TALOS F200X THERMOFISHER TEM

STANDARD OPERATION PROCEDURE

TRANING INFORMATION

The training is divided into four modules. Each module contains sections covered during the session.

Training #1: MANDATORY

Module 1: INTRODUCTION TO THE TALOS F200X

It is essential the user reads and understands the laboratory rules and the general information of the Talos F200X S/TEM before starting training. In this module, staff will characterize a sample using the techniques the user will need. It is recommended to bring your sample to this session as staff can advise on other analytical procedures.

Module 2: UNLOADING AND LOADING SAMPLES

This module includes the information and precautions to take during the loading and unloading of the TEM sample holder. In addition, this module shows how to load a TEM grid into the single-tilt TEM holder.

Introduction for module 3: BASIC CHARACTERIZATION METHODS

Training #2: MANDATORY*

- **Module 3: BASIC CHARACTERIZATION METHODS**
    - **Bright-field TEM imaging: MANDATORY***

Introduction to basic alignments for bright-field images in high resolution. This section is based on the following characterization techniques, and therefore it is mandatory.

Selected area electron diffraction: OPTIONAL*

This section will be trained depending on the user's needs. This characterization technique is linked to bright field imaging.

- **Energy-dispersive X-ray spectroscopy (TEM mode): OPTIONAL***

As in the previous section, this technique will be provided depending on the user's needs.

Training #3: MANDATORY

- **Review of modules 1 to 3.**

The user will use the Microscope under supervision. The supervision could be necessary even after the training sessions, depending on the user's progress.

Training #4: OPTIONAL

Depending on the needs of the user. However, the training on the previous modules (1-3) is mandatory. If needed, Modules 1 to 4 will be shown in three training sessions, with the fourth as review.

Module 4: ADVANCED CHARACTERIZATION METHODS

- **Scanning transmission electron microscopy.**
- **Energy-dispersive X-ray spectroscopy (STEM mode)**

TABLE OF CONTENT

MODULE #1 – INTRODUCTION TO THE TALOS F200X

1 USER SAFETY

- In the event of an audible alarm from the SF_6 detector, you **must exit** the room **immediately**. **Do not re-enter** the room until a manual sweep of the room has been performed by FEMR staff.
- Do **NOT** (i) wander behind the Microscope, (ii) open the microscope enclosure or grey cabinets, or (iii) step on cables.
- Always wear gloves and a laboratory coat.
- In the winter or rainy season, please wear cover shoes or change the pair of shoes. This rule is to keep the laboratory clean and avoid dust inside the laboratory.
- Due to the COVID pandemic situation, wearing a procedural mask is mandatory inside the McGill buildings. Please, always wear a mask inside the FEMR facility.

2 USER INTERFACE WINDOWS

2.1 USER INTERFACE

2.1.1 The user interface windows will appear after initializing the TEM UI and FluCam windows (Section 3.1.4). However, it is important to know the localization of the panels in the interface before starting using the Microscope.

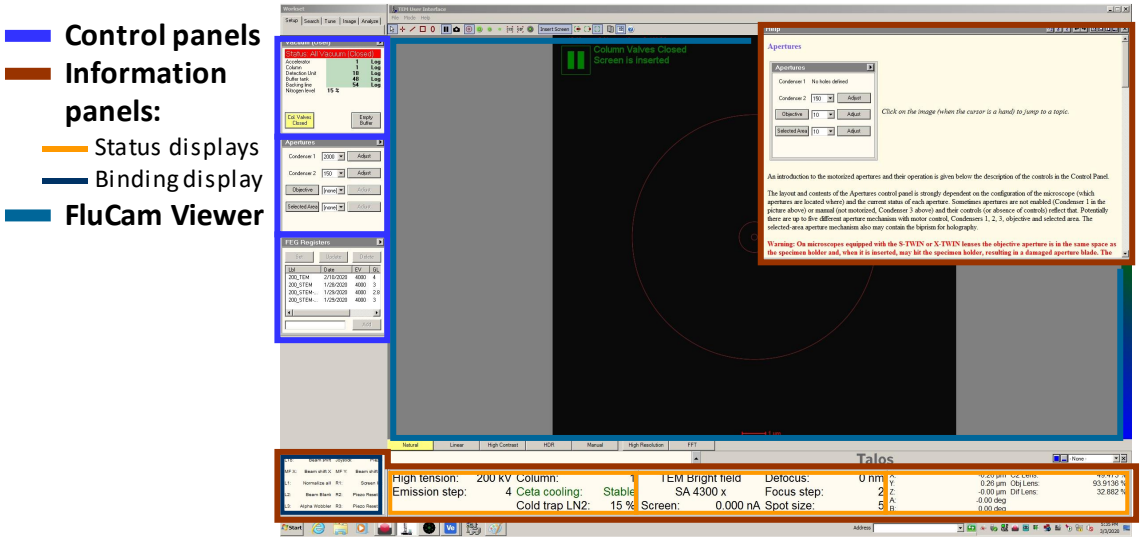


Figure 1: Layout of the TEM User Interface. Note that Thermo Fisher help files use FluCam and SmartCam interchangeably to refer to the fluorescent screen CCD camera.

2.2 COMPUTING SYSTEM

The microscope computing system is arranged in four different monitors and the control-hand pads. The monitors will be named as follows for easier understanding:

1) Bottom-right

   This monitor will display all the software from the Microscope and Flux camera. Also, you will observe the main parameters of the Microscope in this monitor.

2) Bottom-left

   In this monitor, you will observe the Velox-acquisition software.

3) Top left

   The Velox-analysis software will be displayed here.

4) Top-right

   In this monitor, you will observe the window of the TEM launcher system. Also, if you open any folder, it will appear here.

Note. Please, do not try to modify the windows displayed on every monitor. These settings are saved for everyone, and it will confuse every user if these settings are modified.

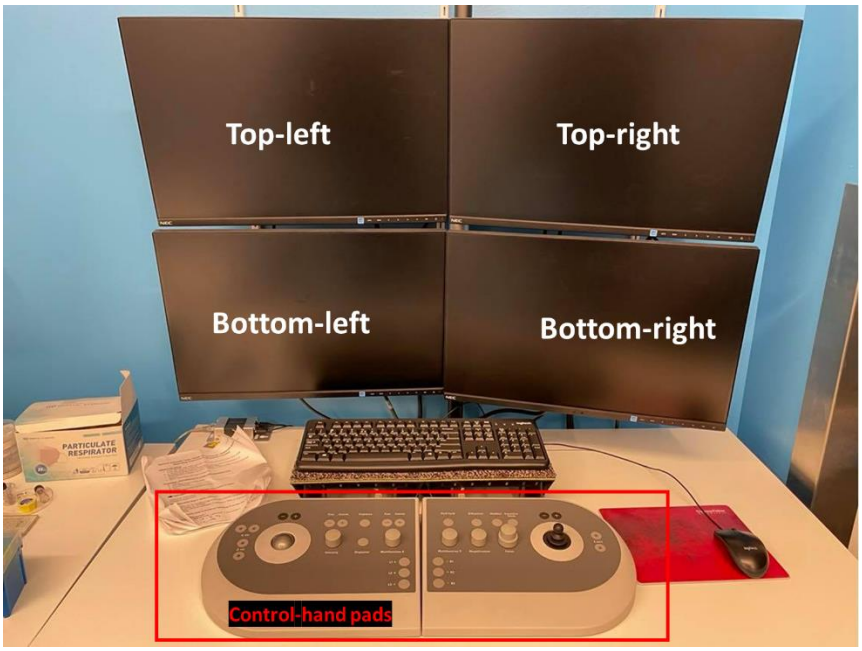


Figure 2 Computing system from Talos F200X

2.3 LEFT AND RIGHT-HAND PANELS

The hand panels will be useful for selecting or changing different camera modes or imaging parameters. In the following steps, we will refer to these panels as right-left hand panels. Please verify this section as needed if you do not find the correct button mentioned through this SOP.

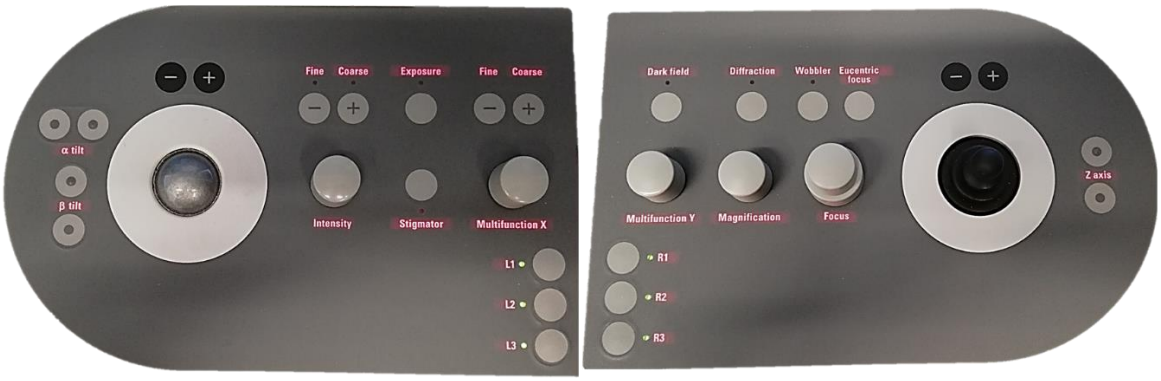


Figure 3 Left-hand control pad. The functionalities of the Multifunction X, L1, L2, and L3 buttons. Right-hand control pad. The functionalities of the Multifunction Y, R1, R2, and R3 buttons.

Note. **Important!** When you decrease or increase your beam intensity, use the intensity knob from the left-hand control pad as follows.

Note. To decrease the intensity, go all through in a clockwise direction. Then, increase the knob slowly counterclockwise to increase the beam's intensity (be aware of the intensity). This procedure is to ensure that you are working on the right sight plane of the Microscope. If you observe that the beam intensity changes contrary to what is written here, please go all through the clockwise direction until the minimum beam intensity is reached and then increase it as needed by turning the knob counterclockwise. **Perform this procedure in the FluCam camera. Never do it on ceta camera to avoid its damage!**

Figure 4 Increasing (red) or decreasing (blue) the beam intensity on the Microscope.

2.4 VELOX SOFTWARE

2.4.1 Acquisition software

Velox software is integrated into two parts, the acquisition and the analysis software. The acquisition part will be displayed on the monitor left-bottom, and it can be divided into three sections:

1) Imaging viewer

   In this section, you will observe the live image of your sample in TEM or STEM modes (with their respective detectors).

2) Imaging options

   This section displays all the options you need to use for the imaging—for example, the acquisition time on live observation, the dwell time, and the image acquisition.

3) Settings

This section displays the image characterization via a histogram of the pixel intensities and FFT. It also shows the detectors' layout from the system and the cameras/detectors that are inserted.

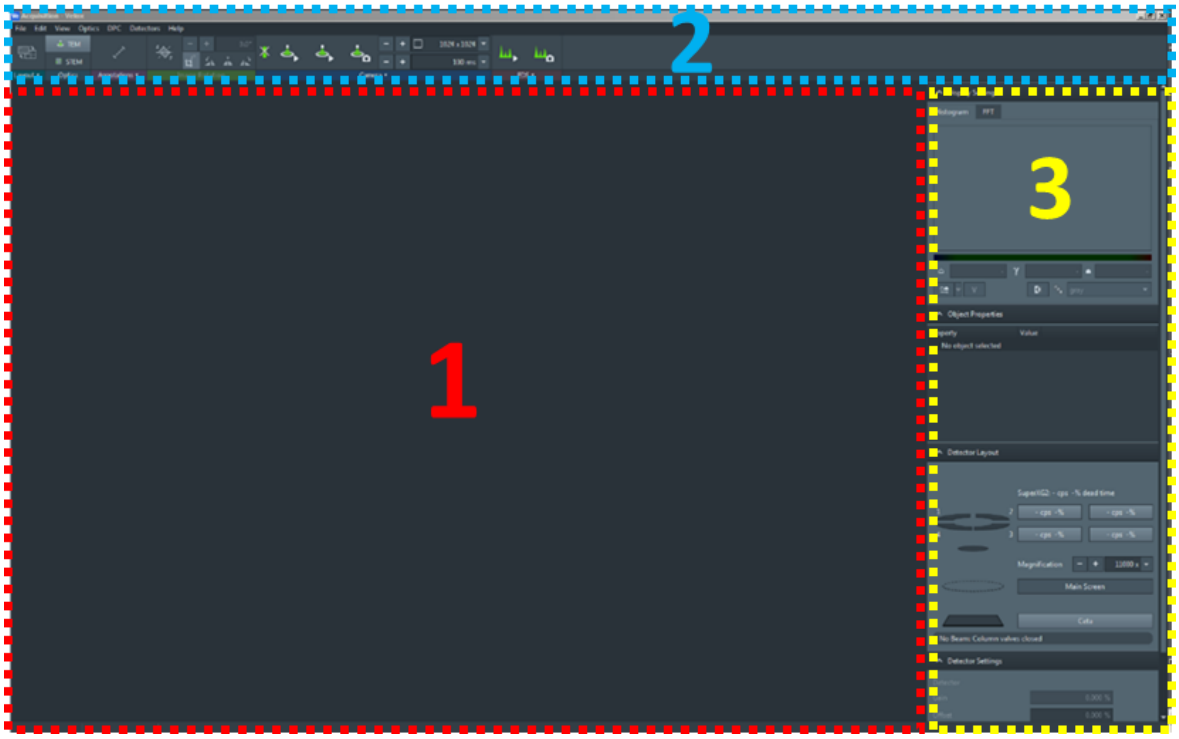


Figure 5 Layout of the Velox software in acquisition mode, showing the three main parts: the imaging viewer (red), the imaging options (blue), and the settings (yellow).

