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1000100ILAB NAME: SEM – APREO 2S LOW VAC – THERMOFISHER

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INTRODUCTION

The ThermoFisher Apreo 2S Lo Vac SEM is an advanced field emission scanning electron microscope equipped with a state-of-the-art set of in-situ modules. This microscope can operate with accelerating voltages from 200 V 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 must be in a safe position.
- The electron beam must remain off *and* the system under high vacuum when not in use.
- The venting gas main valve must be closed when not in use.
- Workstations must remain clean of sample debris.
- Sample containers must be labeled with your name, the date, and specimen information including chemical names.
- Users must 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. Call or use Teams for immediate, real-time assistance.

TRAINING AND QUALIFICATIONS

Training grants access 9-5 during weekdays. For after-hours access, contact the equipment manager.
Basic training includes:

- Virtual overview of foundational SEM topics.
- Demonstration of sample preparation and appropriate operation of SEM with your specimens.
- Guided or supervised practice to build familiarity with the system
- 90 minute “driver’s test” to ensure competent operation of basic SEM functions. Goals include:
  - Safe operation from startup to shutdown, using any notes taken or documentation provided.
  - A basic understanding of controls and tools available to improve images.
- Additional sessions may be arranged by request at the equipment manager’s discretion for practice, before qualification, or for additional options, techniques, or modes (see Appendices)

1. SAMPLE PREPARATION: *Mount, coat, and clean specimens for the SEM*

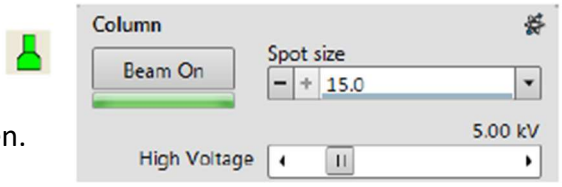
- Always wear gloves when handling anything that goes inside the chamber.
  - ***Do not wear gloves while operating the computer***.
  - Specimens must be **vacuum stable** and **clean**. If possible, secure specimens to a stub.
  - *The MCC has a **plasma cleaner**, a **small benchtop oven**, and a **compressed gas line** from the venting gas available for specimen preparation. Contact staff for assistance.*
  - *Outgassing, debris, or loose particles will contaminate the chamber and vacuum system.*
  - *Powder specimens must use as little as possible and be tapped, dusted, and wiped clean.*
  - *Specimen motion due to poor mounting will become apparent at high magnifications.*
- Bring specimens already prepared or prepare specimens at the bench behind the SEM.
  - *Carbon tape, copper tape, silver paint, and a small selection of pin stubs are provided.*
  - *Contact staff for assistance if you need the sputter coater and have never used it before.*

2. SYSTEM START UP: *Log in and check the system status*

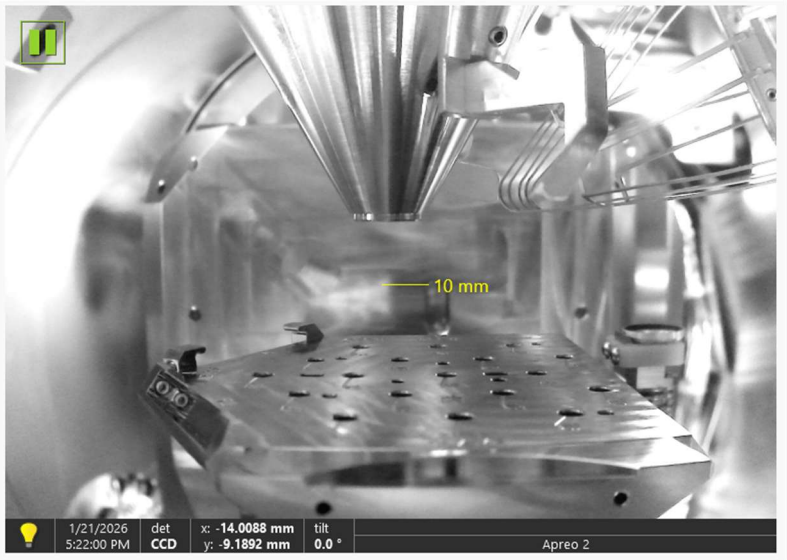
1. Start your session in the iLab kiosk to turn on the two right monitors.

2. Log in to your user profile in the SEM program. (The log in pop-up should be on screen)
   - If somebody else is logged in, you will not see the pop-up. Log them out first {File > Log off.}

3. **Contact equipment manager immediately if the system is not in an operable state:**
   - On the Beam Control tab:
     > The system diagram must be fully green.
     > **Beam On** must be clickable but gray.
     > The bar under Beam On must be fully green.
   - CCD camera must be working and live.
     > Click the **pause icon** to unpause the frame.



4. The stage must start below the 10 mm line.
   - To lower the stage from a live chamberscope image, click and hold the scroll wheel while dragging the mouse down.



5. **Fill out the logbook.**
   - Add comments to the logbook that would be useful for the next user or the equipment manager. Always leave a note if you restart the UI or server.

