Materials Characterization Core

DREXEL UNIVERSITY
Office of
Research & Innovation

ThermoFisher Apreo 2S Lo Vac SEM

Complete Standard Operating Procedure

Last revision: 20 Feb 2026

Version: NB1.2

CONTENTS

ILAB NAME: SEM – APREO 2S LOW VAC - THERMOFISHER

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INTRODUCTION

The ThermoFisher Apreo 2S Lo Vac SEM is an advanced scanning electron microscope equipped with a state-of-the-art set of in-situ modules. This microscope can operate with accelerating voltages up to 30 kV and can achieve a 1 nm resolution at a working distance of 10 mm (with the right conditions and alignments, of course.)

USER RESPONSIBILITIES AND SAFETY

Ensure the system is in a safe state before and after using the microscope:

- Movable parts including stage and detectors should be in a safe position.
- Beam should remain off with the system under high vacuum when not in use.
- The venting gas main valve should be closed when not in use.
- Workstations should remain clean.
- Users should always wear gloves when handling anything that goes inside the chamber.
- Do not wear gloves while operating the computer
- When in doubt, ask for help.

TRAINING AND QUALIFICATIONS

Training consists of 3 parts:

- Session 0: 30 minute Zoom overview of basic SEM topics.
- Session 1: Demo and practice
  - Sample preparation
  - 40 minute demonstration of SEM
  - 1 hour staff-assisted walkthrough of operation
  - Additional time for practice, sample preparation, etc.
- Session 2: Driver's test
  - Trainee operates microscope from startup to shutdown with staff supervision.
  - Sufficient operation of the microscope qualifies users for 9-5 weekday access.
- Additional sessions may be arranged at the equipment manager's discretion for practice, before qualification, or for advanced modes (see Appendices)

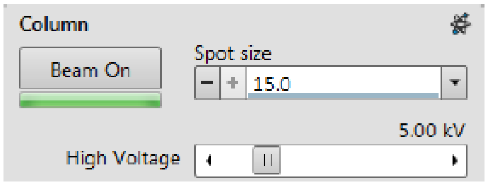
OPERATING PROCEDURES

1. SAMPLE PREPARATION

- Always wear gloves when handling anything that goes inside the chamber.
  - *Please **do not wear gloves while operating the computer**.*
- Specimens should be **vacuum stable**, **clean**, and **secured to a stub**.
  - *The MCC has a **plasma cleaner**, a **small benchtop oven**, and a **compressed gas line** from the venting gas available for specimen preparation. Contact staff for assistance.*
  - *Outgassing, debris, or loose particles may contaminate the chamber and vacuum system.*
  - *Otherwise imperceptible specimen motion may become apparent at high magnifications.*
- Bring specimens already prepared or prepare specimens at the station near the SEM.
  - *Carbon tape, copper tape, and a small selection of pin stubs are provided.*

2. SYSTEM START UP

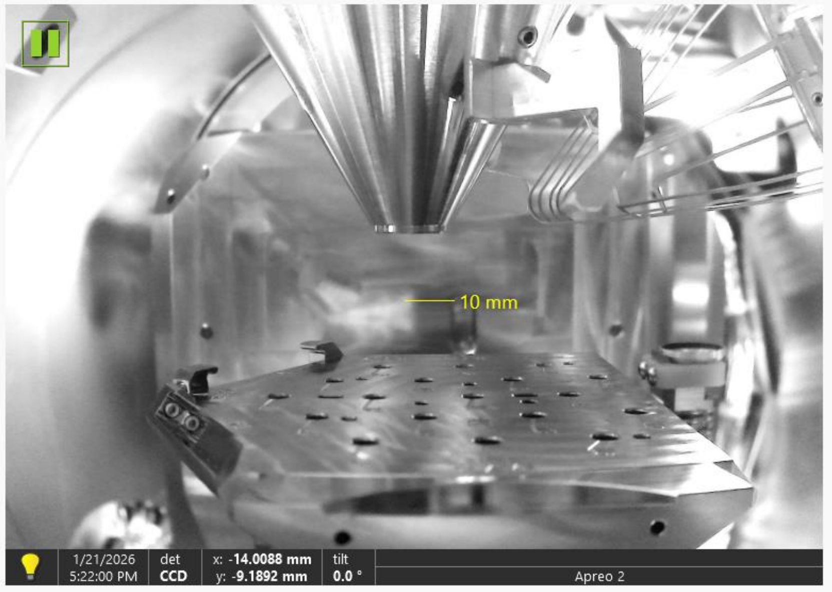
1. Start your session in the iLab kiosk to turn on the monitors.
2. Log in to your user profile.
   - If somebody else is logged in, log them out first.
3. Contact equipment manager if the system is not in an operable state:
   - On the Beam Control tab:
     - The system diagram should be fully green.
     - **Beam On** should be clickable but gray.
     - The bar under Beam On should be fully green.
   - CCD camera should be working and live.
     - Click the pause icon to unpause the frame.
   - The stage should be below the 10 mm line.
     - In the CCD window, click the scroll wheel and drag the mouse down to lower the stage.
4. **Fill out the logbook.**