2.4.2 Analysis software

The analysis part of Velox software is displayed on the left-top monitor. This software extension is divided into four sections:

1) Images from experiment

   These images are the ones that have been taken in Velox-acquisition software. All your images will be displayed here.

2) Analysis area

   In this section, you will be able to manipulate the analysis you want to perform, such as particle size analysis, FFT, or EDS integration.

3) Analysis options

In this section, you will observe the options available for your analysis.

4) Display settings

Here, you will be able to change the pixel intensity for bright-field, STEM, or EDS analyses. In EDS mode, you can select the elements to plot in your spectrum using the periodic table. Also, processing settings are available for EDS analysis.

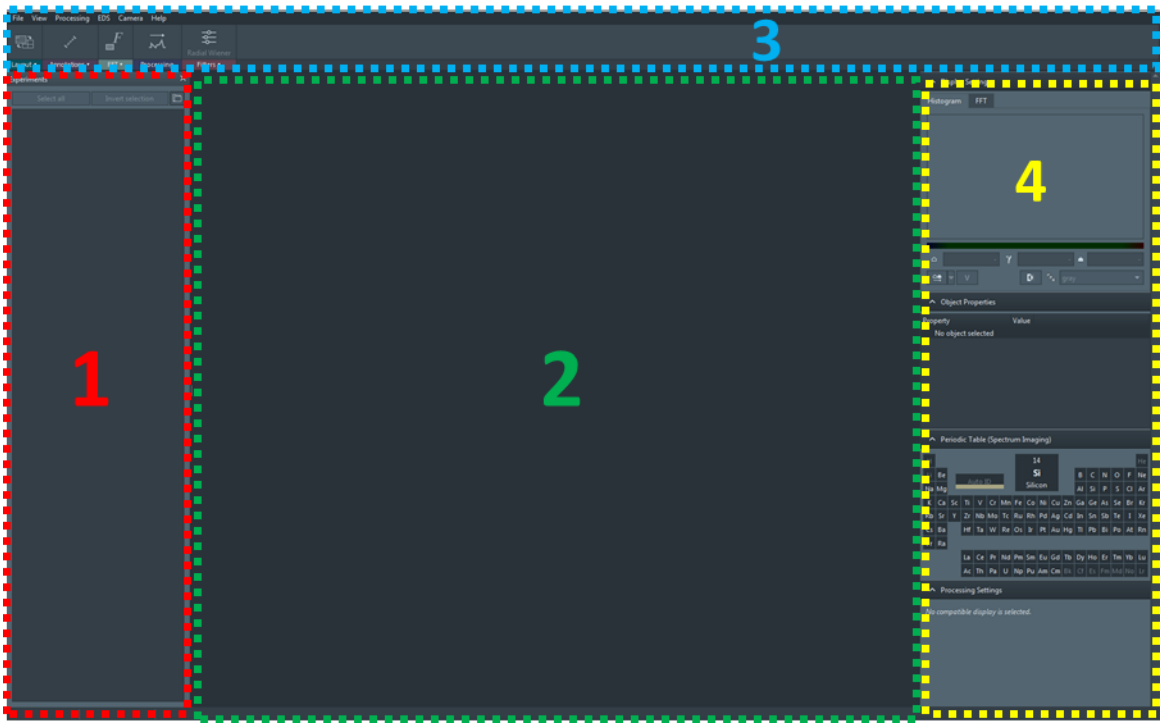


Figure 6 Layout of the Velox software in analysis mode, showing the three main parts: the images from the experiment (red), the analysis area (green), the analysis options (blue), and the settings (yellow).

2.4.3 Velox-sampling

Once you insert the holder inside of the Microscope, there will prompt a "Loading sample" window on the bottom-right monitor. In this window, you will select the path where your images/analyses will be saved. We recommend modifying the "Custom label" and AuNPs, and the software will save your file using this tag. If you change your sample, you can add another tag on the "Custom label" to keep control of the name of any sample. We also recommend selecting "Recommended labels" at least with magnification to observe the real magnification of every micrograph.

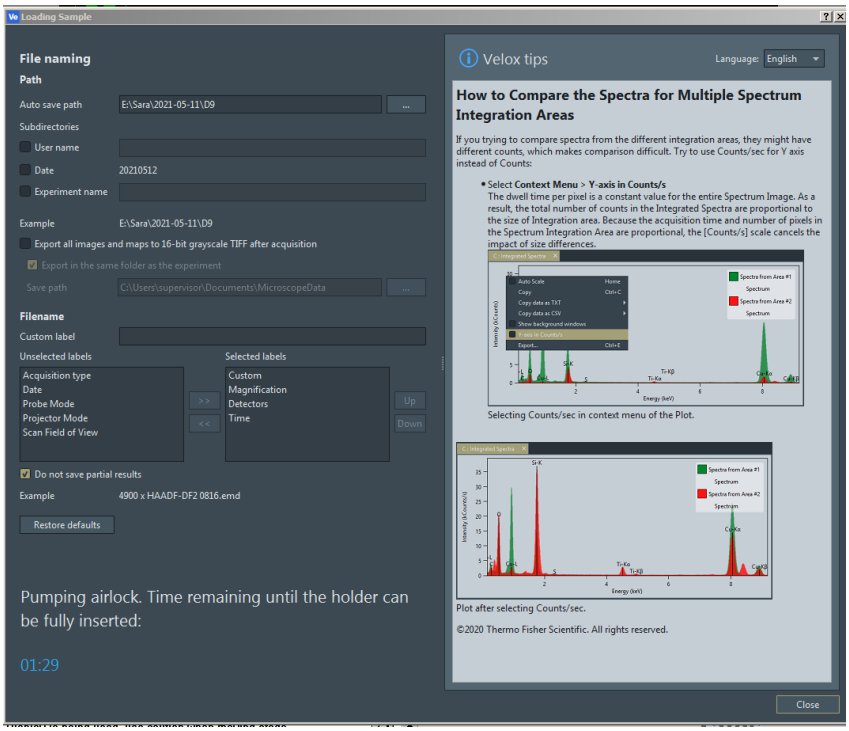


Figure 7 Velox-sampling software. This window will prompt once you insert the holder inside the Microscope.

3 INITIALIZATION AND FINALIZATION OF THE MICROSCOPE

NOTE. **IMPORTANT!** – Avoid system shutdown: The Microscope PC controls the Talos F200X TEM. Shutting down or restarting the Microscope PC will abruptly switch off the 200 kV voltage supply and damage the electron source (i.e., emitter). **Do NOT restart or shut down the Microscope PC**.

3.1 SESSION INITIALIZATION

3.1.1 Sign the log file notebook. It is accessible from the Desktop of the Support PC.

NOTE. If a user was using the Microscope before your session, the "Microscope software launcher" and the camera software would be initialized, and there is no need to follow the next instructions. Follow the instructions to perform the desired analysis. **Always confirm the vacuum readouts (See section 3.1.5) before starting any analysis.**

3.1.2 Log in to the User Account on the Microscope PC, turn on the four monitors and click the *Play* button on the Microscope Software Launcher window. This window is usually located on the top-right monitor.

Figure 8 Microscope Software Launcher window

3.1.3 Wait about 2 minutes for the progress bar in the Microscope Software Launcher to change its status to "started," as shown in Figure 9.

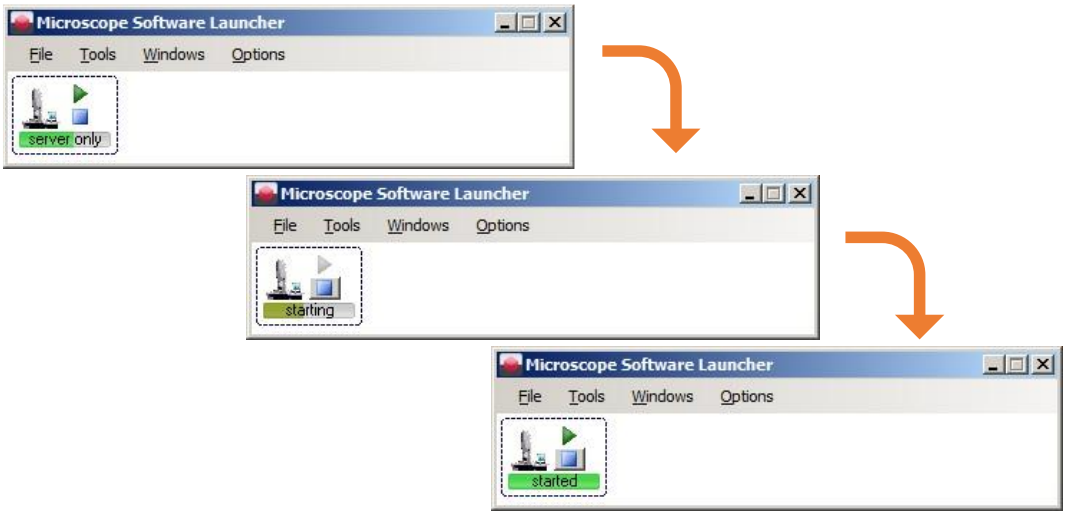


Figure 9 Process of the Microscope Software Launcher when it is started.

3.1.4 Once the Microscope Software Launcher is started, initialize in the following order:

- TEM UI
- FluCam Viewer
- Velox software

NOTE. The TEM UI and FluCam viewer will appear on the right-bottom monitor. The Velox software will appear in the left-bottom (Velox data acquisition) and left-top (Velox data analysis) monitors.

3.1.5 In the Setup tab of Microscope User Interface, verify that the following statements are true. If *any* of the statements is false, please request the assistance of staff.

a. All vacuum readouts are normal, not highlighted in red.
b. Accelerator and Colum values are 1 Log, and the detection unit will be ~18 Log.
c. Col. Valves Closed button is yellow, meaning the column valves are closed.
d. The liquid nitrogen level is 10 % or more.

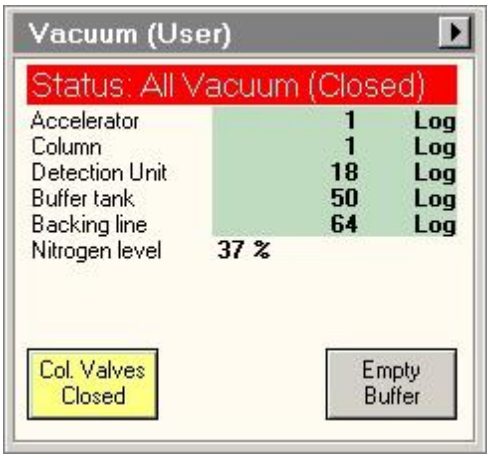


Figure 10 Vacuum control panel window. In this window, the values of the accelerator, column, detection unit, and nitrogen level will be shown as well as the column valves button (Yellow means the valves of the column are closed).

e. Verify that the status display at the left indicates that the high voltage is set at 200 kV and Ceta cooling is stable. Otherwise please request the assistance of staff.

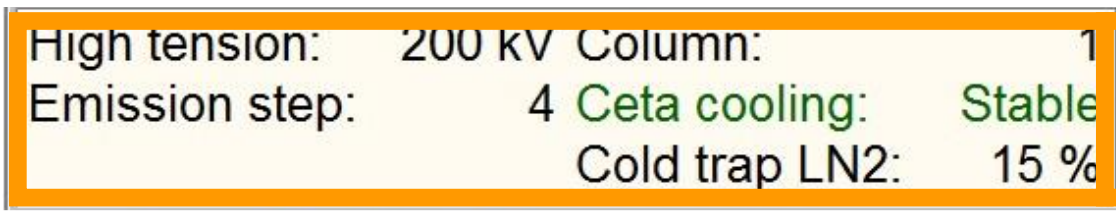


Figure 11 Status displays where the high tension and ceta cooling values and status can be found.

