Talos F200X
Standard Operating Procedure - Short

These instructions are intended for reference only, and will not replace the thorough training required for proper system operation. Contact an electron microscope staff member with questions or to report a system problem.
1. Enable the tool in BADGER

2. LN2: Prior to insertion of the sample holder, liquid nitrogen should be installed. Always use safety glasses when handling liquid nitrogen. If you need assistance contact the lab’s staff.

3. VACUUM: Look at the main vacuum menu for the system. Prior to sample insertion equivalent values (± 2 on the Log scale) should be shown on the screen. Especially important is the value of below 15 on the column reading. Nitrogen level should be > 20%. Ensure that the Column valve is closed (BUTTON IS YELLOW).
4. **SAMPLE LOAD to HOLDER:** Use gloves. After cleaning and degassing your sample select an appropriate holder. Use N₂ gun to clean dirt from its tip.

5. **SAMPLE LOAD to HOLDER cont:** Using the vacuum tweezers insert the 3mm grid sample into the holder cup with area of interest facing down. Lock clamp and use optical microscope to confirm position and lock. Do not use solvents to clean holder. Check the O-ring to ensure that there is no dirt on it. If there is, wipe your gloved finger along it to remove it. If it feels rough to the touch then please add a small amount of formalin oil.
6. **SAMPLE INSERTION: RESET STAGE:** Stage is at the home position: “Search” workset “Stage” pull out menu. Click “Reset Holder”. Look at the X, Y and Z positions and the a and b tilt menus.

7. **On the main ‘FEI User Interface’ Screen check that the sample position (X, Y and Z) and sample tilts (alpha and beta) read near zero values (they may not be exactly at 0.00). Make sure that the objective aperture is out.**

8. **LOAD SAMPLE:** On the microscope touch screen press “load sample”.

[Images and diagrams related to the steps are shown here.]
9. **INSERT HOLDER:** Carefully insert the holder, with the holder pin aligned at the 5 o'clock position, as far as it will go into the CompuStage. Be careful not to scrape the tip. You will feel a slight pressure when the o-ring hits the mating seal. Push firmly but carefully until the holder goes in about 1 inch further and stops at the final position of the mating seal.

10. **HOLDER TYPE SELECTION:** Select the Single Tilt (unless you’re using double tilt and then consult staff) option on the FEI TALOS monitor interface and wait for the load lock to pump down.

11. **PUMPING LOAD LOCK:** The remaining pumping time for the specimen exchange is shown on the “Vacuum Overview” screen.
12. **FINAL INSERTION:** When the pumping cycle ends (red light on the CompuStage turns off), support the surface of the goniometer with one hand and with the other rotate the holder pin clockwise from 5 o’clock to 12 o’clock position. Do not let go of the holder at this point!

13. Gently guide the holder into the column. Tap the end of the holder to ensure stabilization. *(Note: For high-resolution work, you may have to wait 30 minutes or more for the O-ring seal to stop drifting.)*

14. In the event of dumping the vacuum contact EM staff member immediately. They will recover the vacuum.

15. **COLUMN VALVE:** Ensure Column pressure is below log 15 and press “Col. Valves closed” button, so it goes grey.
16. **FLUCAM VIEWER:** A live TEM image will automatically appear in the Flucam Viewer. If image does not appear check that Flucam viewer is in an un-paused position and screen is inserted (“insert screen”). It’s easier to find image at low magnification. If image is too dark (thick sample) use joystick to move sample to thinner area.

17. **SELECT CONDITIONS:** In the FEG Registers window, select aLbl(=label) line for the TEM working Acc Voltage (e.g. 200 or 80KV). Click on “Set” to load optimized working conditions at this voltage.