Spot size 15.0

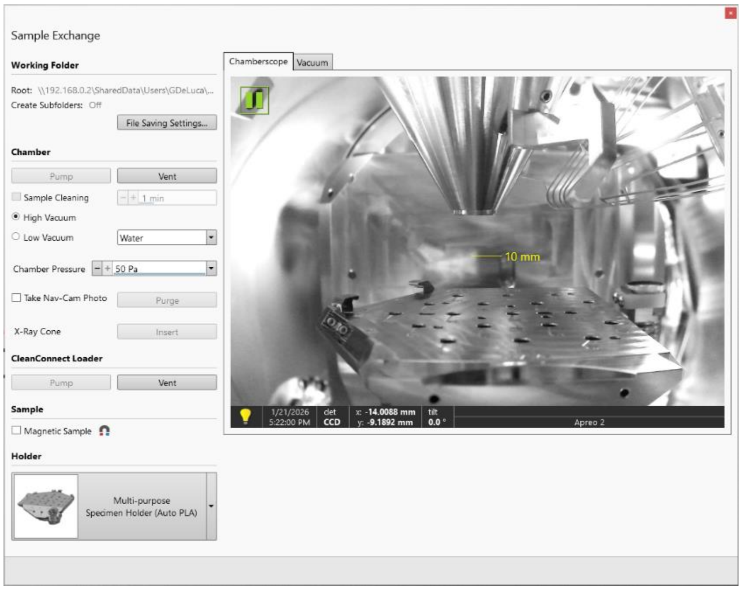
5.00 kV

High Voltage



3. SAMPLE LOADING

1. Open the main valve on the venting gas cylinder.
2. Click ⇄ to open the Sample Exchange window.



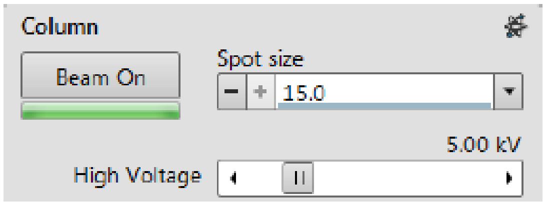
Root: \\192.168.0.2\SharedData\Users\GDeLuca\...
Create Subfolders: Off
☐ Sample Cleaning 1 min
● High Vacuum
○ Low Vacuum Water
Chamber Pressure 50 Pa
☐ Take Nav-Cam Photo
X-Ray Cone
☐ Magnetic Sample
Multi-purpose Specimen Holder (Auto PLA)
1/21/2026 5:22:00 PM det CCD x: -14.0088 mm y: -9.1892 mm tilt 0.0 °

3. Select **Vent** to vent the chamber.
   - Wait for the system to vent.
   - The door will open easily when it is finished.
4. Gently open the chamber door.
5. Arrange pin stubs in the holes on the stage.
   - **Always wear gloves when handling anything that goes inside the chamber.**
   - **Do not lean on the chamber door.**
   - Do not mix flat stubs with cross section stubs – **specimens should be of similar heights.**
   - Finger tighten set screws if loading stubs into set screw locations.
6. Gently close the chamber door while watching the chamberscope.
7. Check the Magnetic Sample box if your specimen is magnetic.
8. Select high or low vacuum then **Pump** to begin pumping the chamber to vacuum.
   - High vacuum is compatible with most specimens. Contact the equipment manager if you want to use a mode you have not been trained to use yet.
   - Select No Accessory or whatever accessory you installed on the pop-up.
   - Select Plasma Clean if you want to clean your specimen. See the Appendix for more information
9. While the system pumps:
   - Raise the stage until the tallest specimen is at the 10 mm line in the CCD camera.
     - > In the chamberscope view, click the scroll wheel and drag the mouse up to raise the stage.
   - Collect a navigation image.
     - > **From the top ribbon, click Stage > Take Nav Cam Photo and wait**.
     - > **Ctrl+shift+Z also starts this process.**
10. The system is ready to proceed when the vacuum icon looks like

4. INITIAL CONDITIONS

1. Select the following settings (See the Appendix for more information.)
    - Voltage
    - Beam current
    - Starting region of interest (double click in navcam image)
    - Imaging mode
    - Detector (possibly multiple)
    - Dwell time
    - Resolution
    - Filtering