3.2 SESSION FINALIZATION

3.2.1 If after your session, there will be more users. Please, just insert the empty holder inside the Microscope to finalize your session.

3.2.2 If you are the last person using the Microscope. Click the stop button on the Microscope Software Launcher and wait for the status to indicate "server only."

MODULE 2 – UNLOADING AND LOADING SAMPLES

4 SAMPLING

4.1 RETRACTING TEM HOLDER

4.1.1 If you are retracting the TEM holder for starting the analysis, verify that the stage is neutralized (x, y, z, and α/β values are 0 um). These values can be seen in the touch screen from the Talos microscope. If values are slightly offset 0 um, press "Reset xyzαβ".

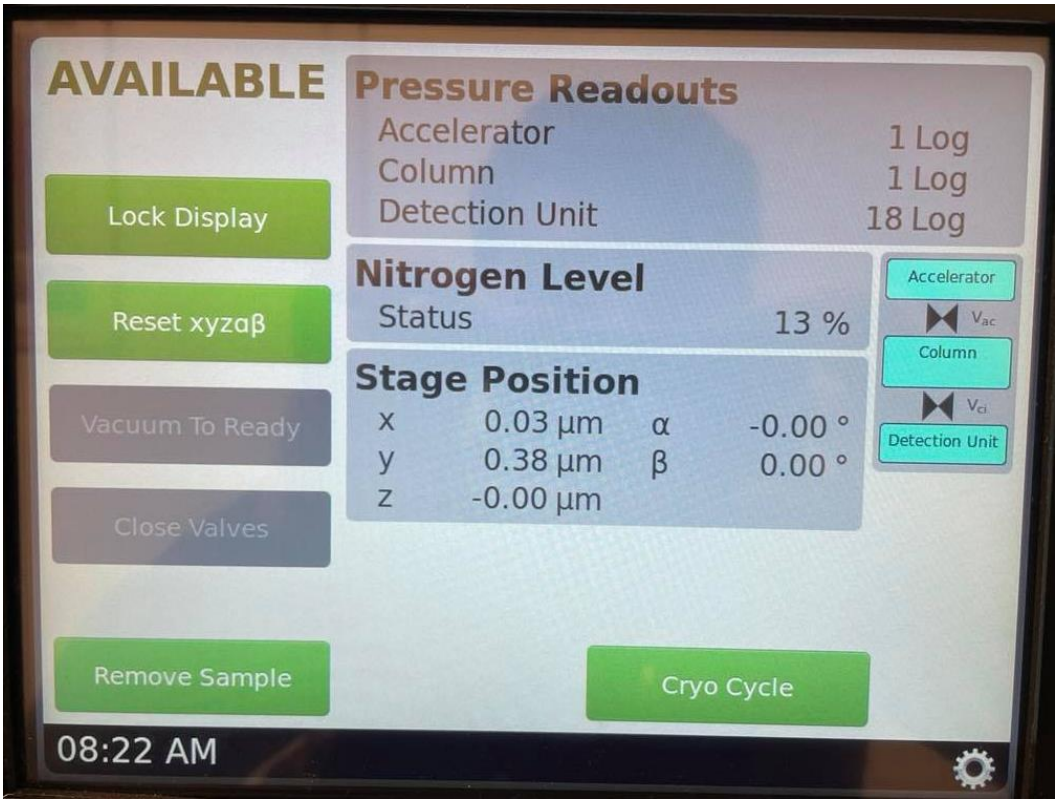


Figure 12 Touchscreen display (On-System Display, On-System User Interface, or On-System UI). Note that pressure readouts must be the same as section 3.1.5. This image is for illustrative purposes only.

4.1.2 If you are retracting the TEM holder for changing, removing a sample, or finalizing your session, **neutralize the stage**. Even though you can neutralize the stage on the touchscreen display from Talos, please, do not perform it this way. Follow the next procedure:

a. Close the column valves. The button should be yellow, as seen in Figure *10*.
b. If the FluCam Viewer shows a "Screen inserted" message, lower the fluorescent screen by pressing the R1 button on the right-hand control pad or just press "insert camera".
c. Retract the objective or selected area apertures if they were inserted.

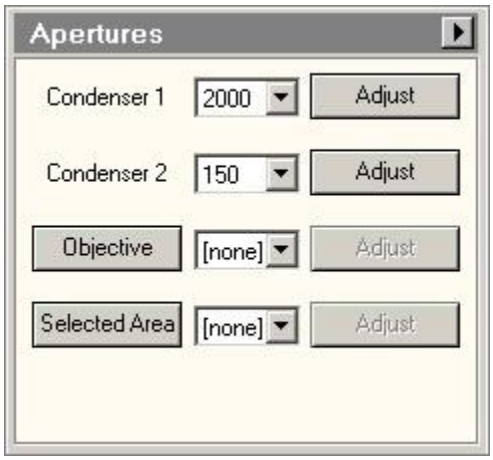


Figure 13 Aperture's window where objective and selected area apertures should be retracted as seen in the figure

d. Neutralize the stage in the Search tab, click the arrow on the Stage² control panel to show the flap-out menu and click the Holder button below Reset.

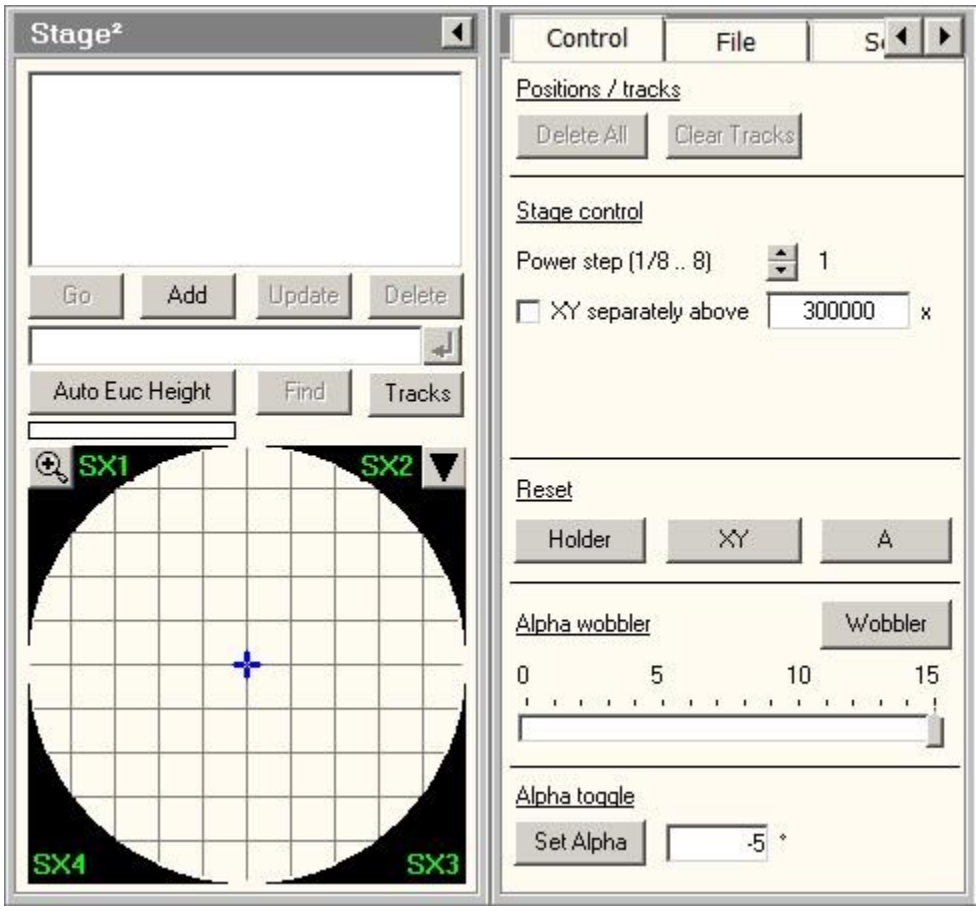


Figure 14 Stage window. The arrow at the top right of the window displays different options. Control option should be selected to reset the holder to its initial position.

e. Retract the holder as in section 4.1.3.

4.1.3 Retract the holder.

NOTE. **Prevent holder and stage damage:** The specimen holder and stage contain delicate, precisely machined components. Never exert significant force during any step of this procedure. Doing so may result in serious damage to the instrument or holder.

NOTE. **Get training:** Do not retract the holder before being trained by staff. If in doubt or uncomfortable executing any of the following steps, please request the assistance of staff.

a. On the touchscreen display, tap the "Remove Sample" button (unlock the touchscreen beforehand, if necessary).

b. Verify *all* Stage position values are zero, V_{ac} and V_{ci} column valves are closed, and the stage LED is off. Open the sound-dampening door in front of the stage.

c. Hold the black base of the holder with one hand (make sure you feel the two slip-prevention O-rings with your fingers), use your thumb, on the other hand, to apply light pressure on the white surface. Pull the holder straight back ***without rotating*** until a stop is felt.

d. Release your grip on the holder and wait for the Pressure readout values to stabilize.

e. Hold the base of the holder again. Rotate the holder clockwise in a controlled and smooth motion until a stop is felt. Release the index finger on the gripping hand and push it against the white surface while holding the holder base, thereby pulling the holder until no resistance is felt. Retract the released holder shaft straight back from the airlock. Be careful not to scrape the holder along the inside of the airlock and not touch the O-ring on or any brass part beyond it.

f. Close the sound-dampening door.

4.2 INSERTING TEM HOLDER

4.2.1 On the touchscreen, tap the Load Sample button (unlock the touchscreen beforehand, if necessary).

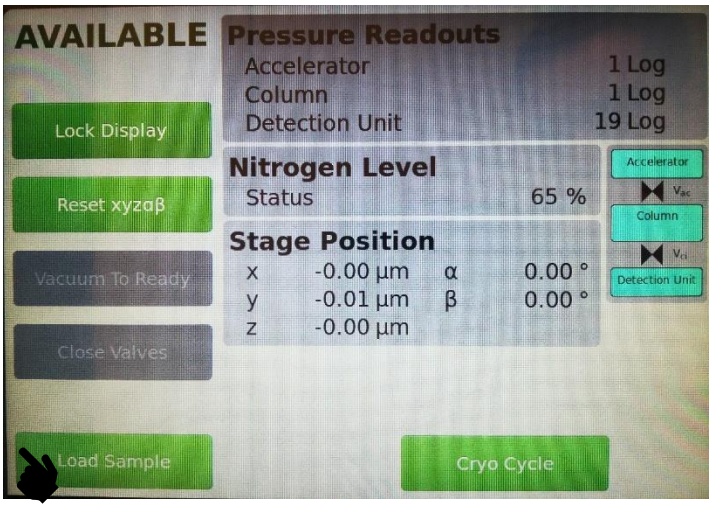


Figure 15 Touchscreen display on the Microscope, ready to load a sample.

4.2.2 Verify all Stage position values are zero, Vac and Vci column valves are closed, and the stage LED is off. Open the sound-dampening door in front of the stage.

4.2.3 Carefully align the blue linear markings on the holder with the blue marking on the stage opening. Gently insert the holder until it stops. Be careful not to scrape the tip on the inner mechanisms of the goniometer. You should feel some resistance as the holder O-ring on the holder enters the airlock chamber.

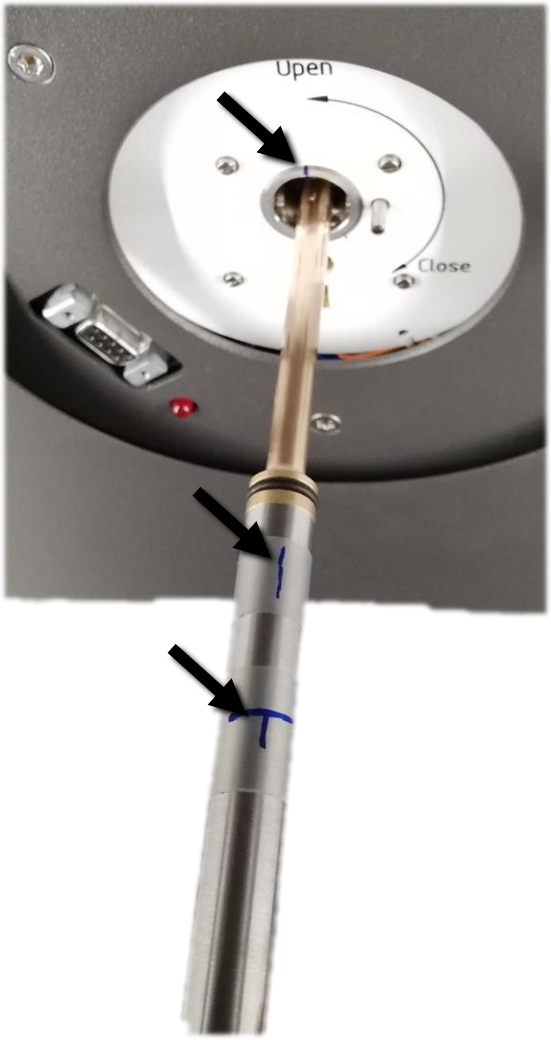


Figure 16 Alignment of the holder before its insertion into the Microscope

4.2.4 Once the sample holder has been inserted, airlock pumping will begin for 120 seconds – Do NOT move the holder during this period. Wait for 120 seconds.