18. **MICROPROBE:** mode is activated as can be seen by the Beam Setting window.
19. **CENTER CONDENSER APERTURE (C2):** Condenser 2 aperture is typically 150 µm, could be 50, 70, 100 µm as well. Select the aperture in Apertures window. Set mag to SA XX (e.g. 130 kX). Use the intensity knob to condense the beam to a spot. Center the beam (in the Flucam Viewer). Expand the beam (intensity knob clockwise) to 4 cm. The beam should cover the circle evenly. If not click on “Condenser 2 Adjust” in the Apertures window and use the Multifunction X and Y knobs to align it with the circle. Repeat these steps until aligned and then press: “Condenser 2 Adjust”.

20. **CONDENSER STIGMATION:** turn the intensity knob clockwise (CW) and counter-clockwise (CCW) and observe its shape. If it’s elliptical click on the condenser in the Stigmator window. Adjust the beam circular and concentrically expanding using the Multifunction X and Y knobs. Click on “None” to save the
stigmator coordinates in column. It might help to write down (or copy to second column) the initial coordinates in case the beam is made worse and you wish to retrace your adjustments.

21. **SET EUCENTRIC HEIGHT:** Go to the search tab and flap out the window next to the stage window. Press the Wobbler button and it should turn yellow. This causes the live image of the Flucam to rock back and forth. Press the Z button on the control panel to reduce the movement until a feature remains steady and is at minimum contrast.

22. **DIRECT ALIGNMENT - Gun Tilt, Method 1:** (at 10-60 kX mag), center the beam in a sample hole, expand the beam within the hole to align with the largest circular target in the Flucam Viewer. Click “Gun Tilt” in the Direct Alignments window. Using the Multifunction X and Y knobs maximize the Screen current (nA) or minimize the Exposure time (s). Click “Done” in the Direct Alignments window to reserve the current Gun tilt conditions.
23. **DIRECT ALIGNMENT - Gun Tilt, Method 2 (alternative to 21, at 2-5 kX mag):** on a hole or amorphous area of the sample insert the beam stopper. Turn the intensity knob CCW to condense the beam to a spot and move the beam to the shadow of the beam stopper (appears as bright blocked spot and 4 diffused satellite spots in the Flucam Viewer). Click “Gun Tilt” in the Direct Alignments window. Using the Multifunction X and Y knobs adjust the bright spot to coincident with the diagonal center of the 4 diffused spots while keeping the bright spot blocked by beam stopper. Click “Done” in the Direct Alignment window to save conditions. Expand the beam and withdraw the beam stopper.
24. **GUN SHIFT (at mag 10-60 kX):**
Condense beam to cross over at spot size 3 and use multifunction X and Y to bring the beam to the center. Now go to spot size 9 and use the track ball to move the beam to the center. Continue this process until the spot remains in the same place and finish on spot size 3. This process does not need to continue beyond magnification 50,000X

25. **DIRECT ALIGNMENT - Beam tilt PPX and PPY (at mag to above 130 kX):**
Turn intensity knob CCW and condense the beam to a spot or small circle and center the beam. Click on “Beam tilt pp X” in the Direct Alignments window to wobble the beam. Use the multifunction X and Y knobs to
minimize the beam movement. If you lose the beam reduce the mag and center the beam. Click “Done” to save. Repeat for Beam tilt pp Y.

26. **BEAM SHIFT**-Click on **Beam shift** in the **Direct Alignments** window. If the beam is out of view, reduce the magnification. Adjust the Multifunction X and Multifunction Y knobs to center the beam. Bring the magnification back up to 130 kX or higher, and repeat. Click on the **Done** button in the **Direct Alignments** window to reserve the current **Beam shift** conditions.
27. **ROTATION CENTER** - Set magnification to 130 kX or higher. Turn the Intensity knob clockwise to fully expand the beam to cover the screen. Use the position joystick to bring the focused feature to the center. Click on **Rotation center** in the **Direct Alignments** window to wobble the beam. Adjust the Multifunction X and Multifunction Y knobs to minimize the image shift (i.e. minimal lateral movement), using the focused feature for visualization of the image shift under the wobbling beam. Click on the **Done** button in the **Direct Alignments** window to reserve the current **Rotation center** conditions.