3. SAMPLE LOADING: *Vent, pump, and set up specimens in the chamber*

1. Open the main valve on the venting gas cylinder (Ar or N2.) Do not touch the other valves.
2. (Optional) Click  to open the Sample Exchange window.
3. Click **Vent** then confirm to vent the chamber.
   - Wait for the system to vent (~3 min. The loading bar is a timer.)
   - Do not use the CleanConnect Vent/Pump options unless using and already trained to use the CleanConnect attachment.
4. Gently open the chamber door (pull slowly and do not lean or push down on the door.)
   - The door is heavy but well balanced. It will glide along its rails easily if vented.
   - If the door does not open, check the gas valve and pressure, then click Vent and confirm again.
5. Arrange pin stubs in the holes on the stage.
   - **Always wear gloves when handling anything that goes inside the SEM.**
   - **Do not lean on the chamber door.**
   - Most pin stub holes are tension fit. Ensure the underside of the stub touches the stage.
   - Do not adhere tapes to the underside of stubs (trim excess or push into the stub groove.)
   - Do not mix flat stubs with cross section stubs – **specimens must be of similar heights.**
   - Finger tighten set screws with a 1.5 mm allen key if loading stubs into set screw locations.
   - If adhering a specimen directly to the stage, do not cover holes with tape. Promptly remove any tape when finished. It will be hard to remove and leave a residue over time.
6. Gently close the chamber door while watching the chamberscope for potential collisions.
   - Ensure the gap between door and chamber is minimal (<1 mm.)
   - If it looks like something will collide, lower the stage in any live chamberscope image by dragging the mouse while holding in the scroll wheel (as if it were a button.)
7. Check the Magnetic Sample box in the sample exchange window if your specimen is magnetic.
   - This restricts Immersion, Optiplan, and other magnetic field-based options.
8. Select high or low vacuum then **Pump** to begin pumping the chamber to vacuum.
   - NOTE: system **may** begin to pump immediately after selecting High or Low Vacuum.
   - Select No Accessory or whatever accessory you installed on the pop-up. Do not use accessories you have not been trained to use.
   - 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 the target pressure only for Low Vacuum before pumping, if known. It can be changed at any time.
   - Select Plasma Clean if you want to clean your specimen. See the Appendix for more information.

9. While the system pumps:

   - Collect a navigation image.
     > **From the top ribbon, click {Stage > Take NavCam Photo}** or press **[ctrl shift z]** then wait for the stage to return to its starting position.
     > If it fails to capture: check the chamberscope for collisions and try again.
     > If it fails to return: type 0 for all coordinates in the stage tab and press enter.

   - After the NavCam Photo procedure finishes, double click the first specimen in the NavCam image to center it under the beam.
     > The green plus or rectangle overlaid on the NavCam image shows the beam position or field of view (respectively) on the specimen.
     > Calibration may be inaccurate for very tall specimens or if the stage was far from 10 mm.
       - Accuracy may be improved by capturing a new NavCam image after raising the stage so the tallest specimen is at the 10 mm line in the chamberscope (see next step,) or by using MAPS (ask equipment manager for details.)

   - Raise the stage until the tallest specimen is at the 10 mm line in the CCD camera.
     > The chamberscope must be live.
     > The chamberscope must be selected (blue border) if using the Quad view frame.
     > In chamberscope view, **[click the scroll wheel and drag the mouse]** to raise the stage.

10. The system is ready to proceed when the vacuum icon is fully green: 
    - The **Beam On** button should now be clickable.

NOTES:

- In Quad View, the active frame (the one being manipulated) is highlighted with a blue border and data bar. Mouse and display controls (e.g. contrast, brightness) will operate on this quadrant.

- A single left click in any quadrant will select that frame.

- Toggle between full screen and quad view with **[F5]**.

- Change the display of any quadrant between electron image, chamberscope, and NavCam image by clicking the icon in its bottom left corner and selecting the appropriate icon.

- In quad view, the NavCam prefers the bottom left frame and the chamberscope the bottom right, but any may be used for electron images.

- Pause and unpause selected quads with **[F6.]** Use **[shift F6]** to pause/unpause all electron image quads simultaneously. The NavCam is a still image and cannot be unpaused.

4. INITIAL CONDITIONS: *Set starting parameters while the system pumps to vacuum*

Select your initial imaging conditions, if known. Most conditions are found on the Beam tab, along the top ribbon, or can be changed from the data bar directly. These settings can be changed at any time (although the system will likely need to be realigned for any beam-related settings including voltage, spot size, and mode.)

- **High voltage:** beam energy: 200V – 30 kV
  - > Higher for EDS, BSE, conductive specimens, bulk specimens.
  - > Lower for delicate samples, surface detail, general imaging.

- **Spot size:** correlates to beam current: 1 – 17 (currents listed in data bar.)
  - > Higher for EDS, BSE, fast throughput.
  - > Lower for delicate samples, high resolution.

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.
  - > **[Ctrl +]** or **[Ctrl -]** to zoom in/out in NavCam, electron, or chamberscope images when selected.
  - > For delicate specimens, start on any object or feature near the specimen surface (e.g. tape, stub, specimen corner.) Ideally the feature should be close in Z to the specimen surface.