5. FIRST IMAGE



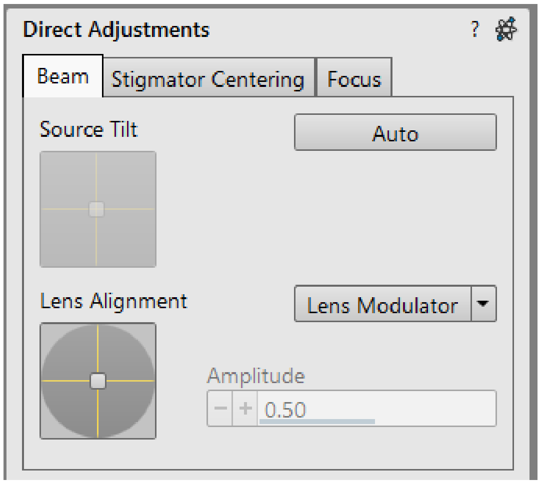
Spot size 15.0

High Voltage 5.00 kV

1. Click **Beam On** to activate the electron beam.
2. Click the pause icon in any frame to un-pause it.
    - All four quadrants can be set to different electron detectors and scan live simultaneously.
3. As needed, select each frame and adjust the brightness and contrast. For any SE detector:
    - Turn the contrast down until the image is noiseless (flat color.)
    - Adjust the brightness until the image is a very dark gray (as close to black as possible.)
    - Turn the contrast up until the image has sufficient contrast.
    - Additional adjustments tend to follow: brightness down, contrast up.
    - If the image appears too dark or bright, try changing the contrast first.
    - For BSE images, or as a first pass, try the auto contrast and brightness adjustment. ◑
4. Navigate the sample as needed to find a feature on the specimen surface. It may be very blurry.
    - Double click in the navcam image.
    - Double click in the electron image.
    - Click the scroll wheel and drag the mouse.
5. Slowly turn the focus knob and watch the features change.
    - If features get sharper, keep going until they appear worse.
    - If features get blurrier, turn the knob the other way until they appear worse.
    - After the image appears worse again, turn the focus knob back to the best image.
    - Note the working distance in the data bar at the bottom of any image.
6. Zoom in at least one step past the target field of view and refocus.
7. Click to link the working distance to the stage height. If it does not look like: press it again.
    - This tells the system where your specimen is for safer movements inside the chamber.
    - When changing the stage height, the working distance will follow.
8. Adjust the stage height until the working distance is 10 mm.
    - This is the optimal working distance for most detectors in this SEM.
9. Make any adjustments to voltage, beam current, and imaging mode before proceeding.
10. If the auto source tilt fails, use the axes in the popup to center the beam spot on the crosshair.