28. **OBTAINING AN IMAGE: Mode 1** (with objective aperture, more contrast less resolution). If you’re not using objective aperture skip to 30: Set mag to higher than 2250 and turn the intensity knob CCW to fully expand the beam. Press “Diffraction” [and “HDR” (High-Dynamic-Range) in the Flucam Viewer if diffraction is difficult to see]. Center the desired diffraction spot, click “Diffraction Alignment” in the Direct Alignments window. Use the
multifunction X&Y knobs to center the direct beam spot. Click “Done” to save alignment conditions.

29. **OBTAINING AN IMAGE** Mode 1 – cont. Select an Objective aperture (e.g. 30 µm) from the Objective drop-down list in the Apertures window. The aperture and central diffraction spot should be observed in the Flucam Viewer. Click on the “Objective Adjust” button. Center the aperture using the multifunction knobs. Click on the “Objective Adjust” button again to save. Press “Diffraction” to exit diffraction mode. Repeat process at higher mag until desired mag is reached. Retract Objective aperture.

30. **OBTAINING AN IMAGE** (with or without an objective aperture): The CCD camera is sensitive to beam intensity. **Never change mag or intensity under camera viewing mode.** Find an area of interest, select mag and center the beam (skip if you used the objective aperture). Expand the beam until Exposure time is >2sec. In the CCD/TV Camera window, press Insert until the button turns yellow (camera
inserted). Click on “Search”, the camera will be automatically inserted and start to scan. In the TIA (TEM Imaging and Analysis) program a live image window will appear. Press R1 to lift the screen. The TIA image should show the Flucam Viewer screen. Focus the beam (using Focus knob) and correct Objective stigmation (click “Objective” in Stigmator window and use multifunction knobs. “None” to save). Click on “Acquire” in the CCD/TV Camera window. Slow scan rate will increase image quality. Choose Binning (1 reading per pixel) and Integration time (1-2 s, shorter if image drifts). Save images in Transfer folder in .emi format or export image (right click) in other formats. Press “R1” to reinsert the screen and “Insert” in the CCD/TV Camera window to withdraw the camera.

31. **UNLOADING A SAMPLE:** Make sure the Objective and Selected Area apertures are out. Condenser 1 aperture should always be at 2000 µm. Close Colum valves (button should appear yellow in Flucam Viewer). Reset the specimen holder: Under Stage2 click on Flap-out, control, “Holder” under Reset. Stage coordinates and tils should
be zero. Unload the specimen holder (support the goniometer with one hand, pull the holder straight back as far as it will go. Stop. Rotate the holder pin CW from 12 to 5 O’clock. Disconnect cable cord for double tilt, stabilize the hand holding the sample holder and pull it back and out of the column), remove your sample and keep the holder in the dry box.

32. **SOFTWARE CLOSING**: should be performed in the following order: TEM Imaging & Analysis (TIA), Esprit, Gatan Digital Micrograph (Click on the Exit without saving.)
| **COLUMBIA UNIVERSITY**  
| **IN THE CITY OF NEW YORK**  
| **COLUMBIA NANO INITIATIVE / CENTER FOR INTEGRATED SCIENCE AND ENGINEERING**  
|  
| **button when asking to Save GIF Settings), Flucam Viewer TEM User Interface. Copy your data to your USB memory and remove your data from the support computer.**  
|  
| **33. Disable the tool in badger.**  
|  
| **34.**  
|
HR TEM set-up

35. **STANDARD TEM ALIGNMENT:** follow the steps necessary for TEM alignment described above. Go to higher mag, e.g. SA 245KX. Click on “Coma Free Pivot Point X” in the Direct Alignments window. Condense the beam using intensity knob and use multifunction X & Y knobs to overlap two spot images. Press “Done”. Repeat for Pivot Point Y.

36. **TEM ALIGNMENT CONT.:** Insert Ceta camera. Set the integration time, lift the screen (R1), press “Search”. In the camera tab change stage piezo moves to Picometer steps.

