Standard Operating Procedure for SEM3

[Note the locations of the various SEM3 components in this **Video: SEM3-00**]
*** Videos have no sound ***

Before beginning, ensure you have an active reservation for SEM3

1 - Loading a Sample:

a) If *Sample Exchange Window* is not open, click  to open it **[Video: SEM3-1a]**
- Click *Chamberscope* tab
- If  icon appears in the Chamberscope image, click to un-pause
- Click 

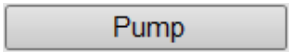
to initiate the vent cycle
- Confirm by pressing the vent button in the pop-up window
- A green status bar will appear at the bottom of the *Sample Exchange Window*… wait for the status bar to disappear, indicating completion of the vent cycle

b) Gently slide open chamber door; place your sample stub in one of the holes in the stage. Make sure your sample is securely mounted to the stub (e.g. with vacuum compatible conductive tape)

Note: If loading multiple samples, limit stubs to one type per session. For example, do not mix flat stubs with 45° stubs or 90° stubs. This is to avoid collisions with the pole piece due to different sample heights. **[Video: SEM3-1b]**

c) Watch the live CCD chamber view and gently slide the chamber door closed **[Video: SEM3-1c-e]**

⚠ Make certain the sample will not contact the pole piece (your sample should be below the 10mm mark on screen)

d) Fully close the chamber door then click 

in the *Sample Exchange Window*

e) Wait for the pumping status bar to reach the 'full' mark and then disappear, indicating the pump-down sequence is complete (~ 5min). Additionally, the chamber/column vacuum status icon at the bottom of the main UI window will appear fully green: 

2 - Preparing to Image:

a) At the top of the UI window, click **Stage** and select **Take Nav-Cam Photo**. Wait for the Nav-Cam operation to complete (~ 1min). An image of your sample should appear in Quadrant 3 (Q3, the lower-left quadrant). **[Video: SEM3-2a]**

b) Lower magnification to its minimum by rotating the Magnification knob on the control panel counter-clockwise. **[Video: SEM3-2b-c]**

c) Navigate to your sample by double-clicking a location in the Nav-Cam photo; the x-y stage will move to the specified location

d) In the *Sample Exchange Window*, raise the sample stage to the 10mm marker by holding the middle mouse button and dragging upward; release the mouse button when your sample reaches the green 10mm marker **[Video: SEM3-2d]**

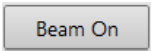
⚠ If your sample is magnetic, check the ☑ **Magnetic Sample** option

e) Click Mode Selection:  and select **Mode 2: Optiplan** in the main UI **[Video: SEM3-2e-i]**

f) Set accelerating voltage and emission current in the drop-down menus; recommended initial values are 2kV and 25pA: 

NOTE: For those accustomed to using SEM1 in SMIF, you may be tempted to use high voltages (e.g. 20kV – 30kV), with the intent of getting high-resolution images. The Apreo does not require high voltages for high resolution imaging; 1kv – 5kV is typical.

g) Click the Beam Control button on the side pane at the right of the UI: 

h) Click 

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

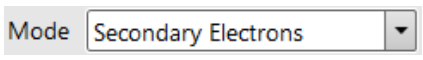
i) Click  to zero the beam shift (this centers the beam within the column)

3 - Basic Imaging:

a) 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 **[Video: SEM3-3a-c]**

b) Click the Detectors button on the side pane 

- Select **ETD**: 

(this assigns the Everhart-Thornley detector to the active quadrant, Q1)
- Select **Secondary Electrons**: 

c) 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: 

d) Adjust Brightness and Contrast manually by rotating the knobs on the control panel -OR- perform the Auto Contrast & Brightness function by pressing:  **[Video: SEM3-3d-f]**

e) Adjust Focus knob, then increase magnification to at least 5,000x and re-focus

f) Click the **Link Z to FWD** icon: 

- 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
- 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
- After linking, the stage Z-coordinate will display the working distance between the pole piece and portion of the sample used during linking.