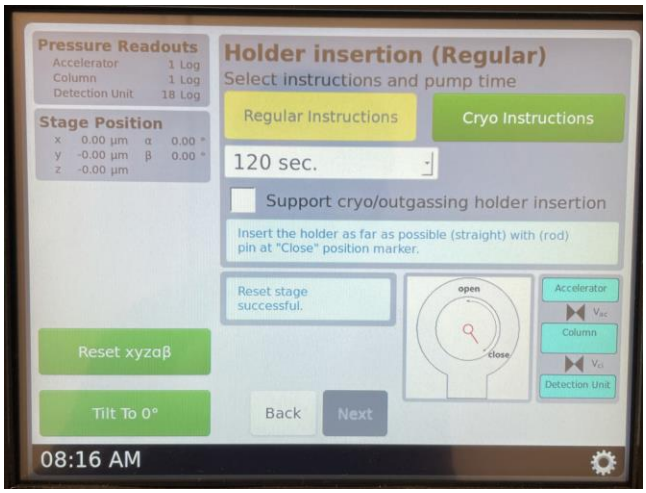


Figure 17 Touchscreen display on the Microscope, showing the airlock pumping time.

4.2.5 Almost immediately to the airlock pumping window, a holder Selection window will pop-up on the touchscreen. Tap to select the Single Tilt holder. A progress bar will appear, showing the remaining pump time. Tap the Next button.

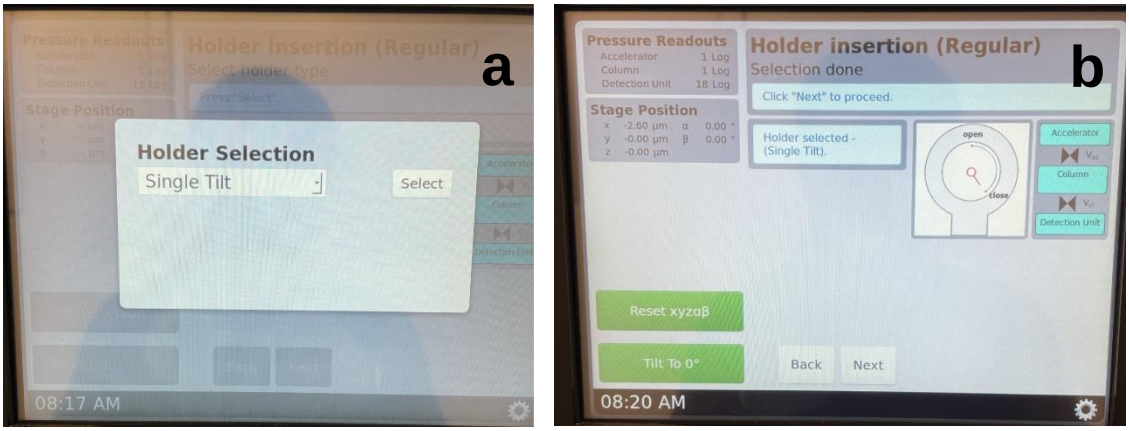


Figure 18 Touchscreen display on the Microscope, showing the holder selection (a) and the window after 120 sec (b).

Note. **Minimize the chance of vacuum break**: The O-ring on the holder cannot hold the vacuum in the airlock for more than 3 minutes. Proceed to step 6 below as soon as the pumping is complete.

4.2.6 When the pumping is complete, the red stage LED is off, support the white goniometer surface with one hand and grip the sample holder securely with the other. Slowly rotate the sample holder counterclockwise until the holder starts moving into the column. Gripping the base, controllably allow the sample holder to slide into the microscope column until it stops. Tap the base of the sample holder to verify it is securely seated.

4.2.7 Tap the Done button on the touchscreen. Close the sound-dampening door in front of the stage.

4.2.8 Wait for the Column vacuum to stabilize at 3 Log or lower.

4.2.9 Start your analysis. First, select your saving path and labels with the window that will prompt up after the insertion of the TEM holder.

Troubleshot. If the column vacuum does not stabilize, unload the holder as seen in section 4.1.3. Verify that there is no dust in the TEM holder. Clean the O-ring with the compressed air located in the laboratory and insert the holder again. If the problem persists, please contact the FEMR staff to solve it.

5 USE OF THE HOLDERS FOR TALOS F200X

NOTE. **Prepare grids appropriately:** TEM grids or self-supported samples must be completely dry and dust-free before they can be loaded onto a sample holder.

NOTE. **Avoid harmful practices with powder samples:** Strewing dry particles directly on a TEM grid is not an acceptable TEM sample preparation method. Grids prepared this way cannot be simply loaded into the Talos. While interacting with the electron beam, particles may detach from the grid and contaminate the holder or the TEM column. Please contact staff for assistance with sample preparation before booking.

NOTE. **Avoid harmful practices with magnetic materials:** Never load samples with magnetic substances (containing Fe, Co, Ni and some REs like gadolinium, dysprosium) in any form onto the single-tilt holder. The magnetic field in the TEM reaches up to about 2 T, and the holder

clamping mechanism cannot prevent magnetic species from contaminating the TEM column. Please contact staff for assistance with sample preparation before booking.

5.1 SINGLE TILT HOLDER

5.1.1 Remove the holder as indicated in section 4.1.3.

5.1.2 Insert the holder into the plastic cover.

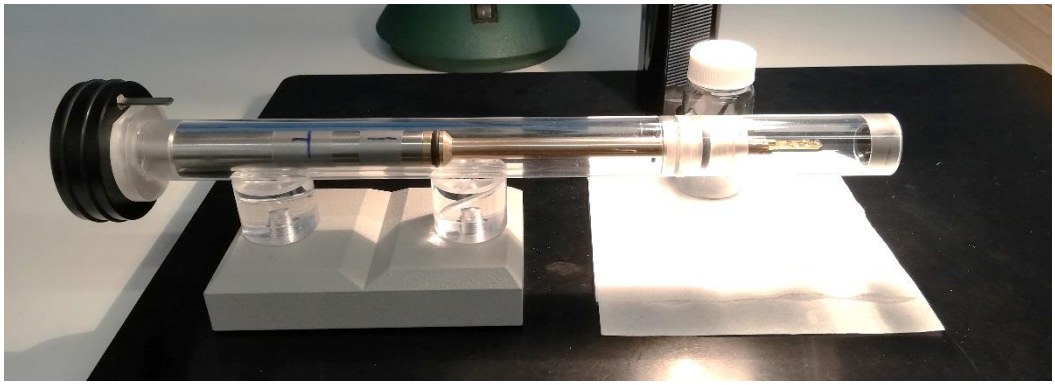


Figure 19 Single-tilt holder deposited into the plastic cover.

5.1.3 Remove the cap at the end of the plastic cover. Use one hand to prevent the holder from slipping out of the stand, insert the loading tool into the hole in the specimen (See Figure 20) clamp and gently raise the clamp straight up until it stops. **Never use tweezers**.

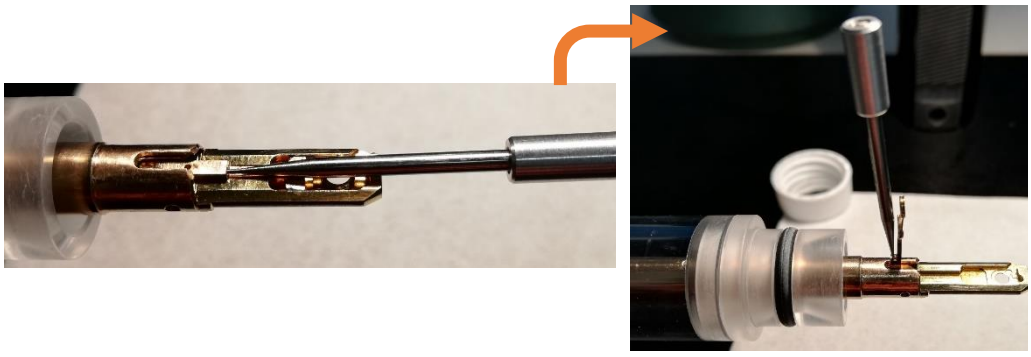


Figure 20 Procedure for the raise of the holder clamp for inserting a TEM grid

5.1.4 Place the specimen grid into the recess at the end of the holder. Make sure the grid is properly centred in the recess.

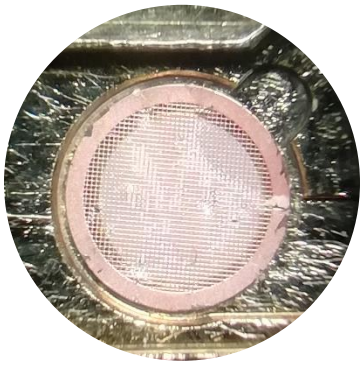


Figure 21 Correct deposition of the TEM grid into the recess of the single-tilt holder

NOTE. For imaging purposes, both sides of the TEM grid are good for analysis. It all depends on where you deposited your material, but both will be OK for imaging. However, we recommend using the dull side (darker, non-copper colour) to deposition your sample if it is in the liquid phase.

5.1.5 Gently lower the clamp straight down to hold the grid securely. Carefully return the tool to the small bottle on the desk.

5.1.6 Retract the holder from the plastic cover. Tap the holder's base several times (before O-ring) and check that the grid is not moving (movement suggests the grid is not properly secured).

NOTE. Do this procedure by having a kimwipe under the holder if the grid falls.

5.1.7 Insert the holder as indicated in section 4.2.

NOTE. Important! After doing any pre-alignment or parameter change in the TEM-UI or FluCam viewer. Always go back to the Setup window and observe from time to time your pressure values. Any change on the pressure value (Column or detector). You must stop your analysis. Close the column valves and retract any objective aperture. Then follow the steps in section 4.1. Then, contact the staff members and verify that the holder is clear, specifically the o-ring part.

6 TEM MODE

6.1 HIGH-RESOLUTION BRIGHT-FIELD TEM IMAGING (HR-TEM)

6.1.1 Initialize TEM as in section 3.1.

6.1.2 Retract and/or insert the holder as discussed in section 0.

6.1.3 Load the 200_TEM FEG (GL3/Spot 5) Register from "FEG registers" window in TEMUI>Workset>Align. Once selected, remember to select the button **"Set"**.

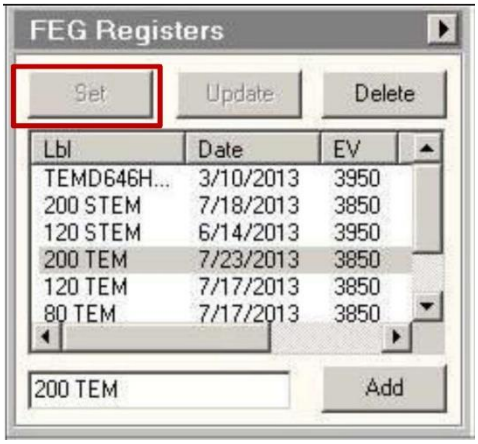


Figure 22 FEG Register window where the registers can be selected.

6.1.4 Insert the Flucam by selecting, Insert Screen located at the top of the FluCam window, and press High contrast at the bottom of the Flucam window.

6.1.5 Verify all vacuum readouts are OK. If so, open the column valve.

6.1.6 In TEM UI>Workset>Tune. Verify that the spot size is in #3 and microprobe is selected on the Beam Settings window.

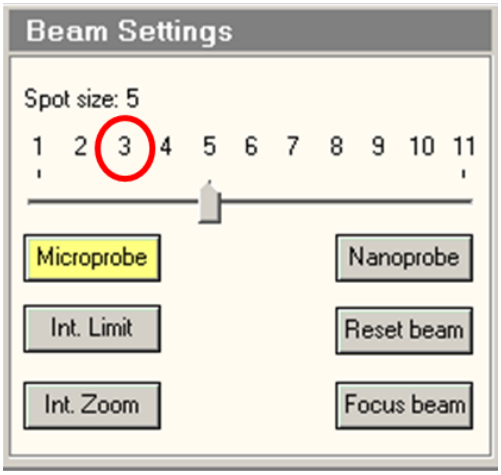


Figure 23 Beam settings window, located at the top of the control panel in TEM UI

6.1.7 Find a region of interest (ROI) with particle(s) in the sample.

NOTE. If you are unable to find the beam. Use the magnification knob on the red-hand pad and decrease the magnification counterclockwise. If you are unable to find it with a lower magnification, please, contact the staff. To move your sample, use the joystick from the right-hand panel, as shown in Figure 24.