6. ALIGNMENT: APERTURE

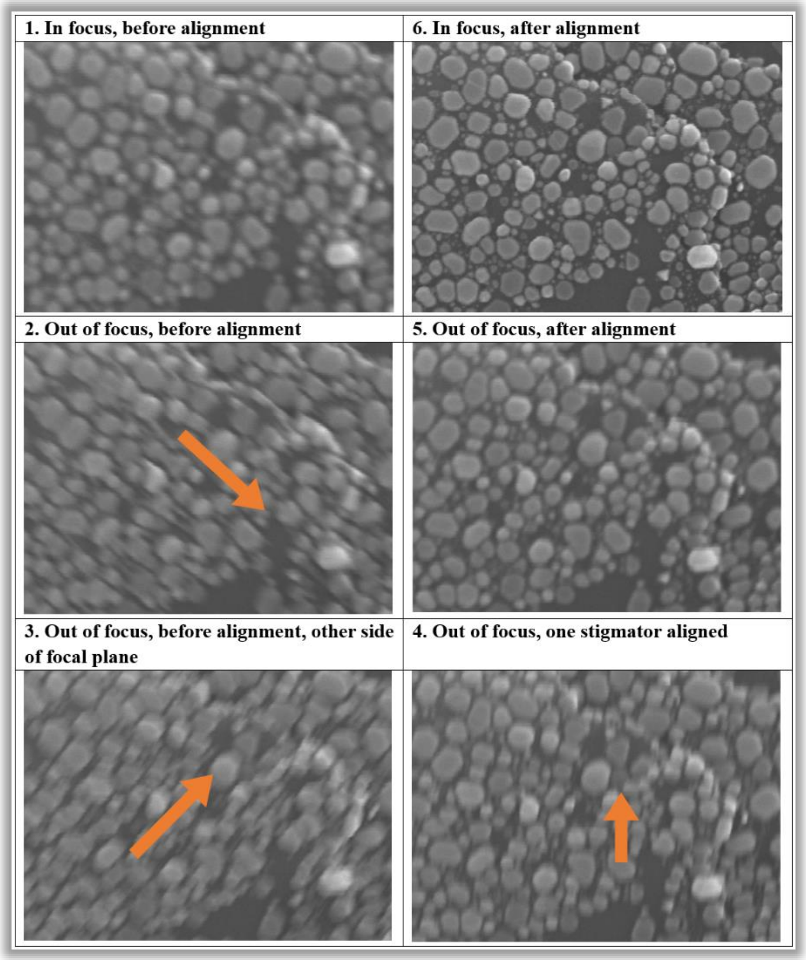
1. Adjust the magnification to at least one step beyond the desired field of view.
   - Magnifications over 2,000X are best. Otherwise, rotation or twisting of the image is normal.
   - If the specimen is sensitive to the beam, do not perform these alignments directly on a desired field of view; acceptable locations are at the same height with small, visible features.
   - Stigmator alignment (next) will be easier with features that are roughly round or random in shape; avoid using fields of view with only parallel/straight lines.
2. **Ensure the image is in focus before proceeding.**
3. Align the aperture: Full frame scan (not reduced area) at a video-rate speed is best here.
   - Go to Direct Adjustments > Beam > Lens Alignment and click on **Lens Modulator.**
   - **Adjust Wobble Amplitude** so you can see the direction features shift.
     - If the image blurs quickly out of focus, reduce amplitude.
     - If the image appears to not move at all, increase amplitude.
   - Use the Axes to align the aperture **until the image pulses in and out of focus without shifting**.
     - If the image shifts along the X axis, grab the Y axis and drag it left-right
     - If the image shifts along the Y axis, grab the X axis and drag it up-down.
     - If the image shifts along a diagonal, pick one axis, then repeat with the second.
   - Click Lens Modulator again to disengage the wobbler and return to normal imaging.
     - The image may be slightly out of focus.



Amplitude 0.50

7. ALIGNMENT: STIGMATORS

1. Align the stigmators: Reduced area scan at a slower speed may be helpful here.
    - Adjust focus above and below the specimen surface. Observe how the features change
        - > If the features blur uniformly, the stigmators are probably aligned or very close to.
        - > If the features stretch opposite directions on either side of focus (or “look different”) proceed with stigmator alignment.
    - Return focus to the midpoint between stretched/blurry images.
    - Adjust X stigmator until the image sharpens. Attempt turning this knob both directions.
    - Adjust focus to confirm the focal plane is on the specimen.
    - Adjust Y stigmator until the image sharpens. Attempt turning this knob both directions.
    - Adjust focus above and below the specimen surface. The features should now blur uniformly.
2. Repeat these alignments as needed throughout imaging, whenever zooming in further than the magnification used for alignment, or after changing beam conditions (voltage, current, etc.)



1. In focus, before alignment

6. In focus, after alignment

2. Out of focus, before alignment

5. Out of focus, after alignment

3. Out of focus, before alignment, other side of focal plane

4. Out of focus, one stigmator aligned

8. REFINING AND SAVING IMAGES

This section is not intended to be step-by-step.

After alignment, the system should be in an easy-to-use state where all you need to do is navigate, zoom, focus, and adjust image settings (contrast/brightness, dwell time, resolution, etc.)

If the image looks worse, beam settings or are changed, or magnification increased:

- **Return to alignment and repeat steps as needed.**

1. Navigate to the region of interest, adjusting magnification, focus, contrast, and brightness as needed.
    - Small adjustments to stigmators may be beneficial if the image does not look sharp.
2. preview the final image quality with the reduced area scan and adjust dwell time and resolution.
    - Remember the reduced area scan settings are independent from the full frame scan.
    - Set the Photo settings under **Scan: Preferences**
    - The contrast and brightness may need to be set differently for slow images.