 Attention: 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.

g) You can now navigate across your sample to locate features of interest. Navigation can be accomplished in several ways: **[Video: SEM3-3g]**

- <u>Mouse:</u> 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)
- <u>Arrow keys:</u> 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 each 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

  ⚠ Attention: 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°

h) Some notes on Working Distance: **[Video: SEM3-3h]**

- At this point, your Working Distance (WD, as displayed in the data bar) should be *approximately* 10mm (you positioned your sample at the 10mm marker in the **Preparing to Image** section above)
- 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
- A good WD for the ETD detector is 7mm
- A good WD for Immersion mode is 5mm
- The MINIMUM WD SMIF allows is 3.0mm; Do NOT use WD smaller than 3.0mm

  ⚠ Attention: 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!

4 - Improving Image Quality:

a) Iteratively adjust Focus → X-Stigmation → Y-Stigmation to improve image **[Video: SEM3-4a]**

b) Perform Lens Alignment: **[Video: SEM3-4b]**

- Make note of some feature in the live image in Q1
- Click the Lens Alignment button: 
- The image will begin to wobble. Drag the crosshairs that appear on the wobbling image in the x- and y- directions. The goal is to minimize the wobbling in the image.
- After minimizing wobbling, click the lens alignment button again

5 - Capturing Images:

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

[Video: SEM3-5a]

b) -OR- users can apply one of three preset scans available by pressing the **s1**, **s2**, **s3** buttons at the top of the UI: 

(in this image, S1 is selected) **[Video: SEM3-5b]**

- **s1** – TV scan rate with a 0.1s frame acquisition time (use for live imaging)
- **s2** – HDTV scan with a 0.2s frame acquisition time (use for reduced area live imaging)
- **s3** – this button can be edited / customized by the user; right click on the s3 button and select edit (use for image capturing by pressing **Ctrl** + **s3**)

  Note: please do not edit s1 or s2… only edit s3

c) 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: **[Video: SEM3-5c]**

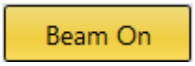
-  The **Snapshot** icon captures a ~1.5MP image in ~ 24 seconds
-  The **Photo** icon captures a ~6.5MP image in ~ 60 seconds

6 - Sample Removal / Ending Your Session:

a) Click  and set Scan Rotation to 0° if not already **[Video: SEM3-6a-f]**

b) 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

c) Click  at the top of the UI to zero beam shift

d) Click  and then 

to turn off the beam

e) Click 

and confirm by pressing vent in the pop-up window

f) If Q3 was changed, set back to Nav-Cam

g) If Nav-Cam zoom was used, click **View** > **Undo Digital Zoom** at the top menu of the UI

h) If Q4 was changed, set back to Camera

i) Wait for vent cycle to complete, then slide open chamber and retrieve your sample

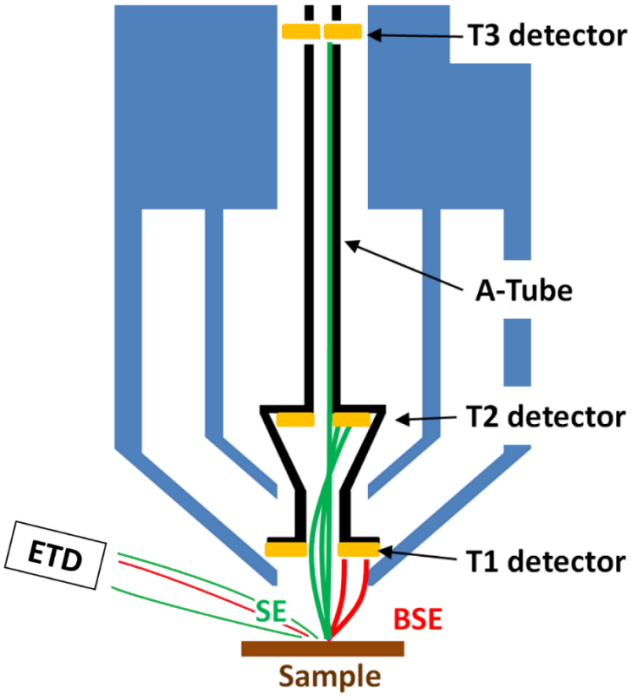
j) Slide chamber closed and press 

[Video: SEM3-6i-j]