- **Imaging mode:** changes detector options, electron filtering.
  - > **Standard**: Most flexible mode. No additional fields applied. Default for Low Vac unless Auto PLA is installed. Large depth of field. Images may appear grainier. SE, BSE signals may be mixed. ETD, T1, T2 available in High Vac. LVD, T1, T2 available in Low Vac.
  - > **OptiPlan**: Most used mode. Filters electrons by energy to the ETD, T1, T2, T3 detectors (see Detectors tab.) Great for short and long working distances. Available in Low Vac with PLA only (ask equipment manager for assistance.)
  - > **Immersion**: Highest resolution mode. Applies additional magnetic field to the pole piece. Great for short working distance, low voltage, small spot size. T1, T2, T3 only. May be available in Low Vac with PLA (ask equipment manager for assistance.)

- **Detector**: Determines signal type used in images and detection efficiency. Preview which signals go to which detectors for a given voltage, mode, and working distance in the Detectors panel on the right side.
  - > **ETD**: Everhart Thornley detector: standard SE detector. Shows BSE + SE in standard mode. Highlights topographic shadow effects due to offset position in chamber. Bias may be tunable.
  - > **T1**: Mounted on outside end of the pole piece. Highest energy electrons (BSE) filtered to this detector in OptiPlan and Immersion modes. Typically sees BSE in Standard.
  - > **T2**: Mounted just inside the pole piece. Mid energy range electrons (SE), with variation by working distance. Most preferred detector for routine, high resolution imaging in OptiPlan.
  - > **T3**: Mounted higher above the pole piece. Lowest energy electrons (surface SE.) May be most effective in Immersion, OptiPlan. Smallest field of view, highest resolution, good with short WDs.
  - > **LVD**: ETD style detector for low vacuum mode only. Bias control must be set below 70%.
  - > **BSE**: Retractable backscattered electron detector. Ask equipment manager for training.
  - > **EDS**: Retractable x-ray detector for elemental analysis. Ask equipment manager for training.

- **Dwell time**: few ns to 10s of us. Amount of time the beam spends on each pixel.
  - > Longer (3 us – 30 us) for smoother images, fine alignments, final captured images.
  - > Shorter (100 ns – 1 us) for faster feedback, rough alignments, navigation, sensitive specimens.
    - Short dwell times may be used with drift compensation and frame integration for high quality images of sensitive specimens (see Filtering below.)

- **Resolution**: image size/number of pixels in an image. Frame time increases exponentially.
  - > Lowest best for quick navigation, lens alignment.
  - > Mid best for most images, fine alignments.
  - > Highest best for large fields of view with fine detail.
  - > Note: The beam size stays the same, but the pixel size changes. So a higher image size resolution does not always translate to finer detail. Zoom in beyond the desired magnification and check alignments for best results.

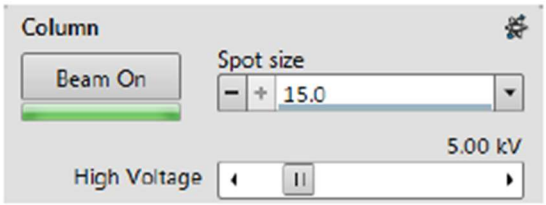
- **Filtering**: Options to aid in imaging sensitive specimens.
  - > **Line integration**: integrates signal from each line N times before proceeding to the next line. Good for images of mildly sensitive specimens with minimal drift. Image may freeze at end of the frame. Useful for live imaging sensitive specimens.
  - > **Frame integration**: integrates signal from a full frame N times. Can be paired with drift compensation. Image freezes at the end of the acquisition. Good for capturing images of sensitive specimens especially when paired with a fast dwell time and drift compensation.
  - > **Scan interlacing**: scans one line then skips N lines through the image before returning to fill in the rest of the lines. Good for very sensitive specimens where specific areas tend to charge strongly. Can be paired with integration modes.
  - > **Averaging**: a live imaging mode that averages the signal of the last N frames, replacing the first frame with the most recently captured frame continuously. Useful for moderately sensitive specimens, but not recommended for alignments due to image lag.

5. FIRST IMAGE: *Turn on the beam, navigate, focus, and zoom to the target magnification*

Before starting the beam, ensure that:

- The chamber is under vacuum
- The NavCam image shows your samples with the green plus or rectangle on your first region of interest
- The sample surface is close to the 10 mm line in the chamberscope (some exceptions.)
- The desired high voltage and spot size are selected
- The desired mode (use case) is selected

1. Click **Beam On** to activate the electron beam.



2. Click the pause icon in any electron image quad to start scanning.
   - All four quadrants can be set to electron images (bottom left icon,) and detectors (select and change detector from the databar) for simultaneous acquisition of different signals.

3. Check to ensure that the UI is responding properly:
   - Reduce the magnification and check the databar. If the databar magnification does not change, restart the software (see the Appendix.)

4. Start at a reasonably low magnification (< 1000 X) and look for any kind of contrast (e.g. edges, features.)

5. Select each frame and adjust the brightness and contrast. (Repeat these throughout imaging as needed.)
   - To adjust all electron images quickly and automatically, press **[shift F9]**
   - If the automatic adjustment is insufficient, manually adjust:
     > 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.
     > If the image appears too bright or washed out, turn the brightness down and contrast up.

   Note: Brightness shifts all intensities linearly. Contrast spreads the range up from the darkest value
   For retractable BSE images, or as a first pass, try auto contrast and brightness adjustment: 

6. Navigate the sample as needed to find a feature on the specimen surface. It may be very blurry. Look for identifying edges. To navigate, you may:
   - Double click in the NavCam image. (Remember, **[Ctrl +]** or **[Ctrl –]** to zoom in/out. Good for long moves if well calibrated.)
     > If the calibration is bad, capture a new NavCam image with the specimen around 10 mm.
   - Double click in the electron image. (good for local moves)
   - Click and hold the scroll wheel while dragging the mouse in an electron image (same as raising the stage but for X, Y. Ensure an electron image has the blue border.)
   - Use the arrow keys (navigates by about 1 frame width per press.)