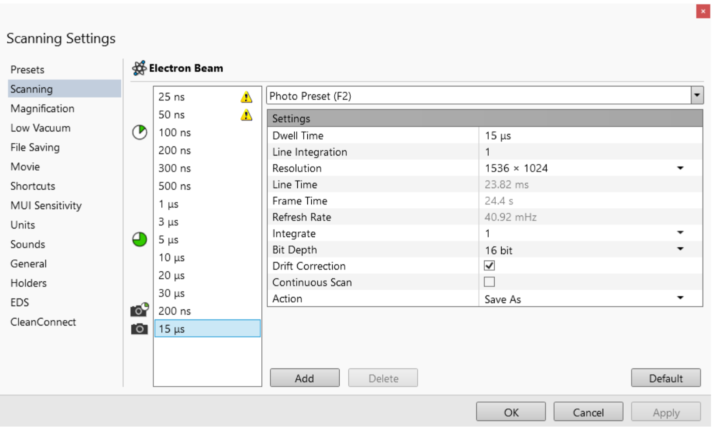
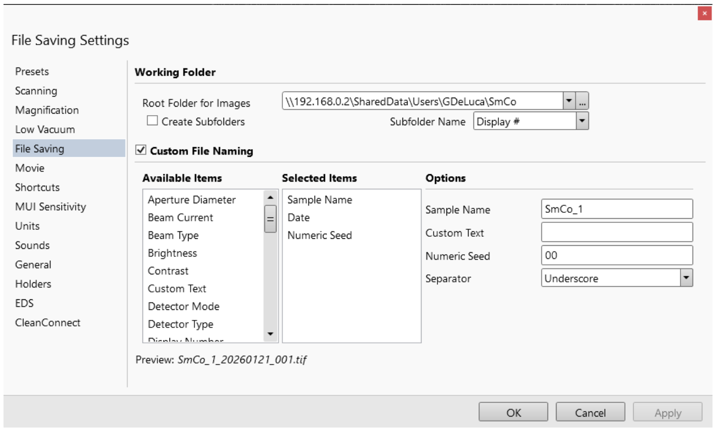


Photo Preset (F2)

Settings	
Dwell Time	15 µs
Line Integration	1
Resolution	1536 × 1024
Line Time	23.82 ms
Frame Time	24.4 s
Refresh Rate	40.92 mHz
Integrate	1
Bit Depth	16 bit
Drift Correction	☑
Continuous Scan	☐
Action	Save As



Working Folder

Root Folder for Images: \\192.168.0.2\SharedData\Users\GDeLuca\SmCo

☐ Create Subfolders

Subfolder Name: Display #

☑ **Custom File Naming**

Available Items	Selected Items
Aperture Diameter	Sample Name
Beam Current	Date
Beam Type	Numeric Seed
Brightness	
Contrast	
Custom Text	
Detector Mode	
Detector Type	
Display Number	

Options

Sample Name	SmCo_1
Custom Text	
Numeric Seed	00
Separator	Underscore

Preview: *SmCo_1_20260121_001.tif*

3. Acquire the image:
    - Use the Photo icon (F2) or Snapshot icon (F4) if you set these under preferences.
    - If using scan presets, simply pause the image then save.
    - If capturing images in multiple quadrants, press shift+F2 or ctrl+shift+[scan preset]
4. Save images in the Users folder on the Support PC.
    - **Do not save images to the microscope PC.**
    - **Do not save images directly to the shared CRF folder.**
    - Save grayscale images as 16-bit and color as 24-bit.
    - Select imaging parameters to be saved with the images.
5. Explore!
    - Navigate, align, capture images, etc. until about 8 minutes remain in your session.

9. SYSTEM SHUT DOWN

1. Check that the CCD camera is live
    - Use either the sample loading window or return one of the quadrants to CCD.
2. Ensure that the specimen is below the 10mm line and tilt is 0.
3. Retract any detectors, if inserted.
4. Turn off any stage bias.
5. Turn off the beam.
6. Vent the chamber.
7. Remove specimens after venting is complete. Clean up the preparation station.
8. Pump the chamber to high vacuum.
9. Close the venting gas valve.
10. Log out of your user profile in the microscope control software.
11. Transfer your data from the user folder to the shared CRF folder.
12. Log out of iLab.
13. Make any final notes in the logbook and contact equipment manager for any significant issues.

APPENDICES [UNDER CONSTRUCTION]

BASIC TROUBLESHOOTING

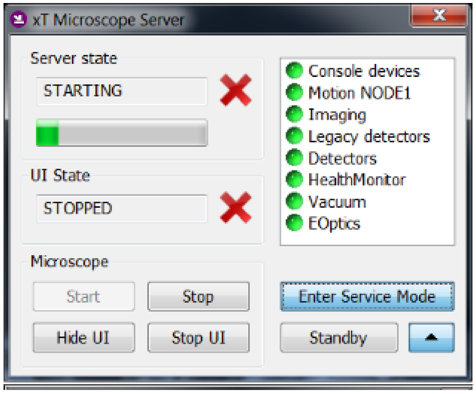
Databar/some part of display is not updating properly: attempt restarting the UI first:

- Find the xT Microscope Server window (below)
- Turn off the beam.
- Click “Stop UI.”
- Wait until “Start UI” becomes clickable.
- Click “Start UI.”
- Turn on the beam after the interface loads.

