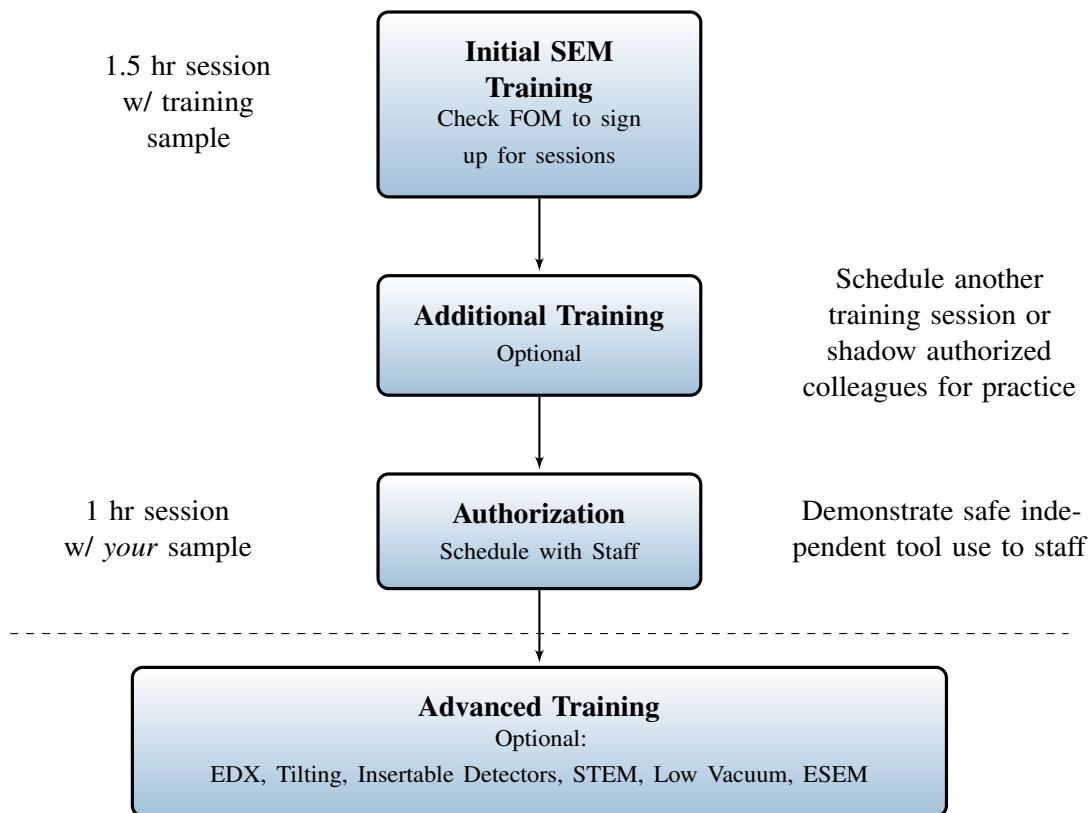


4. Zeiss Sigma 500

4.1 Training Structure

Nano3 has a range of Electron Microscopes to choose from. If you need help selecting a tool, ask staff for help.

- **FEI Quanta FEG250**
 - Recommended for features > 100 nm
 - 1 – 30 keV Beam energy
 - Lateral Sample size limit 50 mm
 - Low vacuum and environmental (ESEM) modes useful for charging and hydrated samples
 - Energy Dispersive X-ray (EDX) capability
 - Insertable Backscatter detector option (additional training required)
- **FEI Apreo**
 - Recommended for features < 100 nm - high resolution system (~ 1 nm)
 - 1 – 30 keV Beam energy
 - Lateral Sample size limit 110 mm
 - Opti-plan and Immersion UHR modes
 - Energy Dispersive X-ray (EDX) capability
 - ETD, Backscatter Electron Detector (T1), Secondary Electron detector (T2)
 - Insertable annular/concentric backscatter detector option (additional training required)
- **Zeiss Sigma 500**
 - Recommended for features < 100 nm - high resolution system (~ 1 nm)
 - 0.1 – 30 keV Beam energy
 - Lateral Sample size limit 110 mm
 - Energy Dispersive X-ray (EDX) capability
 - ETD, Secondary Electron detector (Inlens)
 - STEM capability (additional training required)
 - Insertable Backscatter detector option (additional training required)



4.2 Tool Billing Policy

You will be billed according to the following:

Start time = earlier of the FOM log in or reservation start time

End time = later of the FOM log out or reservation end time

Reservations cancelled within **4 hours** of the start time will be fully charged

It is your responsibility to log out of FOM after your session

Important This tool will automatically log out 1 hour after your scheduled reservation end time.