7. Note the WD value in the databar, then slowly turn the focus knob (coarse or fine) and watch the features change as the WD value changes. The image should be sharpest somewhere between 9 – 11 mm.
   - If features appear sharper, keep going until they appear worse.
   - If features become blurrier, turn the knob the other way until they appear better, then worse.
   - After the image becomes worse again, turn the focus knob back to the sharpest image.
   - If the image appears sharpest in two positions, adjust the focus to a position in between both.
   - Note the working distance in the data bar at the bottom of any electron image again. ***If you are in focus, this value now tells you where the specimen surface is relative to the pole piece.***
8. Click  to link the working distance to the stage Z **only when the specimen surface is in focus.**
   - While imaging, if this icon does not look like:  refocus and press it again.
   - This tells the system where your specimen surface is for safer movements inside the chamber.
   - When changing the stage height (Z), the working distance will follow, but NOT vice versa.

9. Adjust the stage height until the working distance is 10 mm.
   - Enter 10 for Z in the stage coordinates then press enter or click Go To,
   - or hold the scroll wheel while dragging the mouse in an active chamberscope.)

   Note: 10 mm is the optimal working distance for most detectors in this SEM.
   - If going to a shorter working distance: start near 10 mm and ensure it is well focused and properly linked above 3000 X before proceeding to the target working distance.

10. Zoom in at least one step past the target field of view and refocus, relinking if necessary.
    - Click the link icon again at any time if the WD is far from the displayed Z value and you intend to move the stage in Z or T.

11. Make any adjustments to voltage, beam current, and imaging mode before proceeding.
    - You may need to refocus after changing these settings.
    - When changing the voltage and beam current/spot size, the beam may run an automatic source tilt alignment. If this alignment fails, you will see a pop-up requesting that you correct it.
      > There should be a circle visible in an electron image. If it is not visible, adjust contrast and/or brightness.
      > If there is a circle but it is too small/large for easy alignment, use the magnification slider in the pop-up to adjust the displayed size.
      > Click and drag the axes in the pop-up to move the circle until it is centered on the displayed cross.
      > When the circle is centered as best as possible, click ok

Note: the image may look nice now, especially at low magnifications. If imaging only at low magnifications, the following alignments may not be necessary. Aligning the beam will ensure the best resolution for your selected beam conditions. Changing the spot size, voltage, mode, and more will affect the alignment. Lens alignment and stigmator alignment must be checked whenever changing settings. Source tilt may run an automatic alignment (and require manual positioning if it fails) for new voltages. The steps in this operating procedure are laid out in a recommended order, but each step may be completed as needed in response to the image quality.

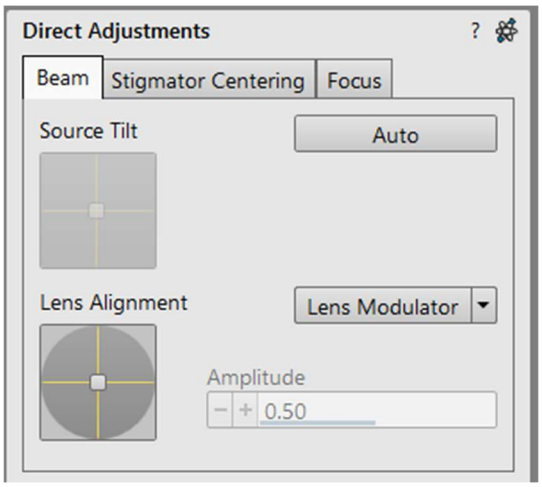
6. ALIGNMENT: APERTURE: *Center the beam for good signal and depth of focus*

Aperture alignment ensures the beam is well centered through the column for even illumination, strong signal generation, and good depth of focus.

1. Adjust the magnification to at least one step beyond the desired field of view.
   - Magnifications over 2,000 X 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, bright 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: Use a full frame scan at a video-rate speed (for most users this is your S1 preset.)
   - Go to {Direct Adjustments > Beam > Lens Alignment} on the right panel and click on **Lens Modulator** (or HV modulator for very low voltages. Always use the recommended setting.)
   - **Adjust Wobble Amplitude** so you can see the direction features shift, if at all.
     > If the image blurs quickly out of focus, reduce amplitude.
     > If the image appears to not move at all, increase amplitude.
   - Click and drag the Axes to align the aperture **until the image pulses in and out of focus without shifting**. The direction you drag the axes directly matches the direction of shift you are aligning.
     > 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, align it until there is no motion in that direction (e.g. only shifting along X after moving the X 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.



7. ALIGNMENT: STIGMATORS: *Make the probe small and round for best resolution*

Stigmator alignment ensures the beam is round in cross section with the fewest aberrations, resulting in the best resolution. See the appendix for a visual representation of this alignment.