Software is closed:

To restart the server:

- Make sure the electron beam is off.
- In the xT Microsocpe server (purple icon), select STOP.
- Wait until all elements have been shut down (the LED icons should all be gray).
- Then, select START to re-launch the server.
- Wait until all elements are fully functional (green – Initialized).
- Log back into your user interface.



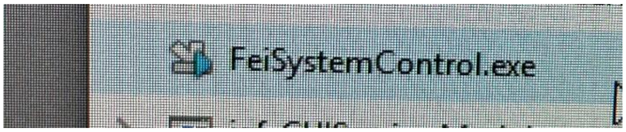
Server state: STARTING ✕

UI State: STOPPED ✕

● Console devices
● Motion NODE1
● Imaging
● Legacy detectors
● Detectors
● HealthMonitor
● Vacuum
● EOptics

If the server says it is already running, is not showing up, or appears hidden in the task bar:

- Make sure the electron beam is off.
- Close the user interface (red xT icon).
- Open task manager ctrl+shift+esc
- End the FeiSystemControl.exe program



FeiSystemControl.exe

- Open the server, purple xT icon.
- Select start UI.
- Log back into your user interface.

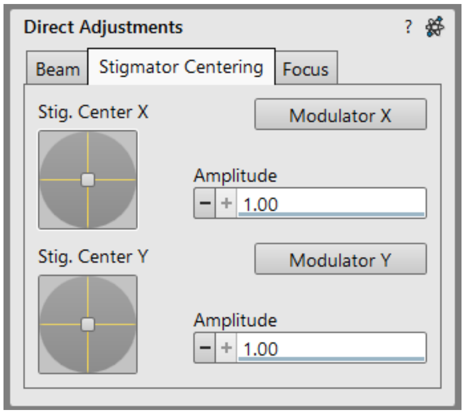
NOTES:

For BOTH computers:

Username: User
Password: Apreo9957280

If the vacuum icon looks like and the door does not open, check the venting gas and click Vent again. Contact the equipment manager if the problem persists.

If the image shifts while adjusting the stigmators, go to Direct Adjustments: Stigmator Centering and one at a time modulate X and Y, treating this like aperture alignment.



Amplitude 1.00

Amplitude 1.00

SAMPLE PREPARATION TIPS

Ensure samples are **clean**, **dry**, **vacuum compatible**, and **securely mounted to a stub**.
For cross section samples, mount specimens with carbon tape on a vertical surface, ensuring the top of the specimen is in line with the top of the holder. If needed, apply a small piece of copper tape to the face of the specimen, then to the stub or stage.
If specimens are not well secured, they risk drifting during imaging. Especially true for cross section imaging.

Supplies:

- Carbon tape: double sided adhesive, conductive. Great for holding samples on stubs.
- Copper tape: single sided adhesive, conductive. Great for providing a flexible grounding path for electrons to reduce charging. Ensure this tape is well secured to the specimen and stub.
- Stubs: basic pin stubs are available. A cross section holder is available. These tools are surfaces that interface the specimen to the stage.
- Double sided copper tape: double sided adhesive, conductive. Similar to carbon tape but useful for e.g. EDS when carbon composition is of interest. Inquire with staff.

Tools:

- Sputter coater: applies thin layer of conductive material to specimen surfaces for improved signal production, charge mitigation, and reduced beam damage. Inquire with staff.
- Plasma cleaner: built in to SEM, external plasma cleaner also available. Reduces hydrocarbon buildup on specimen surfaces for improved image quality, reduced specimen chamber contamination, and potentially reduced charging. Inquire with staff.
- Benchtop oven: Suitable for drying samples, especially overnight. Inquire with staff.

KEYBOARD SHORTCUTS

F5: toggle between quad view and full screen for the selected quadrant
F6: pause/unpause the selected quadrant
Shift+F6: pause/unpause all frames
F7: toggle between reduced area scan and full frame scan.
F2: capture image using camera settings
Shift+F2: capture image from all 4 frames.
Ctrl+shift+z: capture nav cam image
Ctrl+chift+[click a preset]: captures an image at a given preset from all 4 frames.
Shift+[click auto brightness contrast]: adjusts brightness/contrast automatically for all frames.