37. **FFT:** Press “Live FFT” in the CCD/TV Camera window. Go out of focus until you see rings. Adjust objective stigmators using the multifunction knobs to round and concenter the rings.
| 38. **COMA-FREE ALIGNMENT:** Align the beam with the optic axis by clicking on “Coma-free Alignment X” and use the multifunction x knob to minimize shape change and movement of FFT rings (should be stable). Repeat for “Coma-free Alignment Y”.

| 39. **EUCENTRIC FOCUS:** Click “Eucentric focus” and use the Z height to get to optimized focus position at max ring size. Use the focus knob to get to max fine focus (one ring for crystalline material). |
### Diffraction set up

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<tr>
<td><strong>40.</strong> <strong>DIFFRACTION:</strong> Insert a smaller C2 aperture (e.g. 70) and finish your gun shift on a larger spot size (e.g. 6). This will protect the ceta camera from over exposure. Focus the beam and press “Diffraction”. Use the intensity knob to sharpen the diffraction (larger spot size will increase sharpness).</td>
<td><img src="image" alt="Diffraction set up" /></td>
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<tr>
<td><strong>41.</strong> In the Direct Alignments window press “Diffraction alignment”. Use multifunction knobs to center the diffraction pattern. When inserting the ceta camera put the search and acquire buttons on the lowest integration time and carefully monitor the histogram intensity chart to determine acceptable exposure.</td>
<td><img src="image" alt="Direct Alignments" /></td>
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### Dark-Field set up

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<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tbody>
<tr>
<td>42.</td>
<td><strong>DIFFRACTION ALIGNMENT:</strong> Follow diffraction alignment steps described above. Camera Tab: Select Dark Field.</td>
</tr>
<tr>
<td>43.</td>
<td>Click XY button.</td>
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<tr>
<td>44.</td>
<td><strong>CENTER REFLECTED BEAM:</strong> Use multifunction knobs to move the desired reflected beam to center.</td>
</tr>
<tr>
<td>45.</td>
<td><strong>OBJECTIVE APERTURE:</strong> Insert small objective aperture (e.g. 20 µm) and select the desired diffraction spot. Press and unpress “dark field” to toggle between BF and DF image.</td>
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</table>
46. Obtaining Hollow Cone Dark-Field:
In this mode you can select a circular region (like a donut) of the diffraction pattern for obtaining the image. In the diffraction mode, center the transmitted beam, then press “dark field” button on the nub and the “Conical” option on the screen. Insert an objective aperture and center it. Then use the X and Y nubs to tilt the beam and move and select a desired part of the diffraction pattern on the center. Now, press “Dynamic” and “Circle” button. Now, the diffraction pattern will rotate around the center and is obtaining a shape of a donut from your diffraction pattern. Now go to the imaging mode. You will see that some grains in respect to their diffraction condition turn bright and dark.
| By obtaining an image which covers the whole ring, you will get the “Hollow Cone Dark Field” image. You can also change the rotation time of the diffraction pattern in the panel. |   |
47. **STANDARD TEM ALIGNMENT:** perform alignment, make sure camera, objective, and SA apertures are out (“none” in drop down menu), insert screen and select Condenser aperture (e.g. 70 µm).

![Image of apertures](image)

48. **STEM MODE SELECTION:** Switch to STEM mode. In FEG registers window upload previously optimized conditions by pressing “lbl”, e.g. 200 STEM, click on “set”. For HR-STEM use spot size 6-9, EDX spot size 3, both 6.

![Image of FEG registers](image)

49. **CONDENSER:** Center C2 aperture and correct its stigmation (round the beam after clicking “Condenser” in the stigmator window and then “none” after correction). If the beam doesn’t condense and expand concentrically click on “Condenser 2 Adjust” and correct it using multifunction X&Y knobs.