1. Start in a reduced area scan **[F7]** at a slower speed (e.g. > 3 µs) over a group of small round or randomly shaped features.
    - Adjust fine 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.
        > If the features stretch in opposite directions on either side of focus (or "look different") proceed with stigmator alignment:

    - Return focus to the midpoint between stretched/blurry images. It may be the sharpest image or the midpoint between oppositely oriented sharpness.
        > At any point in this process, if there is a significant change or if directional stretching is observed, turn fine focus slightly in both directions to find the best focus.
    - Adjust X stigmator in both directions until the image sharpens, then becomes worse. Turn it back to the sharpest position.
        > If any directional stretching is observed, it means the image is slightly out of focus and the stigmators are not yet aligned. Adjust fine focus to the sharpest image.
    - Repeat with Y stigmator.
    - Adjust fine focus above and below the specimen surface. The features should now blur uniformly.
        > If any directional stretching is observed, make small adjustments to the X and Y stigmators and fine focus.
        > If performing this alignment at a higher magnification than required for final images, some small misalignment is acceptable.

2. Repeat these alignments as needed throughout imaging:
    - Whenever zooming in further than the magnification used for alignment
    - After changing beam conditions (voltage, spot size, etc.)
    - When moving between specimens (may not always be required)
    - If the image looks blurrier than desired.

Note: If the image still looks blurry after alignment, other settings may yield a sharper image. **It is recommended to capture an image before changing settings**. Other settings that contribute to image sharpness include:

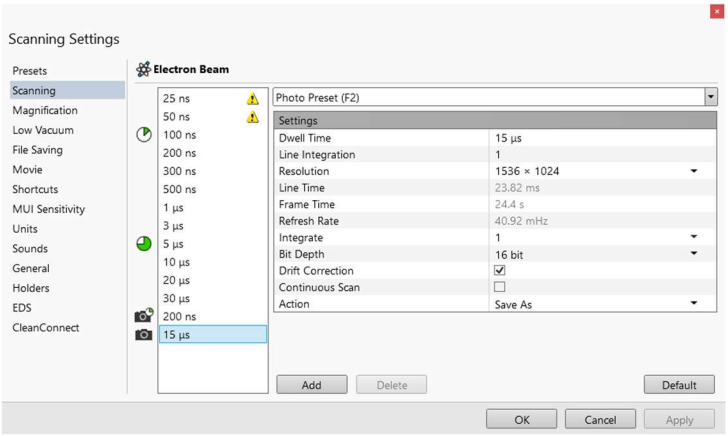
- **Spot size**: analytical spot sizes (10+) tend to yield blurry images at high magnifications.
- **Voltage**: These are specimen specific. Higher voltages have a higher theoretical resolution, but if fine surface details are desired, too high of a voltage may hide fine features.
- **Mode**: OptiPlan is preferred by most users. Immersion may yield better images at short WDs.
- Depending on your specimen, the equipment manager may know of other available options such as beam deceleration, compound lens filtering, additional detectors, sample preparation techniques, or a refinement of alignment procedures.

8. REFINING AND SAVING IMAGES: *Set image parameters and save images*

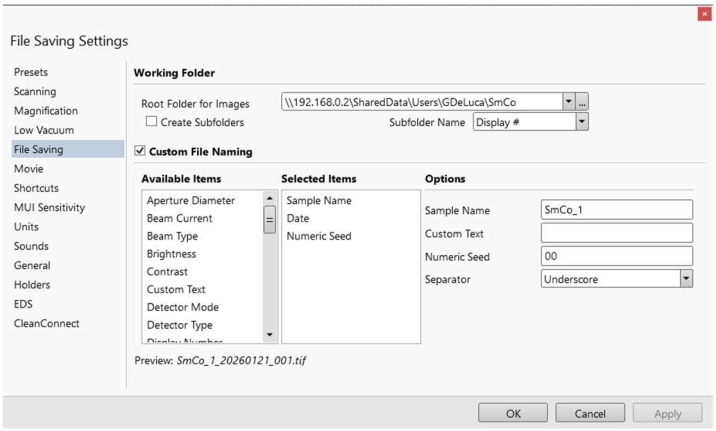
This section is not intended to be step-by-step.

After alignment, the system should be in an easy-to-use state where all you need to do is navigate, zoom, focus, and adjust simple image settings (contrast/brightness, dwell time, resolution, filtering.) Small stigmator adjustments are normal, especially if the lens alignment is not perfect.

1. Check what settings are associated with the camera icon by right clicking on it and selecting {edit,} or from the top ribbon, go to **{scan > preferences > scanning.}**



- In the scanning menu, left click the dwell time beside the camera icon then adjust:
  - > **Dwell time**: find something that captures your image without drift or significant charging.
    - In the electron image, the reduced area scan **[F7]** may be helpful to preview appropriate settings before entering this menu.
    - If the specimen drifts, find a dwell time with minimal drift during a single frame in the full frame scan (as opposed to reduced area,) then increase the number of frames to integrate (N.) If you are aiming for a certain signal to noise ratio seen with a slower dwell time, divide the target dwell time by the single frame dwell time for N (e.g.: an image captured at 10 µs dwell will look very similar to an image captured with 300 ns dwell over ~30 frames.)
  - > **Integration**: line or frame (integrate.) See Initial Conditions for differences between these modes. Set to 1 for no additional integration.
  - > **Drift Correction**: Useful for integrating multiple frames at fast dwell times for drifting specimens.
  - > **Bit depth**: recommended 16 bit. Save images as 16 bit tiff unless color is needed.
  - > **Action**: "Save as:" will prompt user to save images after each capture.
  - > **Optional**: set each camera icon to its own set of combinations for two different image types.
- Alternatively, click the drop down arrow beside the scanning presets in the upper right of the UI (S1, S2, etc.,) to edit the photo settings for scanning presets.



