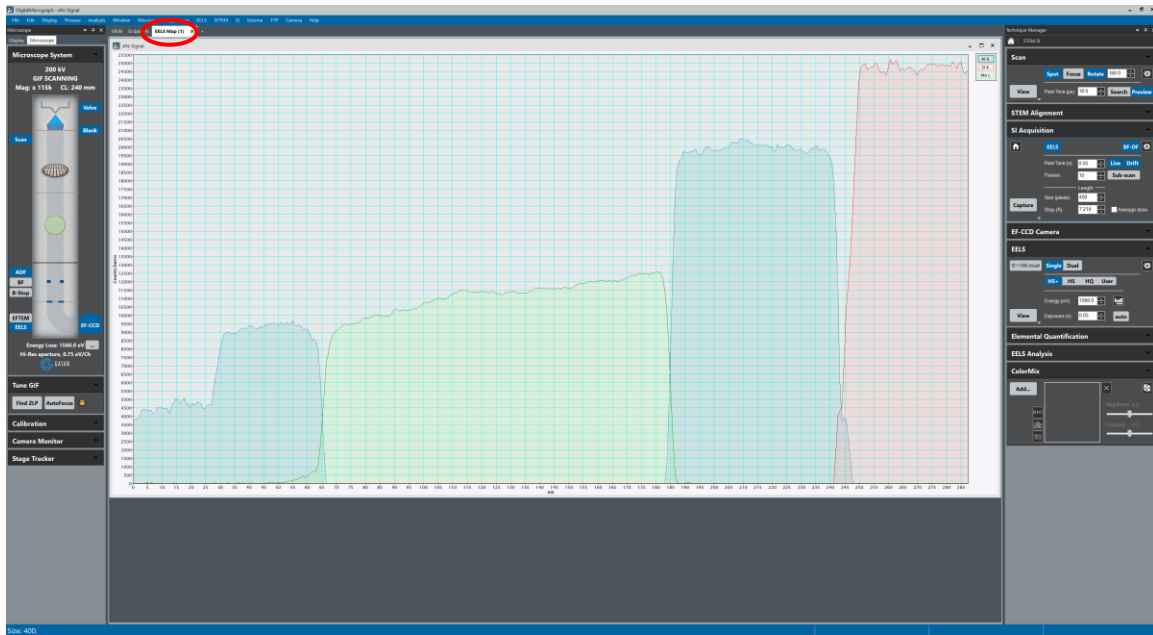
12. Dual EELS

12.1.   In some instances, it may not be possible to obtain two core-loss edges of interest in a single spectrum if the energy difference between the edges is very large. In such instances, Dual EELS can be used to capture "low loss" (LL) and "high loss" (HL) spectra at two different energy offsets with two different exposure times.

12.2.   As described previously, acquire a live STEM image and place a "Focus" box over the area of interest.

12.3.   In the "EELS" (right) side panel, select "Dual"; there is now the option to collect spectra at two different energy offsets and two different exposure times; as described in previously, *adjust each exposure time until the core-loss edges of interest are visible in each spectrum*.

