Basic Operating Instructions for the
SNSF Thermo Fisher Scientific Apreo SEM

Warnings

DO NOT HIT THE LENS PROTECTIVE CAP
Damaging the lens shuts down the system for everyone, and is extremely expensive and lengthy to repair.
Do not make us have to talk to your PI and/or financial officer about the costs for that repair.

Negligent behavior may result in the loss of privileges.

Notes

These instructions are for generic, basic use of the SEM. You should do the appropriate literature search to inform you of parameters and conditions that would be pertinent to your own samples. The system is capable of more advanced work; consult with the trainers if you need access to those capabilities.
Start-up

1) Log into Badger; enable the Apreo SEM (SNL, Nano, SEM: TFS Apreo).

2) Log into your profile within Microscope Control. Check that the system is in a reasonable state before you proceed. The SEM is normally left such that:
   - The system is at high vacuum.
   - The source is on (full green bar under the Beam On button in the Beam Control page).
   - The voltage is off (Beam On is not highlighted)
   - Check that the CCD camera is showing a live image, and there is nothing unexpected in the chamber (i.e., someone else’s sample).
   - Ensure the stage is in the Sample Exchange position (X = 50, Y = 0, Z (red Up arrow) = 40).
     - Navigation page, can double-click on the preset Sample Exchange location if needed.

3) Load the sample:
   - From the Beam Control page, choose “Vent”, then confirm.
     - Venting takes about 1.5 minutes until the chamber door can be opened.
   - **WEAR GLOVES WHEN HANDLING SAMPLES**
   - 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.
   - Close the chamber door carefully while watching the CCD camera. The sample should be well below the lens protective cap.
   - Hold the door shut with gentle, horizontal pressure. Click “Pump” on the Beam Control page.
     - Sample Exchange window will open up
     - Choose “no accessory”, unless the low vacuum attachment is installed.
     - Check the Magnetic Sample box if your sample is magnetic.
     - Wait for the vacuum valve noise, then a slow 3-count, then check that the door is sealed by tugging on the handle gently.
     - Close Sample Exchange window.

4) Take a Nav-Cam image (optional). Click on Stage, Take Nav-Cam Photo.

5) Laterally translate to the first sample, or the middle of the stage.
• Either double-click on your sample in the Nav-Cam image, or
• Go to (0, 0) in X and Y on the Navigation page.

6) Open the Sample Cleaning dialog box (Tools, Sample Cleaning). Wait for good vacuum (green chamber icon in the lower right; below 9 x 10^{-5} mbar). Click “Start” to plasma clean the sample(s) and the chamber.
• Note: if your sample will be destroyed by the air plasma (e.g., graphene, lithium, carbon nanotubes, etc.), you should plasma clean the empty chamber either before or after your experiment. In effect, the chamber should be cleaned once with each sample exchange, either with or without your sample inside.
• Default plasma duration is set to 120 seconds. System automatically pumps back down after the plasma cleaning cycle.

7) After the chamber returns to good vacuum, raise the stage to the proper operating height.
• Bring your sample to the 10 mm line in the CCD window.

8) Ensure your Beam Current and High Voltage are set to your experimental parameters.
• 5 KV, 50 pA is often a generic starting point for conductive samples.

9) Click on Beam On in the Beam Control page to turn on the high voltage.

10) Check that the quad you want to use is configured properly for your imaging.
• Begin with Quad 1.
• Check that you are on the appropriate detector, usually the ETD, in SE mode.
• Check that you are in Standard mode.
• Check your dwell time, resolution, and filtering.
  • 200 ns, 768 x 512, Average 4 is a generic starting set of parameters

11) Un-pause the detector.

12) Check the source tilt and adjust as needed.
• In the Direct Adjustments page, Beam tab, choose “Crossover”.
• Adjust Contrast as needed to see the beam.
• Adjust Crossover Zoom as needed to adjust the size of the crossover image.
• Center the crossover image in the middle of the quadrant using the Source Tilt controls.
• Un-click “Crossover” to return to normal imaging.

13) Set Magnification to 5000 – 8000x.

14) Adjust Contrast and Brightness as necessary.
15) Focus the image.

16) Link Z to the (Free) Working Distance. Stage drop-down menu, Link Z to FWD, or using the shortcut button.

17) Proceed with your characterization experiment.
Basic Imaging Guidelines

1) Ensure the system imaging parameters are appropriate for the characterization to be done. These include, but may not be limited to, the following:
   - Beam voltage
   - Beam current
   - Microscope mode
   - Stage bias
   - Detector(s)
   - Magnification (at least one step beyond your desired image field of view)
   - Tilt correction

2) If you have a particular Region Of Interest (ROI), do your alignments nearby (about a frame-width away), but not directly on it.

3) Ensure the Source Tilt is centered, and that the contrast and brightness settings are appropriate.

4) Focus as best as you can. Always oscillate through focus to ensure you find the best condition.
   - If focusing causes translation of the image, where the direction of image translation correlates to over- vs. under-focus, fix your Lens Alignment.
     - In the Direct Adjustments, Beam tab, Lens Alignment, choose either Lens Modulator (if HV > 3KV), or HV Modulator (HV ≤ 3 KV).
     - Minimize translation during the wobble. Wobble amplitude can be adjusted as necessary.
     - Turn off the Modulator, examine your image as you manually adjust focus.
     - Repeat until the image translation is no longer distracting.
   - For ≤ 3KV, there is a chance the image will still translate even while the HV Modulator shows no movement. In this case, go to the Focus tab, and turn on the Focus Centering Modulator. Minimize motion during the wobble. Check manually and repeat as necessary.

5) If the image skews orthogonally through focus, fix the astigmatism.
   - Approximate best focus (mid-point of skewed images).
   - Adjust one stigmator to find sharpest image. Repeat with the other stigmator.
     - If the image translates as a stigmator is adjusted, similar to the focus image translation, fix the Stigmator Centering.
     - In the Direct Adjustments, Stigmator Centering tab, choose the axis you want to adjust. Turn on the Modulator for that axis; minimize motion. Iterate as needed. Repeat for the other axis.
• Re-focus. If orthogonal skew remains as you go through focus, re-iterate the above steps.
  • Best to use very small objects, and preferably round ones, to optimize your focus and astigmatism.

6) After optimizing for focus and astigmatism, reduce the magnification by one step on the knob. Translate back to the ROI, or move a frame away from the optimization region.

7) Adjust contrast and brightness for the image acquisition scan conditions.

8) Acquire the image using appropriate scan parameters. Save the finished image.
  • Save the un-annotated grayscale image as a 16-bit .tif image.
    • 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.
    • 24-bit images are useful only for color.
  • Annotated images may be saved as .jpg as a version of note-taking. These images are compressed, so data will be lost.
Shutdown

1) Return to Standard mode, ETD detector. Reduce the magnification to minimum. CCD camera should be live.

2) Retract any insertable detectors.

3) Ensure tilt is back at 0°.

4) Turn off any stage bias.

5) Return voltage and beam current to those you want to start with for your next session. Turn off the beam.

6) Return the stage to the Sample Exchange position.

7) Vent the chamber and remove your sample.

8) Pump the chamber down again properly; make sure you obtain good vacuum.

9) Log out of your profile.

10) Set up any data transfer that you need.

11) Clean up the area.

12) Disable your Badger session, close the Badger window.