![Image of stigmator and apertures](image)
50. **CENTER BEAM and DETECTOR:**

Click on “Diffraction”, a Ronchigram should be visible in the beam. Move the beam to the center of the HAADF detector (should look like the image to the right). Click on “Diffraction alignment” in the direct alignments window. Click on “HAADF Detector Area” button in the Flu Cam viewer and use the multifunction X&Y knobs to move the beam to the center of the detector. Click “Done” to save.

Change the height (Z) of the sample on the nub to make the Ronchigram well aligned and then use the condenser stigmation to make the Ronchigram Astigmated (see images below).

An aligned Ronchigram looks like a magnifier with an infinite (in theory) magnification.

51. **DIRECT ALIGNMENT:** If necessary perform direct alignment as described above.
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<td><strong>52. LIVE IMAGE:</strong></td>
<td>press the Z-axis buttons to bring your sample into rough focus. Click “Search” in the STEM imaging window. A live image will appear in the TIA program. If image does not appear check camera length (should be around 220mm depending on the sample), contrast, and brightness (Auto C/B). Fine focus using the Focus knob.</td>
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<td></td>
<td><img src="image1.png" alt="STEM Imaging Window" /></td>
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<tr>
<td><strong>53. ACQUIRE An IMAGE:</strong></td>
<td>Click “Acquire” in the STEM Imaging window to obtain HAADF STEM image. Slow scan rate – higher quality.</td>
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<td><img src="image2.png" alt="STEM Imaging Window" /></td>
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<tr>
<td><strong>54. EXIT:</strong></td>
<td>To exit STEM mode click on “STEM” in the STEM Imaging window and the HAADF detector will be auto retracted.</td>
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<td><img src="image3.png" alt="STEM Imaging Window" /></td>
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EDX Analysis

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<th>55. STEM ALIGNMENT:</th>
<th>Perform STEM alignment and HAADF STEM imaging as described above.</th>
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</table>

| 56. | Press “Search”, “Preview”, “Acquire”, “Focus”, “Scope”, and “Auto C/B” in the STEM imaging window. They should be inactive when using the Esprit software. To ensure they are inactivated you can press the buttons, so they no longer appear yellow. |

| 57. DEPARATOR ON: | Optimum EDX conditions: smallest C1 aperture, 100 μm C2 aperture, Gun Lens 5, and Spot Size 3, giving a probe size of 1-2 nm. Go to the Super X EDX window and click on ► to show the flap-out window. Under the Super X tab if the Pulse processor Status appears Standby, click on the “On” button to start the EDX detectors. For better resolution it should be kept on for about an hour before collecting X-ray data. |

| 58. HYPERMAP: | In the Bruker Esprit program choose the “objects” tab and click on “HyperMap” to activate the HyperMap workspace. |
59. **SPECTROMETER SET UP:** Open the Hardware configuration spectrometer window by clicking on the dropdown arrow. Set the maximum energy at a desired value: 10 KeV is recommended for higher elemental resolution and 20 KeV is for better elemental coverage. Click on “Close” after you’re done.

60. **HARDWARE CONFIGURATION:** Open “Imaging system” (drop down arrow) to open the Hardware configuration window. Set Image resolution [pixel] to a desired value, e.g. 1024. Set Dwell time for Imaging (suggested value 20 µm), Mapping (10-50 µm for fast surveys, 1000 µm or more for trace and quantification), and Line scans.
61. **DRIFT CORRECTION:** click on “Drift correction” from the Hardware configuration window to open the Drift correction window and adjust the correction interval and Dwell time for correction image. Close both windows when you’re done by clicking on “OK” and “Close”.

62. **SAVE:** The measurement parameters by clicking: under “Devices” as a measurement setup that can be loaded (by clicking “Load”) next time.

63. **COLLECT STEM IMAGE:** Open the New Image scan set up windows. Image name and number will appear on image. Click on “Preview” to view the live STEM image. Use the joystick to move your sample to an area of interest, adjust brightness and contrast and click “OK” to close.
64. Click “New” to acquire a STEM image with predefined conditions (in Hardware configuration and imaging), then click on export/import button in image scan window to open Image menu and save the image.