ADDITIONAL CAPABILITIES

These capabilities include:

Pressure limiting apertures (PLAs) & Analytical Techniques
- Low Vac mode
- Energy dispersive spectroscopy (EDS)
- Electron backscatter diffraction (EBSD)
- Electron channeling contrast imaging (ECCI)

Charge mitigation strategies
- Immersion mode
- Compound lens filter
- Stage bias

Heating/Cooling Stages
- TFS microheater stage (RT to 1000°C)
- TFS heating stage (RT to 600°C, 1100°C with shield)
- TFS Cryomat (RT to -180°C)

Mechanical Testing Stages
- Deben MT200VT tensile stage module with incorporated Peltier substage
  - Fibers, biomaterials, thin film, polymers, textiles,
  - heating option –20°C to +160°C
  - loadcell options: up to 200 N
- Deben MT2000VT tensile stage module with incorporated Peltier substage
  - heating option –20°C to +160°C
  - loadcell options: up to 2000 N
- Deben MT300VB vertical 3 & 4-point bending module

Electrochemical Stage
- Solid-state
- Liquid electrolyte

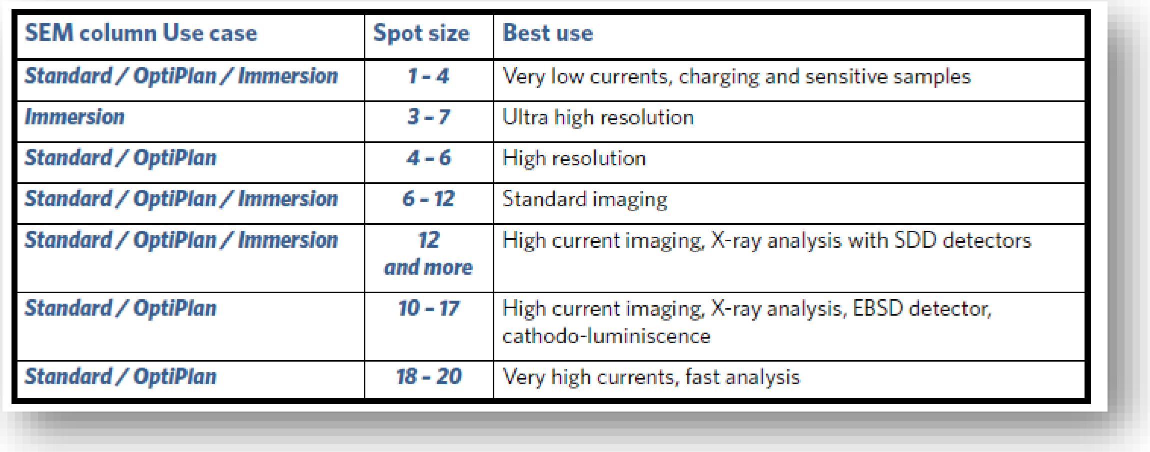
PLASMA CLEANING SPECIMENS IN THE CHAMBER

Use the built-in plasma cleaner to reduce contamination on the specimen, e.g. if raster boxes seem unavoidable, turn off the beam and plasma clean for 1 minute. You may also plasma clean when pumping down, or use the external plasma cleaner and benchtop oven for specimen cleaning.

SETTING (AND ADJUSTING) INITIAL CONDITIONS

Accelerating Voltage: high voltage for analytical work, theoretical higher resolution.
Use low voltages for surface work, sensitive specimens.

Beam Current (spot size): Use the table below for recommendations



SEM column Use case	**Spot size**	**Best use**
Standard / OptiPlan / Immersion	***1 - 4***	Very low currents, charging and sensitive samples
Immersion	***3 - 7***	Ultra high resolution
Standard / OptiPlan	***4 - 6***	High resolution
Standard / OptiPlan / Immersion	***6 - 12***	Standard imaging
Standard / OptiPlan / Immersion	***12 and more***	High current imaging, X-ray analysis with SDD detectors
Standard / OptiPlan	***10 - 17***	High current imaging, X-ray analysis, EBSD detector, cathodo-luminiscence
Standard / OptiPlan	***18 - 20***	Very high currents, fast analysis

Starting region of interest (double click in navcam image): Select an area with visible features. If you cannot see anything in the electron image, try using an edge, the stub, carbon tape, or something else visible at or near the surface of the specimen.
Imaging mode:

- Standard: basic SEM operation. No additional fields applied. The electron beam behaves as expected. Most detectors are available in this mode.
- OptiPlan: Applies 8 kV beam deceleration. Yields high quality images of less- to non-conductive specimens.
- Immersion:
- Detector:
    > ETD: Everhart Thornley Detector. Standard SE detector. Typically shows topographic detail.
    > LVD: Similar to the ETD but for low vacuum mode. Has Bias, Contrast, and Brightness. Try auto contrast and brightness adjustment.
    >
- Dwell time
- Resolution
- Filtering

STIGMATOR ALIGNMENT

Stigmators control the shape of the beam. An ideal electron beam is smaller than the pixel size and round. If the beam is astigmatic, then the beam will be large when in focus, and elliptical when out of focus.

