ThermoFisher Apreo SEM
SOP

BASIC OPERATING INSTRUCTIONS

Log into the tool by using FOM, it will turn on the monitor.

Loading a Sample:

1. If *Sample Exchange Window* is not open, click to open it. 
2. Click **Vent** to initiate the vent cycle. Confirm by pressing the vent button in the pop-up window.

NOTE: Wear gloves when handling samples!

3. Gently slide open the chamber door; place your sample stub in one of the holes in the stage. Make sure your sample is securely mounted to the stub. If the sample is placed in a position that has a set screw, do NOT over-tighten the screw. Only finger-tight. Notify the lab managers if the set screw is getting stripped.

NOTE: If loading multiple samples, limit stubs to one type per session. Do not mix flat stubs with 45 stubs or 90 stubs to avoid collisions with the pole piece due to different sample heights.

4. While watching the live CCD chamber view, gently slide the chamber door closed. Ensure the chamber door is fully closed, then click **Pump** in the *Sample Exchange Window.*
5. Wait for the pumping status bar to reach the 'full' mark and then disappear, indicating the pump-down sequence is complete (~2 - 5min). Additionally, the chamber/column vacuum status icon at the bottom of the main UI window will appear fully green. 

Preparing to Image: All steps must be completed!!!

1. Raise the stage to **10 mm** marker by holding the middle mouse button and dragging upward; release the mouse button when your sample reaches the 10 mm marker.
2. At the top of the UI window, click Stage and select Take Nav-Cam Photo. Wait for the Nav-Cam operation to complete (~ 1min).
3. An image of your sample should appear in Quadrant 3 (Q3, the lower-left quadrant).
4. Navigate to your sample by double-clicking a location in the Nav-Cam photo.
5. Click the Beam Control button  on the side pane at the right of the UI.
6. Set accelerating voltage and Spot Size/ Beam Current in the drop-down menus; recommended initial values are:
   - High Voltage: 5 kV
   - Beam Current: 0.2 nA.
2. Click Beam **ON** to turn on the beam; the button will turn from grey to orange and you will hear a pneumatic valve actuate within the SEM column.

Basic Imaging:

1. Click within Quadrant 1 (Q1, upper left) to activate the quadrant; the data bar at the bottom of Q1 should be blue, indicating Q1 is active.
2. Click the Detectors button  on the side pane at the right of the UI. Select ETD from the dropdown menu, this assigns the ETD to the active quadrant, Q1.
3. Click the green pause button  in Q1 to turn on the detector and begin imaging.

NOTE: You can press the grey/black pause button at the top of the UI at any time to pause or un-pause image acquisition for the *active* quadrant. Please be aware that only two quadrants can be active at the same time.

4. Lower the magnification to its minimum by rotating the Magnification knob on the control panel counter-clockwise.
5. Adjust Brightness and Contrast manually by rotating the knobs on the control panel or perform the Auto Contrast & Brightness function by pressing. 
6. Adjust the Focus knob, then increase magnification to at least 5,000x and re-focus.
7. **Click the Link Z to FWD icon.** 
8. With the sample in focus, pressing this icon will link the position of the sample to the focal length of the microscope establishing the Free Working Distance, which is the distance between the sample and the bottom of the pole piece.
9. Before Link Z to FWD is clicked, the icon appears with a question mark (as shown above); after clicking, the icon changes to  indicating successful linking.
10. After linking, the stage Z-coordinate will display the working distance between the pole piece and portion of the sample used during linking.

NOTE: If your sample is <u>not</u> flat or is mounted such that it is <u>not</u> parallel to the sample stage, you risk collision with the pole piece at smaller working distances. For *non-flat* samples, focus at the highest point on the sample and then Link Z to FWD.

11. You can now navigate across your sample to locate features of interest. Navigation can be accomplished in several ways:

    Mouse: Double-click a point on the Nav-Cam image or the electron detector image to move the stage to that location. NOTE: Nav-Cam navigation is typically used at lower magnifications, to navigate to approximate locations on a sample (due to resolution limits of the optical Nav-Cam image)

    Arrow keys: While imaging the sample with the ETD in Q1 (note: Q1 must be *active*), pressing the left /right /up /down arrow keys on the keyboard will shift the stage in a given direction. The magnitude of the shift is ~ 80% the field of view.

    Stage coordinates: Click the Stage button  on the side pane. You can enter in specific x-and y-coordinates to drive the stage to that location. Sample Rotation and Sample Tilt are also available.

NOTE: The use of Tilt should be done with caution. Tilting samples greatly increases the risk of a pole

piece collision. Always view the LIVE CCD image when tilting. Tilt with caution! Maximum tilt angle allowed is 30°. Or use pre-tilted stubs (45° or 90°).

Some Notes on Working Distance:

You should enable the live CCD view whenever you change WD (the z-coordinate of the sample) or whenever you Tilt; this is to ensure there is NO chance of a pole piece collision.

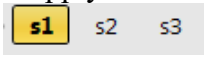
NOTE: If you have initiated stage movement and you fear a pole piece collision is imminent, you can halt stage movement by pressing the ESC key on the keyboard. Be ready to press ESC!

Improving Image Quality:

1. Iteratively adjust Focus, then X-Stigmation and Y-Stigmation to achieve better images
2. Reduced Area mode  is recommended to use, it is handy when focusing and correcting astigmatism, as the imaging update is faster in the smaller area.
3. Perform Lens Alignment:
   a. Make note of some features in the live image in Q1.
   b. Click the Lens Alignment button. 
   c. The image will begin to wobble. Drag the cross-hairs that appear on the wobbling image in the x- and y-directions. The goal is to minimize the wobbling in the image.
   d. After minimizing wobbling, click the lens alignment button again.

Capturing Images:

1. Users can adjust frame averaging, dwell time and scan resolution by adjusting settings from the dropdown menus at the top of the UI: 

   or users can apply one of three preset scans available by pressing the s1, s2, s3 buttons at the top of the UI. 

   - s1 – TV scan rate with a 0.1s frame acquisition time
   - s2 – HDTV scan with a 0.2s frame acquisition time
   - s3 – this button can be edited / customized by the user; right click on the s3 button and select edit. NOTE: Please do not edit s1 or s2, only edit s3.
2. There are two preset image capture buttons; pressing either of these will scan and capture the image and prompt the user to save the image file:
   - The Snapshot icon 
   - The Photo icon 

NOTE:

- Save the un-annotated grayscale image as an 8-bit or 16-bit .tif image, 24-bit images are useful only for color. This file format is as uncompressed as the system can provide, while also including the metadata of the system parameters in the footer of the file.
- Annotated images may be saved as .jpg as a version of note-taking. These images are compressed, so data will be lost.

- Sample Removal / Ending Your Session

1. Click  and set Scan Rotation to 0° if not already.
2. Click  and set WD to 10mm or greater; If Tilt or Rotation were used, set both to zero; set stage translation X=0 and Y=0
3. Click  and then to turn off the beam.
4. Click Vent and confirm by pressing vent in the pop-up window.
5. If Q3 was changed, set back to Nav-Cam view, if Nav-Cam zoom was used, click View > Undo Digital Zoom at the top menu of the UI.
6. If Q4 was changed, set back to Camera view.
7. Wait for vent cycle to complete, then slide open the chamber and retrieve your sample.
8. Slide chamber closed and press Pump.
9. Logout in the FOM from the SEM.