4.3 Introduction to Scanning Electron Microscopy

Scanning electron microscopes (SEMs) are used to acquire high resolution images of micro and nano-structures that aid in the characterization of surface morphology and topography, as well as the analysis of sample composition using energy dispersive x-ray spectroscopy (EDX). SEMs are utilized in academic, industrial, and commercial contexts across a multitude of disciplines including material science, electronics, aerospace, medicine, biology, chemistry, geology, and even forensics. The versatility and resolution capabilities of the SEM have led it to be a crucial proponent in the advancement of modern day nanotechnology.

Electron vs. light microscopy

It is useful to first discuss the basic concepts of light optics, in order to understand the fundamentals of electron microscopy. Optical microscopes focus a light source into an area by using glass lenses. Optical microscopes can achieve magnifications up to $\sim 1000\times$ and spatial resolution of $\sim 1\text{ }\mu\text{m}$. It is important to note that optical microscopes gather an image over an entire field of view simultaneously requiring further optics to e.g. focus an image on a camera detector. On the other hand, in an SEM electrons are used as the source instead of light. The instrument focuses the electron beam using electric and magnetic fields to a nanometer sized **probe** or **spot** that scans over the sample area. The image is formed very quickly, one pixel at a time from electrons that scatter from the sample. One definition of resolution is the minimum distance two structures can be separated and still be physically observed as two distinct features. In light optics the wavelength of the illumination source dictates the limit of resolution. Although electrons in an SEM have a wavelength on the order of 10^{-2} nm , practically it is not the limiting factor for obtaining high resolution images. The best possible resolution in an SEM is the probe size ($\sim 1\text{ nm}$) but the electron-sample interaction often decreases the achievable resolution. For this reason, correct **sample preparation** is key to optimal imaging results.

The main components of the system

The SEM consists of a ‘column’ which contains the electron optics (source and lenses) and a chamber where the sample is placed. The system is maintained at very high vacuum levels to maintain beam and imaging quality. An electron source is positioned at the top of the microscope. A beam is formed by ‘dragging’ electrons from the source using an effect known as field emission. The electrons are then accelerated toward the sample with an applied voltage allowing control of the electron **beam energy (keV)**. Moving down the column of the SEM we find the main optical elements: **focus** lenses, **stigmation** lenses, and **apertures** which control the probe size. Some systems allow control of **beam current** directly. This can be achieved on any system by changing aperture size (usually unchanged for high resolution imaging). Focusing and stigmation lenses control the cross-sectional shape of the beam. Ideally the beam should have a circular profile, otherwise the beam is said to have astigmatism which can be corrected for by adjusting the stigmator lenses. Stigmator lenses aren’t typically present on light microscopes since the lenses are fixed and can therefore be manufactured very precisely to account for astigmatism. In an SEM there is a choice of the beam energy, and therefore we need the freedom to control stigmation. For optimal system performance, alignments are required to ensure the electron beam goes through the center of all the optical elements. Some systems have more alignments than others and in addition to aligning the beam/apertures, alignment of the stigmator lenses may also be needed. The last stop before moving down to the main chamber are the scan coils. The scanning (or raster) of the electron beam is controlled in x and y via precise beam deflection from the scan coils. Finally, in the main chamber there is a stage where samples are placed. At the top of the chamber there is a cone known as the **pole piece** which is part of the final objective lens. The distance between the

pole piece and sample surface (when the beam is in focus) is known as the **working distance** or WD. The other hardware inside the chamber are electron detectors used to form images. As the beam is scanned over the sample, scattered electrons are collected by the various detectors. To understand the choice of detectors and image formation, we need first to discuss the electron-sample interaction.