k) End your reservation if necessary

- **APPENDIX 1 - Utilizing Multiple detectors (ETD, T1, T2, T3): [Video: SEM3-A1]**
    - In addition to the ETD described above, the Apreo is equipped with a set of *Trinity* detectors, located within the column: T1, T2 and T3
    - Following the procedure described in the **Basic Imaging** section above, users can assign different detectors to different quadrants (Q1, Q2, Q3, Q4)
    - Users can opt between any of the detectors, depending on imaging needs
    - Simultaneous imaging of up to 4 detectors (one in each quadrant) is possible

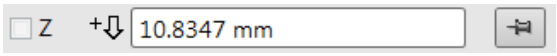
      Note: Holding **Shift** and pressing a button will perform that action on all imaging quadrants:
        - Shift +  captures a snapshot from all imaging quadrants
        - Shift +  (un)pauses all imaging quadrants
        - Shift +  performs auto brightness/contrast on all imaging quadrants



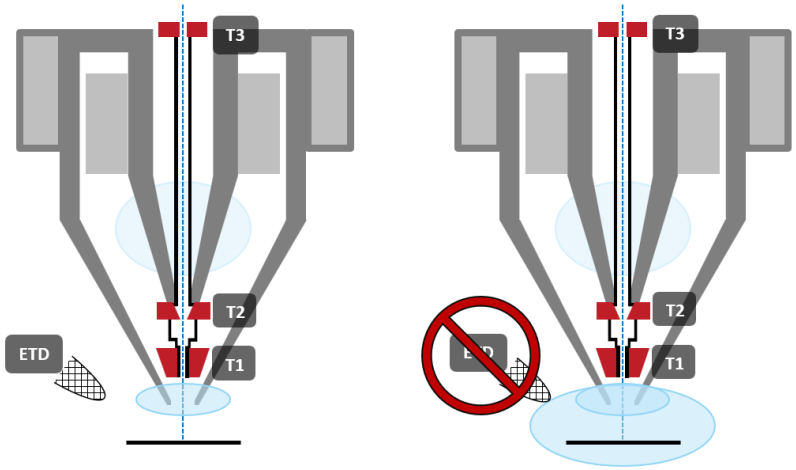
- **APPENDIX 2 - Immersion Mode:** [Video: SEM3-A2]

  In the **Preparing to Image** section, **Optiplan** was selected as the imaging mode. Optiplan is suitable for a variety of samples and is capable of high resolution imaging. When ultra-high resolution is desired, users can enter **Immersion Mode** as follows:

  - Verify your sample is NOT magnetic (if it is, you should have checked the ☑ **Magnetic Sample** in the **Preparing to Image** section above); do NOT proceed with immersion mode if your sample is magnetic!
  - Ensure the CCD view is live (i.e. no green pause button in the upper left of Q4)
  - Click the Stage button on the side pane:  .
  - Ensure that you have performed the Link Z to FWD operation (as described in the **Basic Imaging** section above); if linking was performed, there should be a down-pointing arrow beside the z-cordinate:

    If not, perform Link Z to FWD as described in the **Basic Imaging** section
  - With the CCD image live, change your working distance to 5.0mm (this is a good starting point for immersion mode)
  - Click the down arrow on the Mode Selection icon:  and select **Mode 3: Immersion**
  - Assign your detectors (T1, T2, T3) to your desired quadrants, as described in the **Basic Imaging** section; Note: The ETD is not intended for Immersion mode.



OptiPlan
Electrostatic lens

Immersion Mode
Compound final lens