   12.3.1.   The LL exposure time *will be the value used for spectrum imaging* and should be substantially smaller than the HL exposure time.
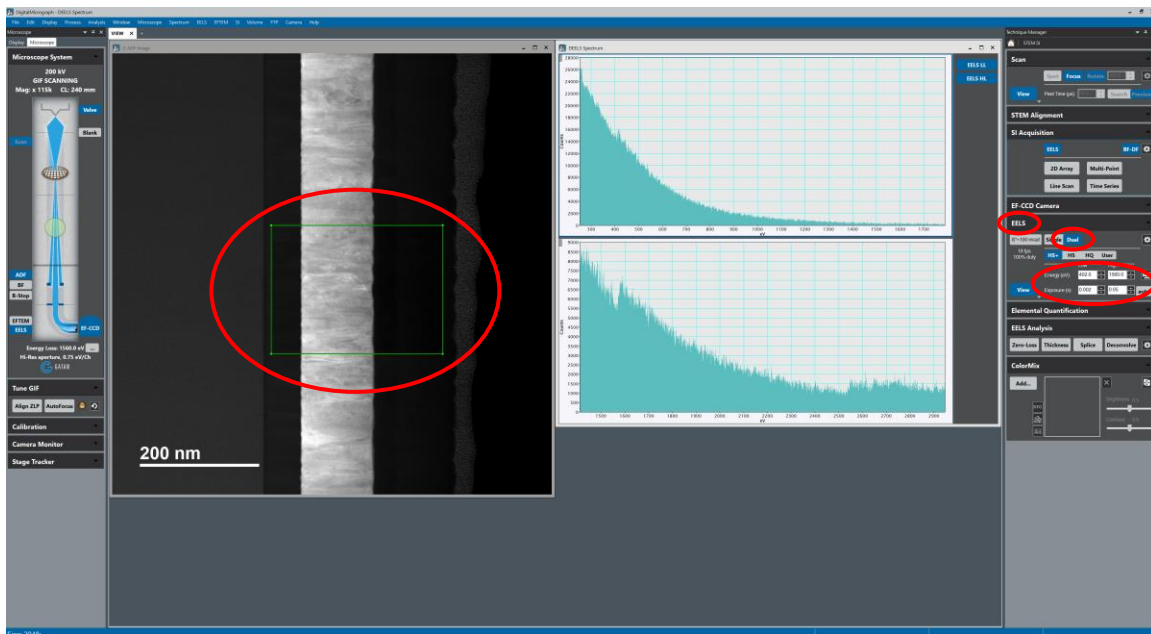
12.4. Setting up and collecting the spectrum image when using Dual EELS is essentially identical to as described previously (a 2D array is shown here). Use the LL exposure time determined in 10.3 for the "Pixel time"; set "Passes" equal to the ratio of the high loss exposure time to the low loss exposure time (e.g. 0.05 s / 0.002 s = 25 passes).
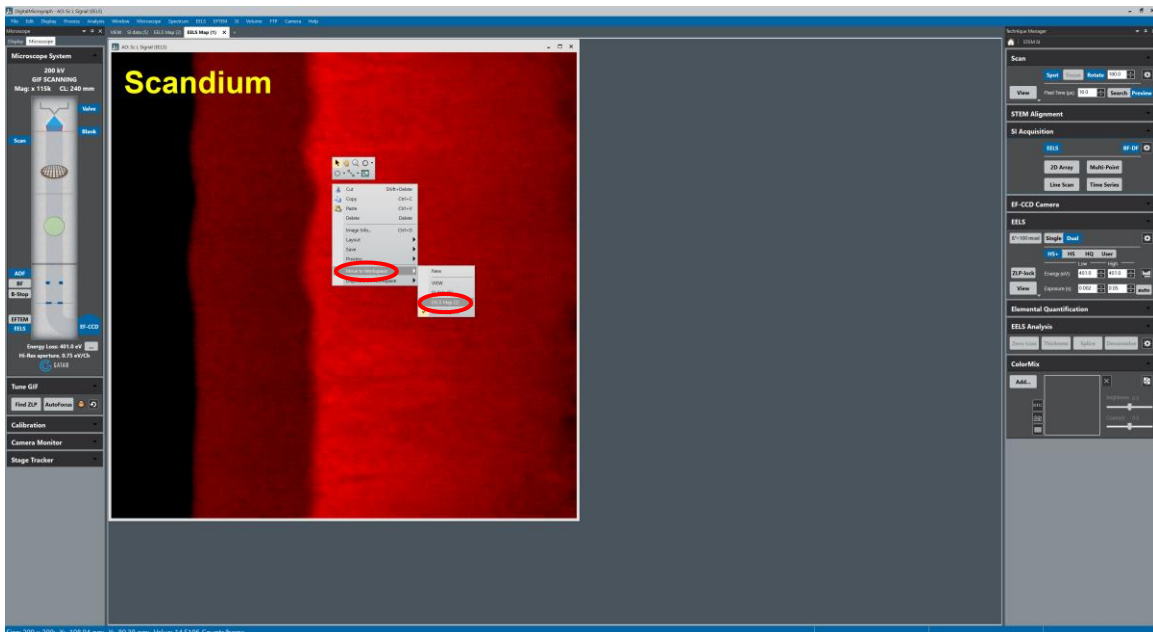
12.4.1. Setting the "Passes" to this value ensures sufficient HL signal will be collected.