Figure 24 Image of the joystick for moving the sample. Use (-) or (+) to decrease or increase the movement, respectively.

6.1.8 Press the Eucentric focus button on the right-hand control pad.

NOTE. We recommend a 14 kX magnification while doing the eucentric point alignment. Higher magnifications will be difficult to find the eucentric focus. Remember that during this procedure, the sample will move a bit. The important thing here is that the movement is minimized as much as possible.

6.1.9 On TEMUI>Workset>Search, select the arrow at the top-right of Stage² control panel to show the flap-out menu and click on Wobbler.

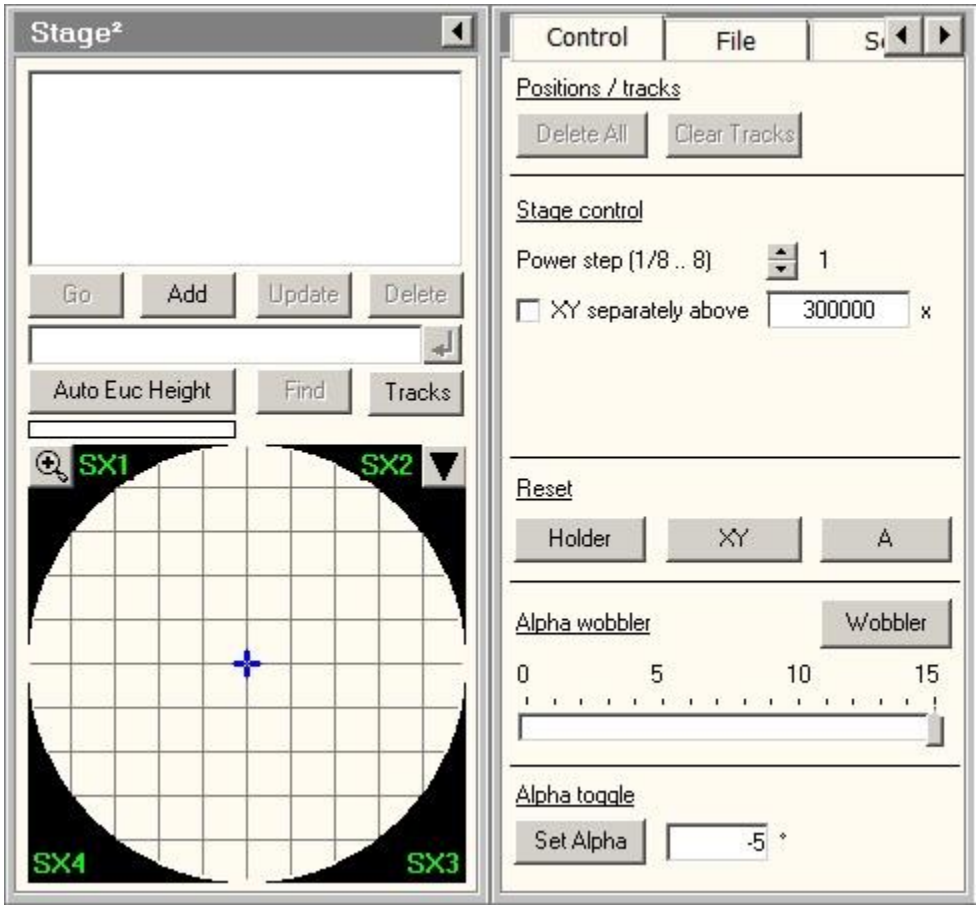


Figure 25 Stage window where the position of the holder can be observed. By clicking the arrow, a control settings window will appear. The control setting is the only tab you will use.

6.1.10 With z-axis buttons on the right-hand panel, try to reduce the shifting of the image. By doing this procedure, you are trying to find the optimal eucentric height of your sample. If the z-axis movement is too fast or too slow, please use the buttons (-) or (+) located at the top of the joystick (See Figure 24) to increase or decrease the movement as needed.

6.1.11 Alignment of the beam shift. On TEM UI>Workset>Vacuum/HT and Direct Alignments window, select beam shift with a magnification of 14 kX. The goal in this alignment is to "center" the beam in the FluCam. Use the same magnification as the eucentric point.

6.1.12 Use the multifunction X&Y on the right/left-hand panels to center the beam in the FluCam. The circles on FluCam are helpful to observe if the beam has been centred correctly. Spread or increase the beam to fit one of the circles. **IMPORTANT!** Click "Done" after you finish.

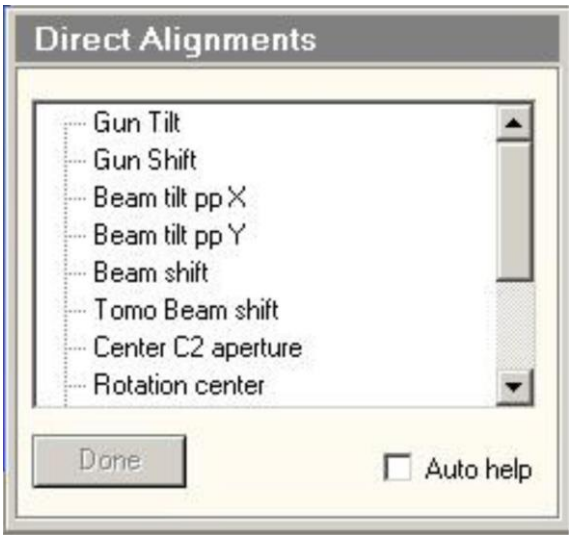


Figure 26 Direct alignments window located at the left side of the control panels of TEM UI.

6.1.13 Rotation center. It is in the same window as the beam shift. Once selected, the image will move; by using multifunction X&Y on the right/left-hand panels, try to observe an image as that wobbles into its same position. Perform this procedure at 46 kx magnification. Usually, this alignment is very stable, and it is not needed to align it. However, verify this alignment.

6.1.14 Condenser aperture alignment. Go to TEM UI>Workset>Tune and select the aperture for C2. For high-resolution TEM, we recommend a condenser aperture 2 of 100. Use magnification of 10 kX. First, you will focus the beam, and then you will spread it. If you see that the beam is not expanding or focusing on the same place, follow the alignment of C2 aperture. The comparison of a bad and good C2 alignment is seen in Figure 27.

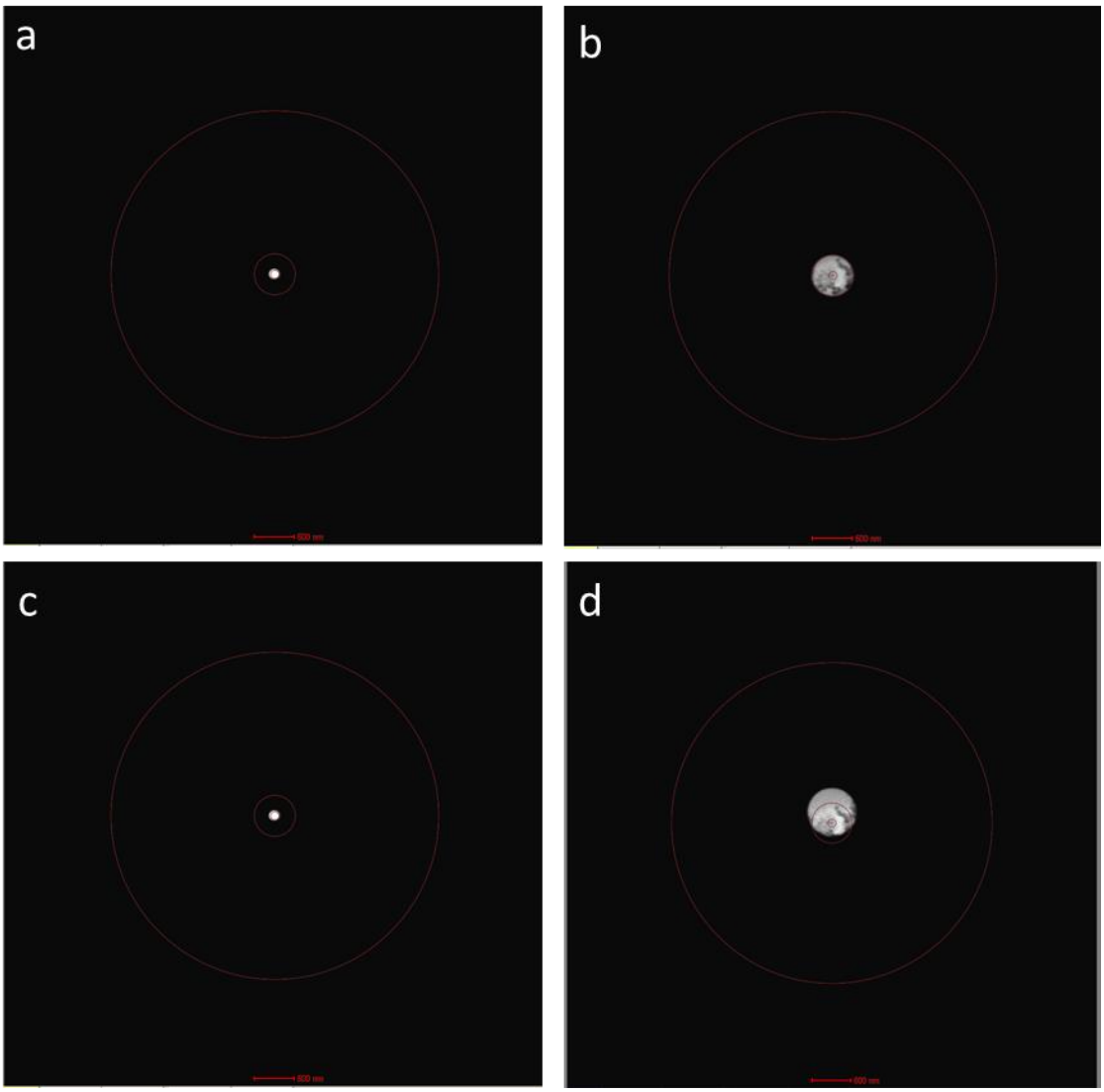


Figure 27 Observation of the alignment of C2 aperture. A good C2 alignment is observed when the beam is focused (a) and when it is spread, the beam stays at the same place (b). A bad C2 alignment is when the beam is focused (c), and after its spread does not stay at the same place (d), there is a shift in any direction. The red circles of the FluCam are used as a guide.

- Alignment of C2.
  - Center the beam with beam shift (in case it was not centred before).
  - Focus the beam at the first red circle of the FluCam. Spread and focus the beam regularly to observe if the beam stays at the same place.

- If the beam shifts when it is spread, select "Adjust" on the aperture settings of the condenser 2 option. Center the beam with multifunction X&Y on the right/left-hand panels.
- Focus and spread the beam again to observe the beam's behaviour, try to use the multifunction X&Y to fix the shift as much as possible.
- **Important!** Click on "adjust" again to finish this alignment.

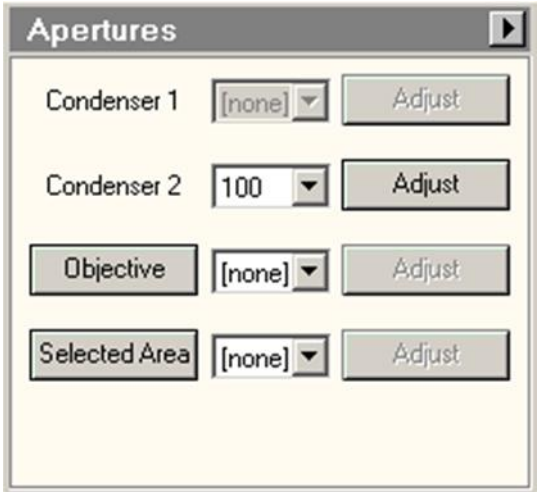


Figure 28 Apertures windows located at the left side of the TEM UI

NOTE. In our Microscope, Condenser aperture #1 is selected by default (2000). The use of smaller condenser aperture #2 will be weak the beam, but if you are looking for HR-TEM at very high magnifications, the number 100 will be good for this purpose.

Optional. If you observe white shadows in your particles (usually on the nanometric scale), you may use an objective aperture. However, you must align the objective aperture.