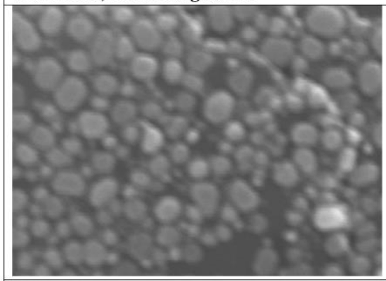
The elliptical beam effect is visible when out of focus as stretched or smeared features. (2, 3, and 4 below.)

If you observe stretching in perpendicular directions as you adjust the focus, your focal plane will be in between these positions. The image may not look sharp, but the lack of directional blurring is good. Start here.

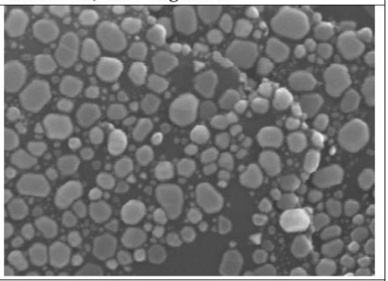
With the specimen in focus, adjust first one, then the other stigmator, aiming for the sharpest image with each. After adjusting each stigmator, play with focus again to verify whether there is any stretching. Stretching tells you there is astigmatism AND that you are out of focus.

Note: these effects are most visible at higher magnifications (e.g. >5000 X) and in fields of numerous small round or random features. Avoid aligning on straight lines.

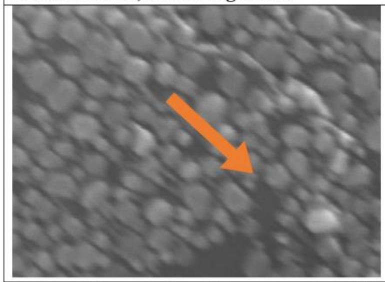
1. In focus, before alignment



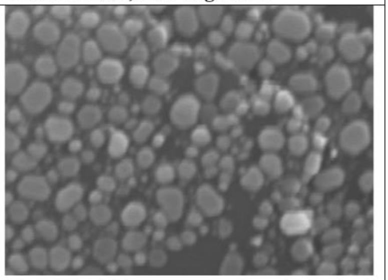
6. In focus, after alignment



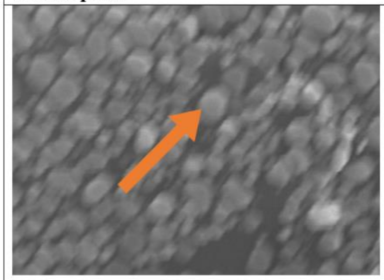
2. Out of focus, before alignment



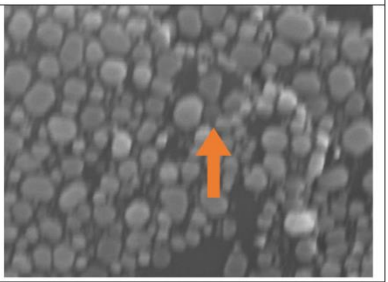
5. Out of focus, after alignment



3. Out of focus, before alignment, other side of focal plane



4. Out of focus, one stigmator aligned



USING SCAN PRESETS

Scan Presets are labeled S1-S6 in the upper right corner of the UI.

- Customize these according to your own use and needs; they are independent for each user profile
- Select a preset and click the dropdown arrow on the far right.
- Select the dwell time and resolution as desired
    - Other settings include frame/line averaging/integration.
    - Use of averaging/integration reduces noise for short dwell times. Especially useful for beam sensitive specimens to reduce the electron dose on the specimen.
    - Continuous: tells the system whether to stop at the end of a frame or scan until paused.
    - Action: allows you to select whether to save or prompt to save images.
- Preset ideas:
    - Navigation: Fast scan/TV rate: short dwell time (200 ns,) low resolution (768.)
        - Good for navigating, zooming, etc.
    - Rough focus, stigmators: Slow scan: longer dwell time (3 us,) low resolution (768.)
        - Good for focusing, stigmators, contrast/brightness
    - Fine focus, stigmators: slower scan: longer dwell time (5-20 us,) mid resolution (1500.)
        - Good for fine focusing, stigmators, especially when used with reduced area
        - May also be good as “image preview” to check settings for high quality images
    - Lens align: very fast scan (100 ns,) low resolution (768.)
        - Good for lens align!
        - Or any other modes that use a wobbler (e.g. stigmator centering.)
    - Imaging: long dwell time (10-30 us,) mid to high resolution (1500+.)
        - Best for capturing images.
        - For beam sensitive specimens, using faster speeds