2. File save settings (file path, naming conventions,) are accessible from the Sample Exchange window, **{Scan > Preferences > File Saving}** on the leftmost side of the scan/photo settings, or at the bottom of an image save pop-up after capturing an image.
    - Save images in the **Users** folder on the Support PC (this folder is pinned on the left side of the file explorer when selecting "root folder for images.") Select your individual/specimen folder within "Users."
        > **Do not save images to the microscope PC.**
        > **Do not save images directly to the shared CRF folder. Transfer after saving from the support PC.**
    - **Create Subfolders** (optional) to save images from different quadrants/detectors in their own folders.
    - Select "Custom file naming"
        > Drag and drop items between the left two lists to include/exclude system parameters
        > Type in values under "options" for text.
            - **Add at least 2 leading zeros to the numeric seed to avoid a software bug.**
    - *Image file name preview is shown at the bottom of this window.*

3. When you want to capture an image:
    - Click the camera icon **[F2]** or Snapshot icon **[F4]** if you set these under preferences.
    - If using scan presets, hold **[ctrl and click the scan preset number.]**
    - If capturing images in multiple quadrants simultaneously **[hold shift while clicking either of the above options.]**
    - Save grayscale images as 16-bit and color as color-tiff.
    - Open at least the first image captured during a session on the data PC to verify that the databar is accurate. If it is inaccurate, you may need to restart the UI (see Appendix.)

If the image looks worse, beam settings are changed, or magnification is increased:
- **Repeat alignment steps as needed.**

9. SYSTEM SHUT DOWN: *Remove specimen, log off, leave system in safe state*

1. Turn off the electron beam (Click Beam On again – it is gray when the beam is off, yellow when on.)

2. Check that the CCD camera is live
   - Use either the sample loading window or return one of the quadrants to CCD and unpause.

3. Ensure that the specimen is below the 10 mm line and tilt is 0. Lower the stage if needed. It will lower further while venting.

4. Retract any detectors, if inserted, including EDS, retractable BSE, x-ray cone/auto-PLA

5. Turn off stage bias, if used.

6. Vent the chamber. If it fails to vent after 3 minutes, check the main gas valve and pressure, then vent again, clicking confirm in the pop-up.

7. Remove specimens after venting is complete. (Wear gloves!)

8. Pump the chamber to high vacuum.

9. Log out of your user profile in the microscope control software. {File > Log off user.} (May be completed after pumping initiates.)

10. Log out of iLab.

11. Transfer your data from the user folder to the shared CRF folder or your preferred destination.

12. Make any final notes in the logbook and contact equipment manager for any significant issues.
    - **Logbook notes must include**: any computer or software restarts + reason; any problems encountered while imaging, even if you resolved them; anything a future user should now; anything you want the equipment manager to know.

13. Close the venting gas main valve.

14. Clean up the specimen preparation area.

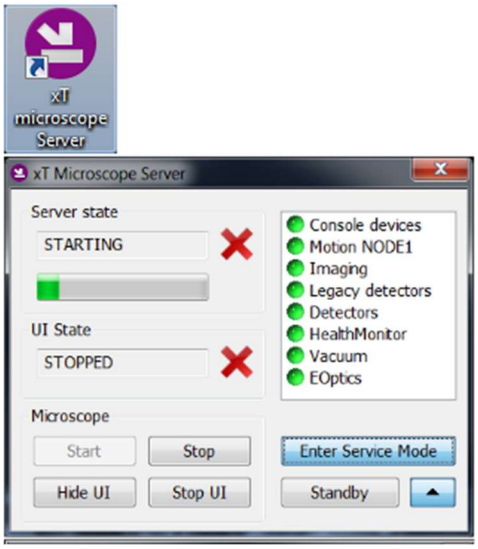
APPENDICES

BASIC TROUBLESHOOTING

This section outlines a selection of basic troubleshooting procedures that resolve most of the common problems encountered on the SEM. If you do not feel comfortable performing any of these procedures independently, feel free to contact staff for assistance.

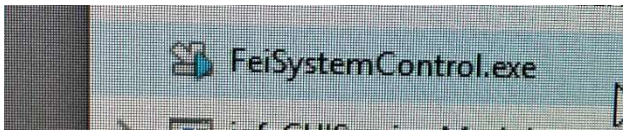
Most software problems can be resolved by restarting the user interface only:
e.g.: the databar/some part of display is not updating properly:

- Find the xT Microscope Server window (purple icon, likely open.)
  - > If the server claims to be open but there is not window visible, check the bottom right of the center monitor for the small icons menu under ^. Look for the microscope server icon (purple,) right click, and select "open."
- In the main UI, turn off the beam.
- In the server, click "Stop UI."
- Wait until "Start UI" becomes clickable.
- In the server, click "Start UI."
  - > The user interface will now restart.
  - > You will need to log in to your profile again.
  - > The beam will be off.
  - > Most settings will be preserved.
  - > Some options, especially in ChemiSEM/EDS, may need to be reselected.
  - > Most alignments should be preserved.
- Turn on the beam after logging back in.
- Leave a note in your iLab reservation that there was an issue with the approximate duration of time the system was not usable.
- Leave a note in the logbook so we have a record of why you needed to restart the UI.