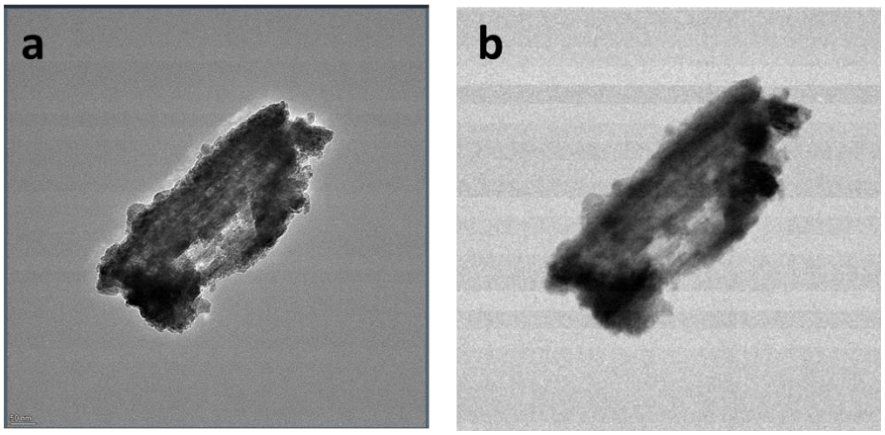


Figure 29 Effect of the objective aperture on bright-field imaging. The white shadows (b) are removed by using a small objective aperture (b).

- Objective aperture alignment
  - With the intensity knob from the left-hand pad, increase or decrease the beam intensity to make an electron dose rate around 3 e/A^{2}s. This number is located at the bottom of the FluCam window.

Note: To increase the dose rate, increase the magnification around 22000X and increase the intensity of the electron beam until it reaches 3 e/A^{2}s.

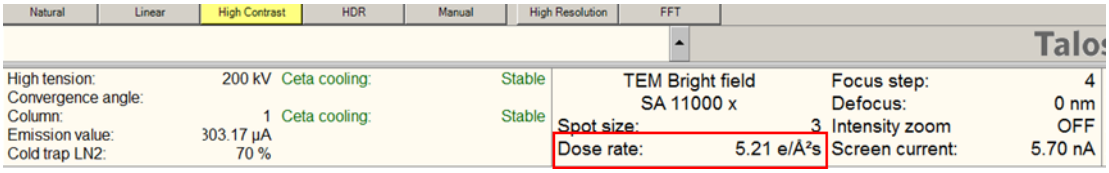


Figure 30 Location of the dose rate parameter on the FluCam window.

  - Change the camera mode to HDR. Then click of diffraction button on the right-hand control pad and verify that the camera length is around ~330 mm.
  - Alignment of the central spot. Select "Direct align" from directs alignments window (on Vacuum/HT tab) and center the central spot with multifunction X&Y on the right/left-hand panels. **Important!** Click "done" after you finish this procedure.

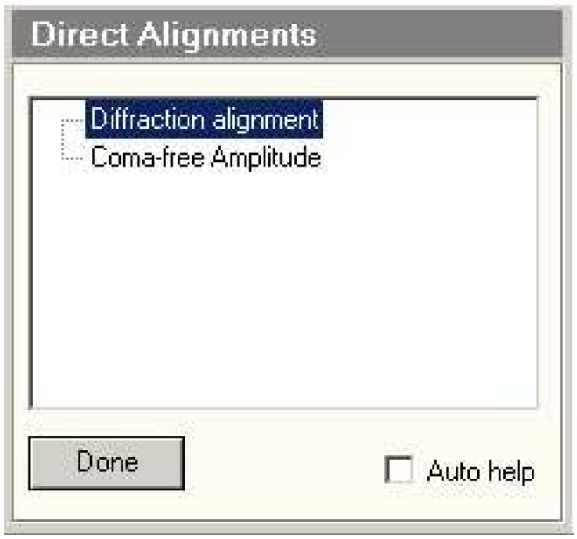


Figure 31 Direct alignments window

- Insert the objective aperture and then center the aperture (the dark circle must be centred) with adjust button on the "Apertures" window. Remember to press "adjust" again to stop the alignment.
- Click on the diffraction button on the right-hand pad to change the imaging mode to High contrast again.

Tip. If you are not looking for a high-resolution TEM image (i.e., lattice fringes observation, phase observation, etc.), do not spend too much time on the pre-alignments having only a well-centred beam and optimal pre-alignments is enough, and you will save time for your analyses.

6.1.15 Ensure that there is no saturation of the flux camera. This observation is crucial to avoid damaging the ceta camera once it is selected. This information can be seen on the right intensity bar of the Flux Cam viewer.

Figure 32 FluCam interface. The beam intensity is shown at the right of the window. Red means a higher intensity, and blue for a lower intensity.

6.1.16 In Velox-acquisition software (left-bottom monitor), insert the Ceta camera.

Figure 33 Ceta camera settings are shown in Velox-acquisition software in the settings section.

6.1.17 Lift the FluCam by selecting Insert Screen and start the View mode of Ceta.

6.1.18 Watch the *detector saturation indicator* on the Display Settings side panel on, Histogram tab on Velox-acquisition software. Make sure the Ceta camera is not oversaturated.

6.1.19 Switch to the FFT tab and use the focus knob on the right-hand control pad to change the image focus until 1 or 2 Thon rings become visible. This knob is composed of a top and bottom knob. The bottom one will change the step, and the top will change the focus.

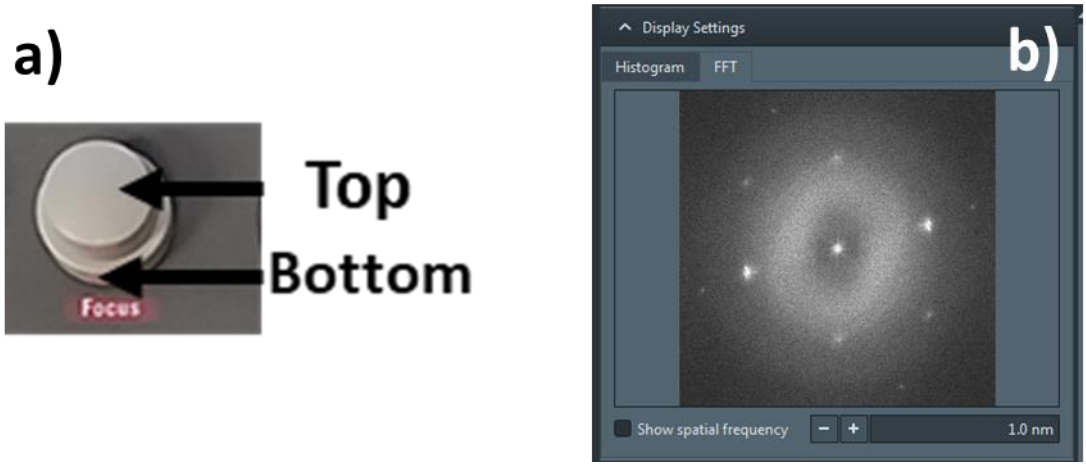


Figure 34 (a) Focus knob and its composition into the top and bottom knobs and (b) effect of the focus change on the FFT pattern.

6.1.20 Sigmate the OL to make the rings as round as possible with a stigmator button located at the right-hand pad and using multifunction X&Y.

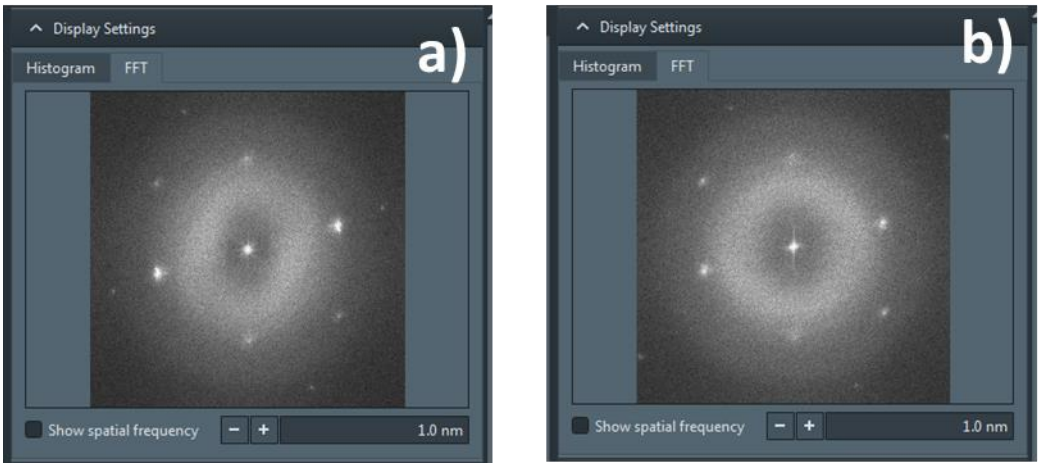


Figure 35 Effect of the variation of stigmatism before (a) and after (b) the change. Please note that the first ring is modified only with the focus knob. The stigmator button only modified the "shape" of the ring.

6.1.21 Ensure the lattice of your particle(s) of interest shows minimum details by defocusing the image so that the FFT shows roughly only the first Thon ring.

6.1.22 On the imaging option toolbar, click on "Camera" to display the imaging settings. Here, you can modify the "live" mode (Figure 36-red) by changing the image's resolution and exposure time. We recommend having a frame combining of "1 frame". You also have the option of modifying the acquisition settings (Figure 36-green). Remember, all these modifications are in Camera options (you must click on the camera button). Click on to see the  live mode again, and you must click the icon at the top of the camera button.

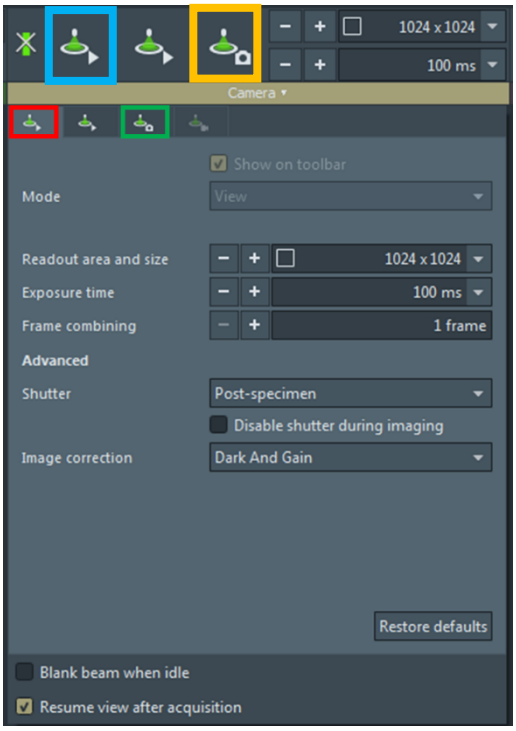


Figure 36 The acquisition and live mode settings options on velox-acquisition software.

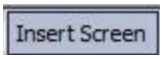
6.1.23 Acquire images by selecting  located at the top of Velox-acquisition software (See **Figure *36***), in the imaging options toolbar.

6.1.24 If higher magnification is required, increase magnification and repeat steps 6.1.186.1.18 to 6.1.23.

6.1.25 For a different ROI, stop the Ceta camera, insert the fluorescent screen and decrease magnification. After finding a new ROI, repeat the steps 6.1.18 to 6.1.23.

6.1.26 Save your images in Velox analysis software (Top left monitor).

6.1.27 Finalization of the bright-field imaging.

- Insert the FluCam by selecting 

on the top of the FluxCam viewer.
- Retract the Ceta camera by clicking on the button on Velox software.
- Decrease the magnification at least with a scale between 1 or 5 um.
- Remove your sample by following the steps in sections 4.1.2 (removing a holder) and 4.2 (inserting the holder without sample).
- Finalize your session by following the steps in section 3.2.

NOTE. Remember to retract an objective aperture before unloading the holder.

NOTE. Remember that the holder should be inserted again into the Microscope without any sample.

6.2 SELECTED AREA ELECTRON DIFFRACTION (SAED)

6.2.1 Initialize the Microscope following the steps in section 3.1 and load your sample into the Microscope as discussed in section 0. If you already have your sample loaded inside the Microscope, go to step 6.2.4.

6.2.2 Pre-align the Microscope as in section 6.1-HIGH-RESOLUTION BRIGHT-FIELD TEM IMAGING (HR-TEM).

NOTE. If you previously used an objective aperture, retract it before inserting a selected area aperture.

6.2.3 On TEM UI>Workset>Tune> Aperture window, choose the selected area aperture and change your magnification around 30 to 40 kX.

NOTE. The smallest aperture (10) will provide an area of 270 nm^2. Usually, for nanoparticles, this aperture is the correct one to perform SAED.

6.2.4 After choosing the desired selected aperture, center the beam by clicking "adjust" for the selected area aperture on the aperture window. For centring, you will use the multifunction knob X&Y from the control pads. Remember to select "adjust" again to exit the alignment of the aperture.