65. **DRIFT CORRECTION** (if needed): can be activated by clicking on:

66. **MAP SET UP**: In the map setup window select desired map size (mapping area of interest in pixels as showed by green frame in Image scan window). Please note that there isn’t enough memory for the full size (1024x1024). Image filter is for digital image processing. The QMap resolution allows converting up to 8 pixels of spectrum data to 1 pixel in the final EDX maps during quantified mapping to shorten measurement time. Quantified mapping can be perform using HyperMap workspace post data collection. Map filter changes elemental map digitally.
67. **MEASUREMENT SET UP**: In Map data open the Acquire window (drop down arrow) to adjust measurement set up. If “Manual” is checked, the measurement will require user’s second click on the Acquire button to stop. If Measuring time [s] or Cycles is checked, the measurement will be automatically stopped when reaching the set values. **Never** check HV off in the Switch off microscope drop down menu.

68. **LOAD METHOD**: Open the Load method window (from either the QMap or Quantify). LinemarkerTEM.mtd is the recommended selection for beginners.

69. **START EDX MAPPING**: Click on “Acquire” to start the mapping. Click again and the Acquire turns
to “Stop” and the mapping will stop after finishing the current frame. Click on “Stop” to stop immediately.

70. **SELECT ELEMENTS:** Click on to select elements to monitor. The “Auto” will eliminate option that should not be detected.

71. **IMAGE OVERLAY:** In the Element images window, click on the check box below each image to overlay/no-overlay the image in the Map data window. Click on the color button to change color for an image.

72. **SAVE DATA:** Once the acquisition is done click on the export/import button in the Map result tab of the Map data window to Save the acquisition result under Database. If this is the last acquisition turn off the super X detectors by changing their status to: “Standby” (the button “On” should be available but not yellow at this status).
73. **DATA ANALYSIS:** Click on the pipette button to choose the marker you wish to use and use it to select an area on the map. Analyze the elements as described above and obtain composition by clicking on “Quantify” (composition in % will be described at the bottom of the Map data window). Click on “QMap” for concentration maps.
74. **SAVING OPTIONS:** Element maps can be saved under Map data to image files, e.g. .bmp format. Various data and processing results can also be added to project and save all together as one Project package.

75. **BACKGROUND CORRECTION:** Calibrate the spectrum. Usually use zero peak and another element peak. If want to be more accurate on low energy range, can choose, i.e. N; if need to calibrate the whole spectrum, can choose, i.e. Cu.
76. **Set up Cliff-Lorimer factor.** Go to “database”. Create a new library. Input the information needed. Click OK. This will generate the corresponding K factors. You can check the factors by clicking “cliff-lorimer factors”. Then save this library for your reference.

77. **Quant the spectrum.** Load “Interactive TEM.mod” for quantification method. Press “Quantify”. Identify all the peaks. Click Continue.

78. **Setup background windows.** Click continue.
79. See deconvolution results. Here, if you see some peaks not filled with colors, go back and identify that peak. You can do this back and forth to make sure all the peaks are identified. Then click the button shown in the red circle, and select “Build automatic” to save the quantification method.

80. Select elements which are only for deconvolution. Click OK. This will deconvolute these elements during the quantification, i.e., fit peaks, but the concentration of them will not be included/calculated.
81. Check other settings. Choose 3 sigma for error.

82. Save the method, i.e. BN_test.mtd. Click accept.

83. Go back to load “BN_test.mtd”. Click quantify and then you will get the quant result. B, N ~ 50at%.
**84. PROPER LINESCAN PROCESSING:** Draw a line in a raw hypermap data. Go to Linescan. Click “Add to project”.

**85.** Go to “Linescan”. Drag the spectrum from Project to the window as shown.

**86.** Right click and drag the line in the reference image, you can get the sum spectrum from that region.

**87.** You can identify the elements; load the quant method and quant the line profile. On the bottom-right corner, you can set up how many spectra per point for quant, which is more like binning. You will lose the resolution but get smoother line profile.