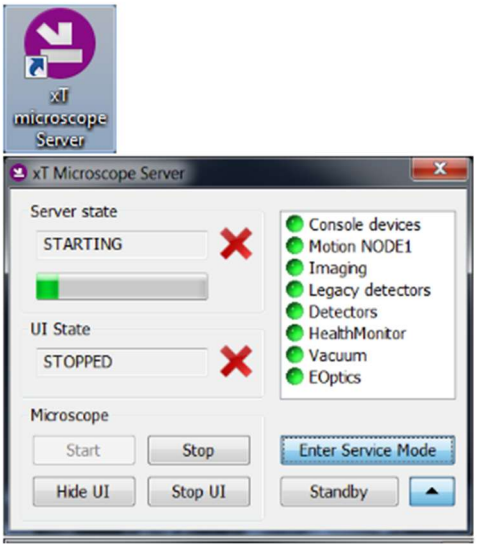
If the server says it is already running, is not showing up, AND appears hidden in the task bar/small icons (^):

- **DO NOT DO THIS IF THE IT IS POSSIBLE TO STOP THE SERVER ANOTHER WAY (see below.)**
- 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
- Open the server, purple xT icon.
- Select start UI.
- Log back into your user interface and continue imaging.
- Leave a note in the log book.



For problems not resolved by restarting the UI, or if the problem seems to be in the communication between the computer and the microscope, the server itself may need to be restarted. To SAFELY restart the server:

- Find the microscope server.
- Ensure the beam is off.
- Click “Stop UI.”
- Click “Stop” to stop the server itself.
- Wait for the server state to update to “STOPPED.”
- Click “Start” to relaunch the server.
- Wait for “Start UI” to become clickable.
- Click “Start UI” to relaunch the user interface.
- Log back in to your profile.
- Most settings and alignments should be preserved. It will be worth checking alignments, etc. after restarting the server.
- Make a note in the logbook recording the incident that caused the server restart and that the server was restarted.
- Leave a note in your iLab reservation with an estimate of time lost due to the server restart.



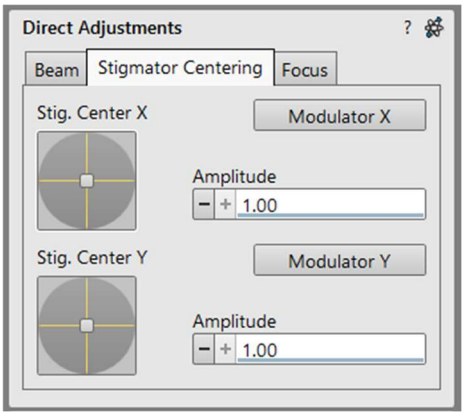
NOTES:

For BOTH computers:

Username: User
Password: Apreo9957280

If the vacuum icon looks like  and the door does not open, check the venting gas and main regulator pressure, then click Vent and confirm 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 (lens) alignment.



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 shift [click a preset]]: captures an image at a given preset from all 4 frames.

[Shift F9]: adjusts brightness/contrast automatically for all frames.

ADDITIONAL CAPABILITIES

This SEM is equipped with *in situ* options and options to aid in routine imaging that are not always covered during basic training.

DO NOT ATTEMPT TO USE THESE MODES UNLESS YOU HAVE BEEN TRAINED BY THE EQUIPMENT MANAGER

These capabilities include:

Low vacuum capabilities

- Low Vacuum mode: good for non-conductive specimens that cannot be coated.
- Pressure limiting apertures (PLAs): enable use of additional imaging modes and more spatially precise EDS analysis when operating in low vacuum mode.
- Higher pressures available with additional apertures and pumping.
- System is not capable of environmental (wet) SEM

Analytical capabilities

- Energy dispersive spectroscopy (EDS): may be covered during basic training. Analytical elemental analysis using characteristic x-ray signal generated while imaging. May be semi-quantitative with standardless quantification (common for SEM.)
- Electron backscatter diffraction (EBSD): good for understanding grain size, orientation, and distribution in crystalline materials. Very surface sensitive – polishing required.
- Electron channeling contrast imaging (ECCI): good for understanding relative grain size and orientations, less time consuming than EBSD. Not compatible with magnetic specimens.

Charge mitigation strategies

- Immersion mode: enables use of very low voltages at short working distances in high vacuum mode
- Compound lens filter: aids in filtering more electrons to detectors higher in the column (T3, T2) to reduce APPEARANCE of charging.
- Beam Deceleration: applies a bias to the stage that reduces beam landing energy to reduce impact on specimen while maintaining high resolution benefited from a higher voltage.

In situ options: mechanical testing and electrochemical setups may be used ex-situ.

DO NOT USE THESE OPTIONS UNLESS YOU HAVE BEEN TRAINED BY THE EQUIPMENT MANAGER

Heating/Cooling Stages

- TFS microheater stage (RT to 1200°C): suitable for VERY small specimens, high or low vacuum
- TFS high vacuum heating stage (RT to 600°C, 1100°C with shield): suitable for larger specimens, high vacuum only. Setup easier than microheater setup.
- 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, (5 N option available for users in Gogotsi group, with Gogotsi group permission.)
- 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.