6.2.5 Find an ROI using the joystick from the right-hand pad.

6.2.6 Click on the "Diffraction" button from the right-hand pad. If the electron diffraction pattern is not centred, use diffraction alignment from the direct alignment window and then use multifunction x-y to center it, then click "done".

6.2.7 Then add the beam stop button  located at the top of the FluCam window with a small outline.

NOTE. **Important!** Never switch to the Velox-acquisition software before adding the beam stop. Please, double-check the beam stop was inserted correctly.

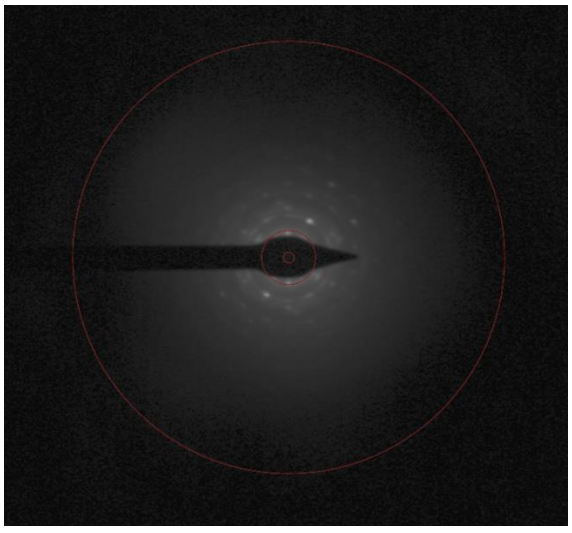
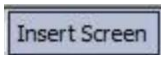


Figure 37 Beam stop observation on FluCam window. Please, verify it is inserted. Sometimes the contrast is not that high to observe the beam stop.

6.2.8 In Velox-acquisition software (left-bottom monitor), insert the Ceta camera.

6.2.9 Lift the FluCam by selecting 

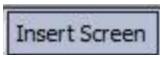
and start the View mode of Ceta.

6.2.10 Once you observe the pattern, take a picture in velox-acquisiton software as usual by clicking on the icon 

NOTE. **Important!** Never switch to the Velox-acquisition software before adding the beam stop. Please, double-check the beam stop was inserted correctly.

6.2.11 If you need to change your ROI, retract the ceta camera, insert the FluCam, retract the beam stop by selecting first  and then  . Then press the diffraction button from the right-hand pad to observe the area with the inserted area aperture.

6.2.12 Change the ROI again with the joystick from the right-hand pad and follow the steps from 6.2.6 to 6.2.10.

6.2.13 To finalize the electron diffraction characterization. First, retract the ceta camera and insert the FluCam by selecting again 

, then retract the beam stop by clicking first  then  on the top of the FluCam window.

6.2.14 Click on the "Diffraction" button on the right-hand pad. You will now be in imaging mode on FluCam.

6.2.15 Remove the selected apertures. Spread the beam and be sure that the beam is observed in FluCam.

6.2.16 Finalize your session by first retracting the holder and removing your sample as in sections 4.1.2 and 4.1.3. Then, insert the holder without any sample, as in section 4.2. If you are the last person using the Microscope, finalize the TEM session as in section 3.2.

NOTE. During SAED experiments, α-tilt must be used to find the correct plane. However, this step will be explained as need it by FEMR staff.

6.3 ENERGY-DISPERSIVE X-RAY SPECTROSCOPY (EDS)

6.3.1 Initialize the Microscope following the steps in section 3.1 and load your sample into the Microscope as discussed in section 0. If you already have your sample loaded inside the Microscope, go to step 6.3.2.

NOTE. Retract any objective or selective aperture if you were analyzing a sample before EDS measurements.

6.3.2 In TEM UI>Workset>Tune. Verify that the spot size between #7-9 (preferably 8) and microprobe is selected on the Beam Settings window.

6.3.3 Pre-align the Microscope as in section 6.1-HIGH-RESOLUTION BRIGHT-FIELD TEM IMAGING (HR-TEM), remember to use the preferred spot size before the pre-align.

NOTE. During EDS experiments, the magnification is up to the user, and it depends on the type of material to be analyzed. However, use the specific magnifications to perform the alignment.

6.3.4 To perform an EDS analysis, go to Velox-acquisition software (left bottom monitor)

6.3.5 Click on "  " to display the options and parameters. Here, the most important parameter is the acquisition time (to have enough electron counts). Then, click on  to perform the analysis.

6.3.6 On Velox-analysis software, you will observe an EDS spectrum. Select the desired elements from the periodic table from the display settings (right panel).

6.3.7 To save the spectrum, right-click on the image and export it as CSV/TXT to plot the spectrum by yourself or export it as an image file (TIFF or PNG).

NOTE. If you want to continue with bright-field imaging again, change the spot size to 3 and pre-align the Microscope as in section 6.1-HIGH-RESOLUTION BRIGHT-FIELD TEM IMAGING (HR-TEM).

6.3.8 To finalize your session, follow steps 6.1.27.

MODULE #4 – ADVANCED CHARACTERIZATION METHODS

7 STEM MODE

7.1 SCANNING TRANSMISSION ELECTRON MICROSCOPY (STEM) IMAGING

7.1.1 Initialize the Microscope as in section 3.1 and load your sample as in section. If you already performed a previous analysis in TEM mode, proceed to the next step. **Ensure that the objective aperture is retracted!**

7.1.2 Open the column valve and load the 200_STEM_FEG GL:6 spot size:9 Register from "FEG registers" window in In TEM UI>Workset>Tune>FEG Registers. Once selected, remember to click the button **"Set"**.

NOTE. If the purpose of the STEM analysis is to perform STEM-EDS, select the register 200_STEM_FEG GL:3 spot size: 6. This register has a less spatial resolution for STEM analysis, but it is optimal for EDS measurements on STEM mode.

Tip. The register from this section is optimized for both STEM and EDS analysis. To save time, use this register if you will perform STEM-EDS analysis.

NOTE. Perform all these changes in the FluCam.

7.1.3 In TEM UI>Workset>Tune located at the control panels in the bottom-right monitor. Verify that the spot size is in #9 and nanoprobe is selected (This spot size is only if you are using STEM analysis only, use spot size #6 if you load the register for STEM-EDS). Please wait for normalization every time you change the spot size.

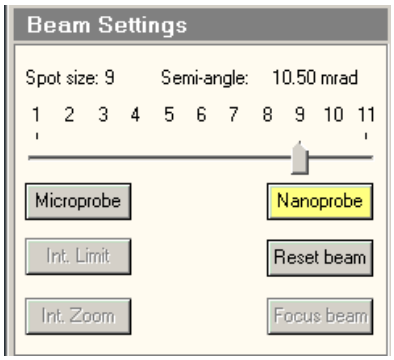


Figure 38 Beam settings windows for STEM mode. If your analysis is for STEM-EDS, select the corresponding register and verify the spot size is on #6.

7.1.4 Then, in TEM UI>Workset>STEM/CCD, change the camera to 98 mm on the STEM Detector window. Please wait for normalization every time you change the camera length.

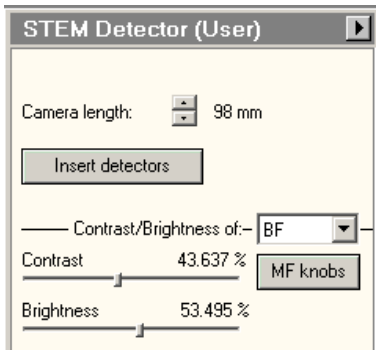


Figure 39 STEM Detector window where the length of the camera should be changed to 98 mm.

7.1.5 In the Velox Detectors toolbar, activate the HAADF detector. Velox opens an Image Display tab for each activated detector and starts scanning the beam in View mode.

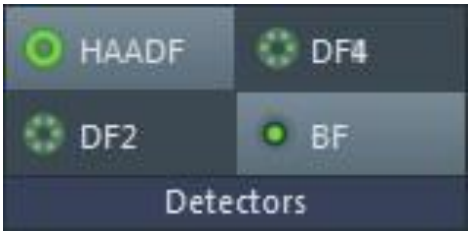


Figure 40 Detectors available for STEM analysis. These detectors are shown in Velox-acquisition software (bottom-left monitor) in imaging options.

NOTE. Our Microscope has different Dark-field and bright-field detectors. If you need them, please select the necessary detectors.

7.1.6 Click on the STEM Imaging dropdown menu, make sure the beam scanning presets for View mode are set to adequate values: for example, 512×512 scan size and 1.5 µs or 2 µs dwell time.

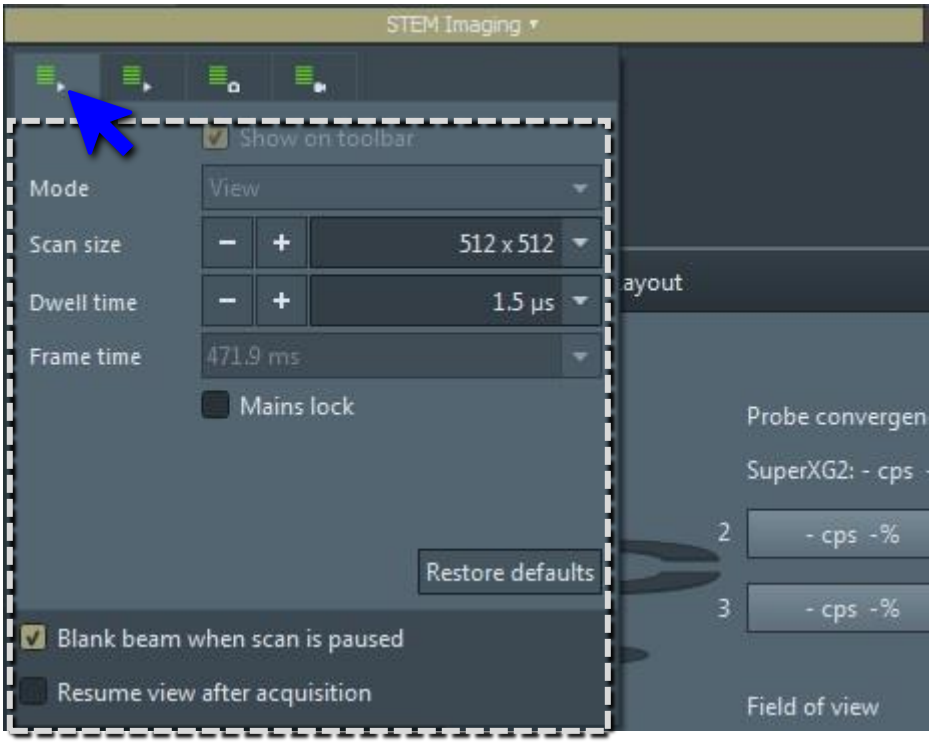


Figure 41 STEM imaging settings in live mode (view mode). This window is shown in Velox-acquisition software in the imaging options section.

Note: If you changed the mode from TEM to STEM, your eucentric focus will be the same and there is no need for alignment. However, if you start with STEM directly, find the optimal eucentric focus by pressing the alpha wobbler (R2-right panel) and pressing Z-axis buttons from the right panel until you reduce the shifting of the image. Your dwell time must be fast to see the shifting (approx. 200 ns)

7.1.7 If the HAADF image is improperly exposed, namely appears too dark or too bright, gain and offset adjustments are required. Click on the HAADF Image Display and watch the *detector saturation indicator* on the Display Settings side panel, Histogram tab (outlined gray below).

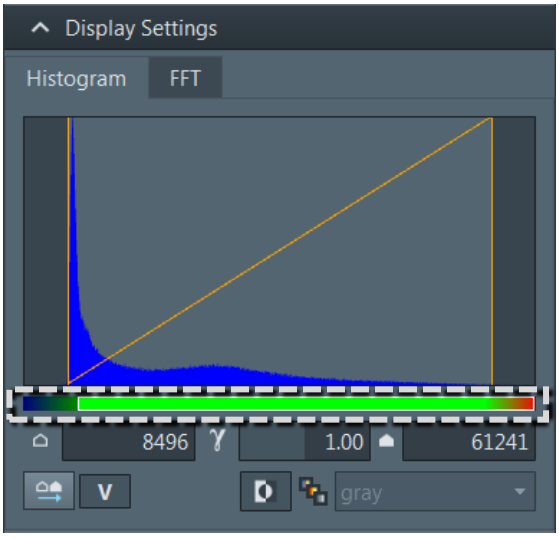


Figure 42 HAADF image settings. This window is in Velox-acquisition software in the settings section.

7.1.8 In the Detector Settings panel, click and drag slightly the percentage bar next to the gain setting to change the gain value. This will expand or shrink the indicator. Click and drag the offset bar to change the offset value. This will shift the indicator left or right.