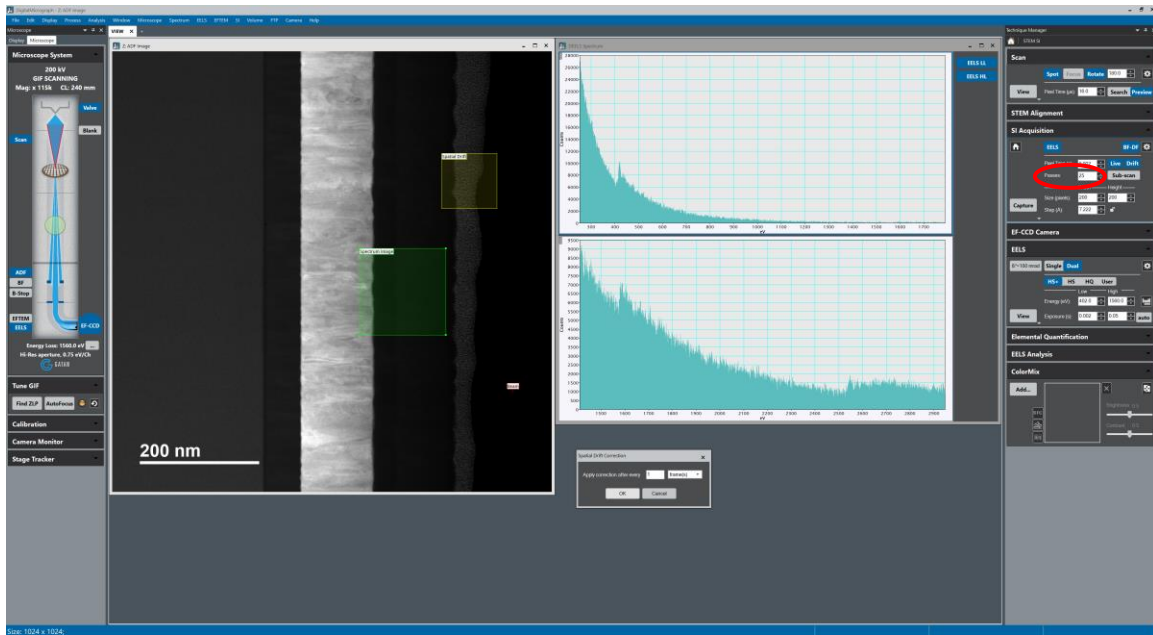
12.5. Selecting core-loss edges for mapping and generating the maps is essentially identical to as described previously; the only difference is that _this must now be done for both LL and HL spectra_.
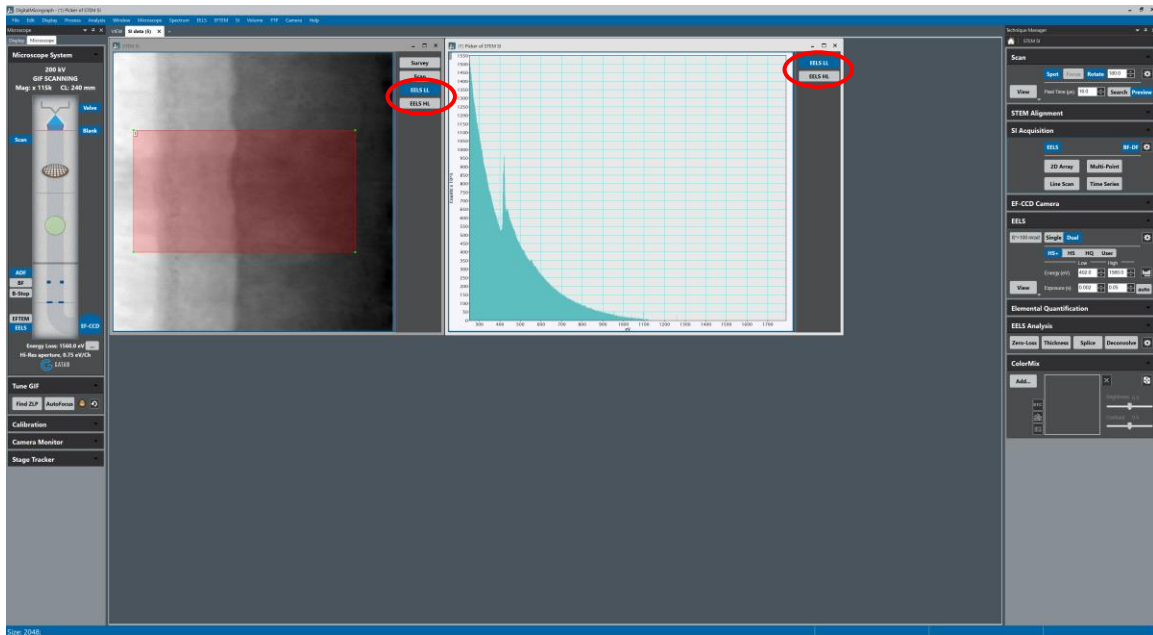
12.6. _Due to both LL and HL spectra being present, maps will be created respectively in two different workspaces_. To create a composite, _all maps must be in the same workspace_. To move a map to another workspace, right click on the map, select "Move to Workspace" and then select the appropriate workspace. Once all maps are in the same workspace, the composite may then be created as described previously.