ADJUSTING INITIAL CONDITIONS

- **High voltage**: beam energy: 200 V – 30 kV
  - > Decrease to see more surface detail or for sensitive specimens. Reduces interaction volume.
    - May require shorter working distances. To reduce WD, focus on specimen, link stage Z to WD, then type in the target WD in the stage coordinate list and hit enter.
  - > Increase to sharpen images or for better analytical yield (BSE, EDS.)

- **Spot size**: beam current: 1 – 17 (can be displayed as current. Ask equipment manager for details.)
  - > Decrease for sensitive specimens, fine detail
    - Decreasing will increase image noise. Increase dwell time, averaging, or integration to compensate for increased noise.
  - > Increase for more efficient signal generation, especially for EDS, BSE.
    - Increasing will blur high magnification images and may increase charging. Compensate for charging by decreasing dwell time and adding averaging or integration. If beam is well aligned and image is still blurry, try a different mode or reduce spot size.

- **Imaging mode**: changes detector options, electron filtering.
  - > **Standard**: Most flexible mode. No additional fields applied. Default for Low Vac unless Auto PLA is installed. Large depth of field. Images may be appear grainier. SE, BSE signals may be mixed. ETD, T1, T2 available in High Vac. LVD, T1, T2 available in Low Vac.
  - > **OptiPlan**: Most used mode. Filters electrons by energy to the ETD, T1, T2, T3 detectors (see Detectors tab.) Great for short and long working distances. Available in Low Vac with PLA only (ask equipment manager for assistance.)
  - > **Immersion**: Highest resolution mode. Applies additional field to pole piece. Great for short working distances, low voltage, small spot size. T1, T2, T3 only. May be available in Low Vac with PLA (ask equipment manager for assistance.)

- **Detector**: Preview which signals go to which detectors for a given voltage and mode in the Detectors panel on the right side.
  - > Scan with multiple detectors simultaneously to determine which detector yields the best image for your needs.
  - > The Detector tab preview shows estimates of signal types filtered to each detector based on working distance and voltage. Changing the voltage in this menu does not update the system beam voltage unless you click “Apply.”

- : few ns to 10s of us. Amount of time beam spends on each pixel.
  - > Reduce if specimen is moving, charging, or for operations requiring fast feedback
  - > Increase for reduced noise and visibility of fine details.
  - > Use the reduced area box **[F7]** to try different dwell times without waiting for the full frame.

STIGMATOR ALIGNMENT

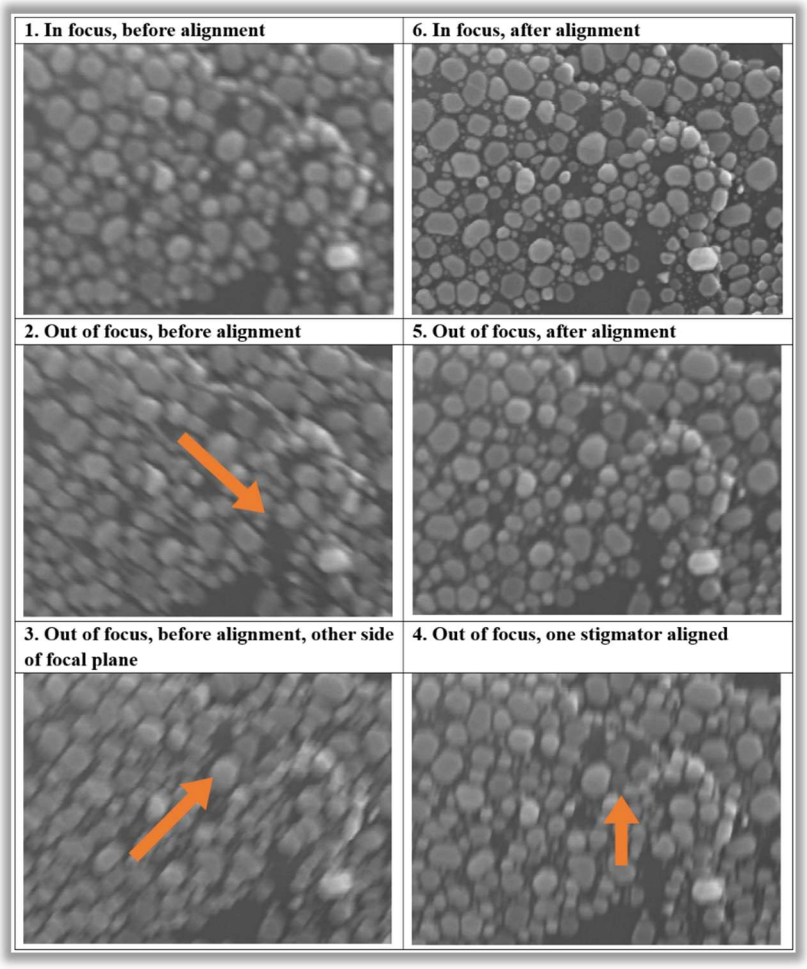
Stigmators control the shape of the beam. An ideal electron beam is smaller than the pixel size and round. If the beam is astigmatic, then the beam will be large when in focus, and elliptical when out of focus.

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.



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