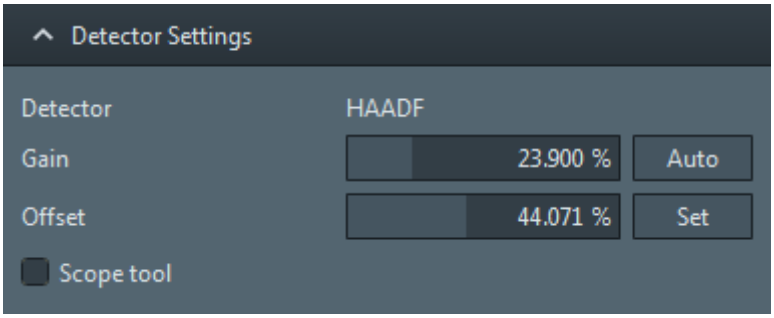


Figure 43 Detector settings window. It is located at the bottom of the HAADF image settings in the settings section.

7.1.9 Readjust the gain and offset until the image is properly exposed on HAADF image settings. The indicator is then centred and covers about 80 % of the green area. 

7.1.10 Change the magnification as needed and find a region of interest (ROI) by navigating across the sample using the joystick on the right-hand control pad.

7.1.11 If the images look out of focus (blurry), you can modify three parameters:

1) If you did not perform the optional step of performing a fast pre-alignment on bright-field mode, then use the Z-axis buttons on the right-hand control pad to bring the sample closer to focus. Otherwise, proceed to the next step.
2) Finetune the focus with the Focus knob, adjust the focus step as needed. The main parameter to improve the image quality is the Z-axis. The focus adjustment should be minimum.
3) If the images still appear blurry, click on stigmatism on the stigmator button in the left control pad. Then use the multifunction knobs to make the image as sharp as possible. The change should be minimum.

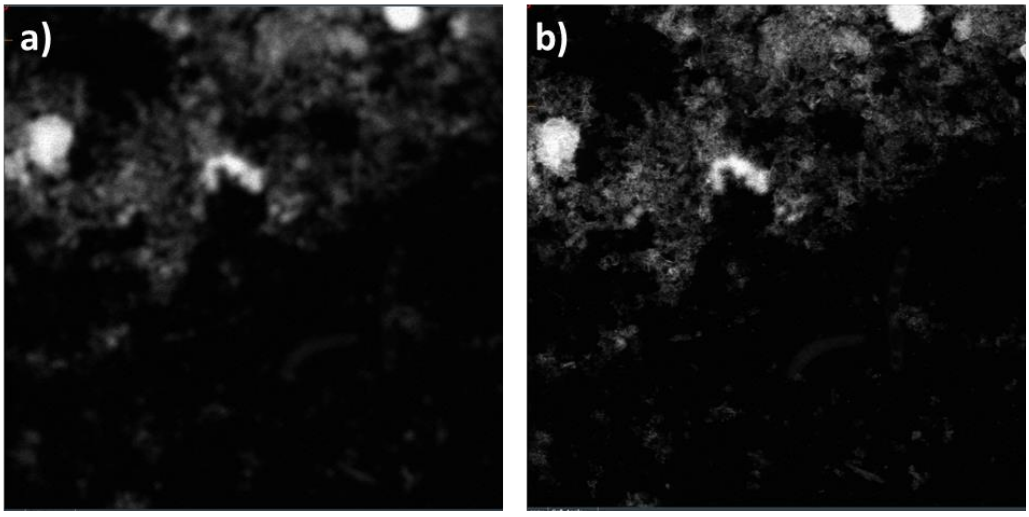


Figure 44 STEM image before (a) and after (b) the modification of z-axis, focus and stigmatism. Remember that the z-axis will be the parameter to provide a better and sharp image.

NOTE. Every time you move from a different ROI, modify steps 2 and 3 from section 7.1.11. And you can use a reduced area to observe the changes better. This reduced area will decrease the dwell time, and you will be able to observe in a better way the differences in steps 2 and 3 from section 7.1.11. If your sample is a flat substrate (e.g., SiO, Si_3N_4, graphene), the eucentric point will be similar when you change the ROI, and there is no need to modify Z-axis every time you change your analysis area if the substrate is not flat (e.g., carbon fils, lacey carbon, holey carbon). However, focus and stigmator must be verified every time you change the ROI.

7.1.12 When parameters are changed on STEM mode, you can use the reduced area option to improve your visualization. The reduced area button is  which is in the STEM imaging window in Velox-acquisition software. With this option, you can observe the real-time change (inside the blue square) with the previous image without change (outside the blue square).

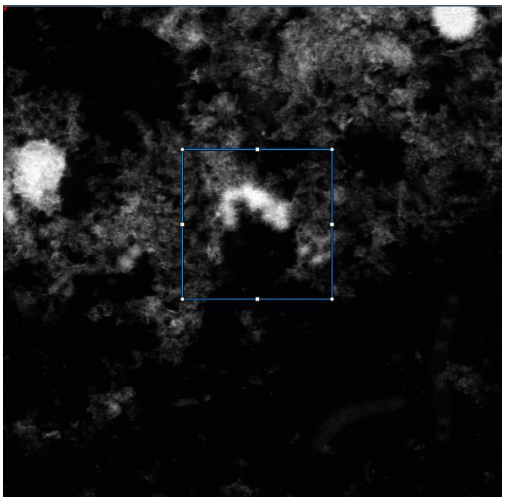


Figure 45 STEM image showing the reduced area selection. This option is helpful for focus and stigmatism changes due to its minimal change on the image.

NOTE. Be aware of deselecting the reduced area button once you finish with steps 2 and 3. Then, changes will apply to all your observation area.

NOTE. You may find that you will have a brighter area surrounding your particle during your acquisition in STEM analysis. This problem may be due to carbon contamination. Please, contact the FEMR staff for guidance.

7.1.13 Select image acquisition parameters: click on the STEM Imaging dropdown menu, make sure the beam scanning presets for Acquire mode are set to adequate values. Generally, scan size should be 1024×1024 or 2048×2048 and dwell time from 5 to 40 µs. Be aware that every time a user changes these parameters, they will be preset for the next user. We recommend writing down your parameters for future analysis.

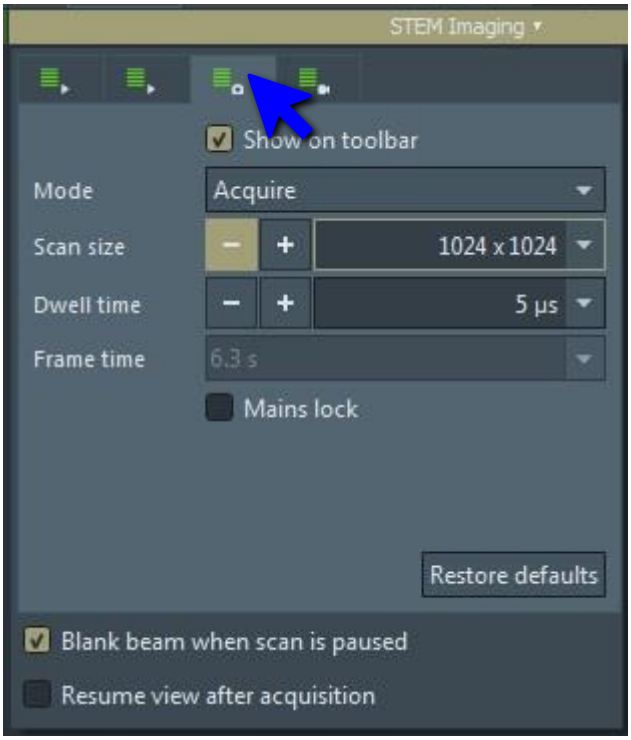


Figure 46 STEM imaging acquisition window. In this window, you will be able to modify your parameters for the desired image.

NOTE. The acquisition time depends on your sample. Usually, the drifting problem will be higher on a nanometric scale, and you will need a faster dwell time. For higher scales, drifting is minimized, and you can select a slower dwell time. However, if you have a faster dwell time, the resolution (image sharpening or small features below 10 nm) will be lost.

Tip. If you have a nanometric particle or sample to analyze, wait a couple of minutes to stabilize and minimize the drifting. If your sample is beam-sensitive, block the beam by selecting the button "L2" on the left-hand pad, deselect it to observe your image again.

7.1.14 Click Acquire. 

7.1.15 If needed, apply annotations in the Toolbar of the Processing window of Velox-analysis software (top-left monitor). Click File ► Save.

7.1.16 Proceed to your next analysis or stop the STEM imaging mode.

7.1.17 To exit STEM mode, blank the beam by selecting the button "L2" on the left-hand pad and insert the fluCam screen. Load a TEM bright-field alignment you used earlier to initially set up the instrument. Then un-blank the beam.

7.1.18 Find the beam and bring it to the Center. This procedure will help to avoid beam loss in the future. There is no need for the alignment of other parameters.

7.1.19 Finalize your session as shown in sections 4.1 (retract the beam) 3.2 (finalizing your session)

7.2 ENERGY-DISPERSIVE X-RAY SPECTROSCOPY (EDS) ELEMENTAL MAPPING

7.2.1 To perform a STEM-EDS analysis (usually for mapping purposes). Please, follow the steps from section 7.1, and be sure to select the 200_STEM_FEG GL:3 spot size: 6 register.

Tip. Before doing your STEM/EDS elemental map, take a STEM image. Sometimes during the EDX mapping, your sample may be damaged or contaminated with carbon, and you will not be able to take a good image again.

7.2.2 Once you find an ROI, go to the EDS panel in Velox-acquisition software (bottom-left monitor) in imaging options and select the window "SI" to display the EDS mapping options.

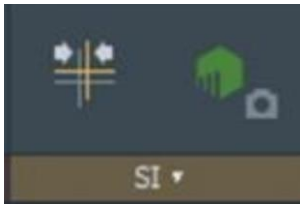


Figure 47 EDS-SI window, located in imaging options on Velox-acquisition software.

7.2.3 In SI options, you will need to modify four important parameters:

1) Dwell time

   Here, the dwell time is the time that will take one scan to analyze your area. For nanometric or single particles, we recommend using a dwell time of 2.5 us. Also, this time is good if your sample drift (even with drifting correction).

2) Auto-stop

   This option is selected if you want a specific analysis time. It will stop the analysis, depending on the number of frames you select. We recommend not select Auto-stop if you do not know how strong the EDS signals from your sample are. This section is useful when you want to compare an EDS analysis with two different samples. When comparing qualitatively two EDS analyses, you must use the same parameters, including the acquisition time (dwell time* number of frames).

3) Drift compensation

   This option will help in correcting the drift problem of your sample during the acquisition time. It is recommended for nanometric analysis.

4) Blank beam.

   Please avoid damages to the camera or if you have long acquisition times during the EDS analysis. We recommend selecting this option if you have long experiments.

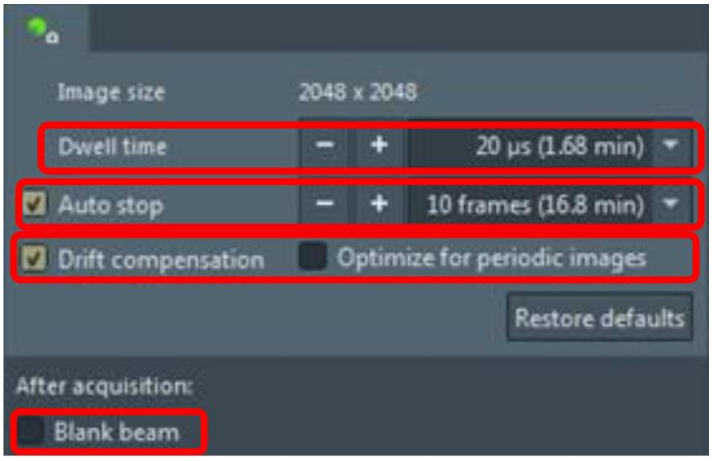


Figure 48 EDS-SI options window.

7.2.4 Once parameters have been selected, go to Spectrum Imaging Area, and draw an area of interest.

7.2.5 Then, select Drift Measurement Area and draw a drift area in the STEM image.

NOTE. We recommend drawing this area inside or outside the Spectrum Imaging Area. Try to find "sharp" well-defined structures or features to help with the drifting corrections.

7.2.6 Select Spectrum Imaging  to start the acquisition.

7.2.7 Select Spectrum Imaging again to stop acquisition if Auto Stop was not selected.

7.2.8 To exit STEM mode, blank the beam by selecting the button "L2" on the left-hand pad and insert the FluxCam screen. Load a TEM bright-field alignment you used earlier to initially set up the instrument. Then un-blank the beam.

7.2.9 Find the beam and bring it to the Center. This procedure will help to avoid beam loss in the future. There is no need for the alignment of other parameters.

7.2.10 Finalize your session as shown in sections 4.1 (retract the beam) 3.2 (finalizing your session)