13. Finishing

13.1. Insert the viewing screen to block the beam from entering the GIF; verify no STEM image or EELS spectrum is actively being collected.

13.1.1. Navigate to the "Scan" (right) side panel and verify "View" is gray.

13.1.2. Navigate to the "EELS" (right) side panel and verify "View" is gray; if Dual EELS was performed, switch back to "Single" mode.

13.2. In Microscope Control, select the "EFTEM" tab; navigate to the "Filter" control panel and select "EFTEM".

13.3. Deactivate "High Resolution" mode for the FluCam.
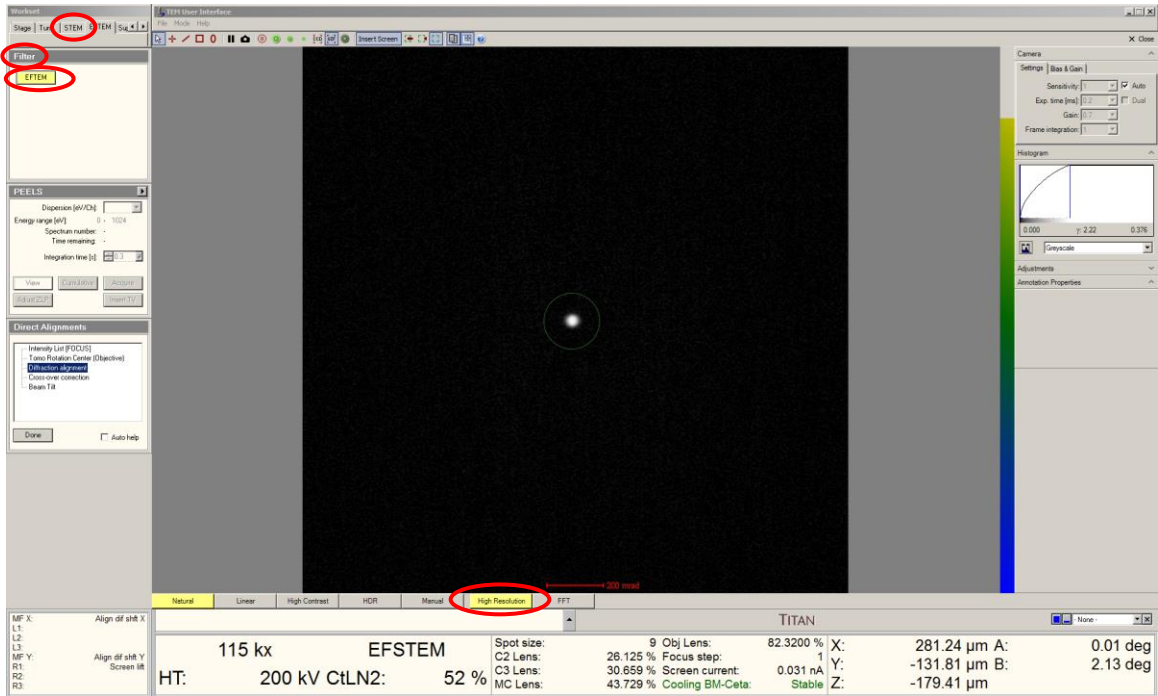
13.4. Locate the gray scan input box on the workstation desktop; select the "INT SCAN" button; this returns control of the STEM signal back to the microscope.