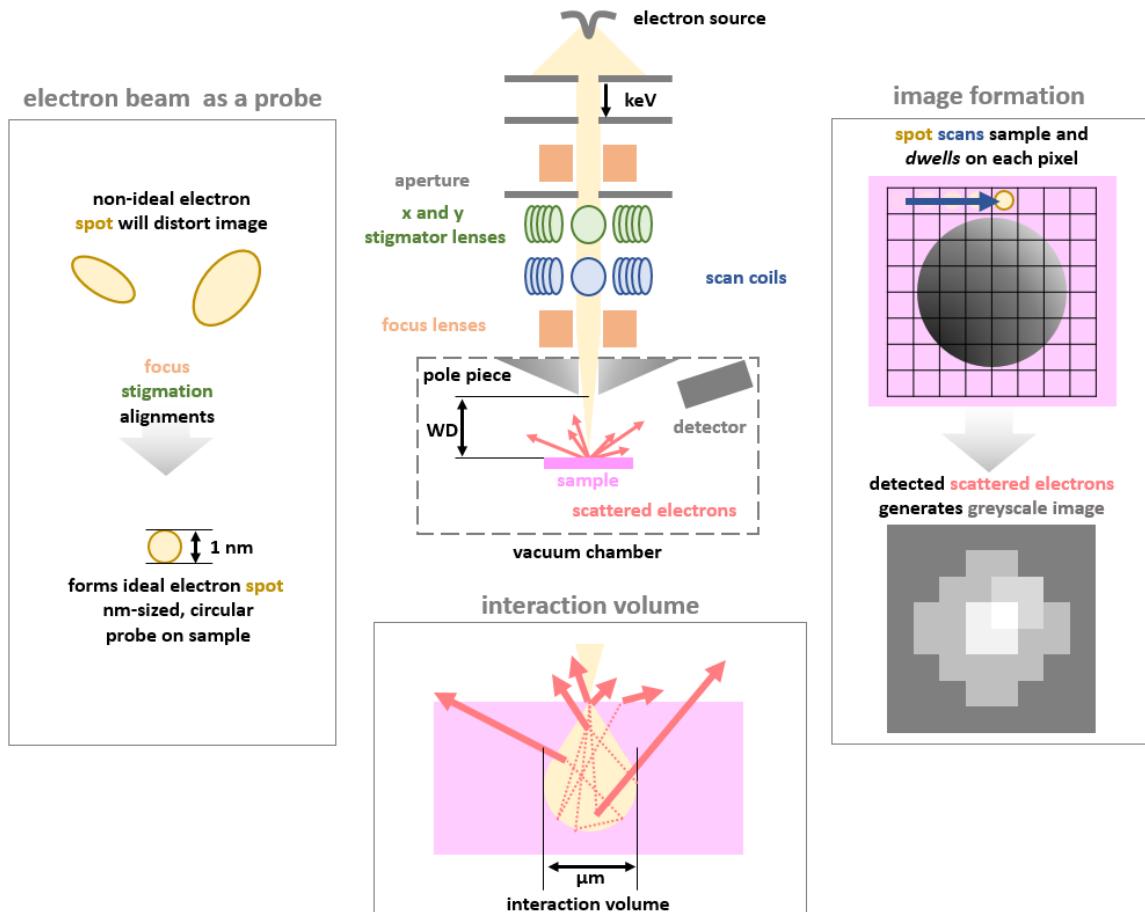


Figure 4.1: SEM system overview. Left: Characteristics of the electron probe tuned with focus, stigmation, and alignments. Center Top: Main components of a typical SEM. Center Bottom: electron-sample interaction. Right: Image generation.

Electron-sample interaction and electron detectors

The electron beam is incident on the sample with \sim nm probe size. The beam penetrates the sample surface and interacts over a volume that may be many μm^3 in size depending on the beam energy (keV) and material properties. This region is known as the **interaction volume**. Since electrons scatter throughout this volume, they may make their way back to the surface μm 's away from where the beam entered. This is why the probe size is a limit to achievable resolution. There are two types of electron scattering relevant for SEM: Elastic and Inelastic. When undergoing elastic scattering, electrons maintain their energy and are deemed to be **backscattered electrons** or BSEs. Backscattered electrons can give qualitative information on material composition (Z number) since heavier elements will scatter more electrons than lighter elements. When undergoing inelastic scattering, electrons lose

energy and are deemed **secondary electrons** or SEs. Secondary electrons are characterized by their low energy (< 50 eV). Secondary electrons are only able to be detected if they are generated within the top 1 – 2 nm of the surface (otherwise they don't escape the material). Therefore, secondary electrons provide insight into the surface details and morphology of the sample. The type of detector being utilized will dictate the qualities of the image being acquired. Detector position (angle) can also affect the image qualities. The most ubiquitous detector is the ETD. Images from this detector are usually a mixture of BSEs and SEs. More importantly, this detector is often angled with respect to the sample stage resulting in a **shadowing** effect - producing images that seem 3D, showing depth more clearly. Specialized detectors are often ‘in-lens’ or viewing top-down generally omitting any shadowing effects.

Image formation and signal processing

Finally, how do we get an image from the SEM? The image is formed by scanning the electron beam over the sample. Although the scanning looks smooth, the beam actually pauses for a short time on each pixel for a duration known as **dwell time** allowing signal in that pixel to be collected before moving to the next. Shorter dwell times result in noisy image that refreshes quickly, whereas longer dwell times result in higher signal images that refresh very slowly. On some systems this parameter is controlled via **scan speed**. Operationally, short dwell times are used for optimization and longer dwell times are used for image capture. Remember the wavelength of electrons is a few orders of magnitude lower than the visible light wavelength, so none of the images can physically be generated in true color. Consequently the images are greyscale 0 – 255, 0 being associated with black and 255 with white. The greyscale value is assigned proportional to the number of electrons being detected per pixel. You can control **brightness** and **contrast** of the detector allowing the aesthetic of the image generated to be changed. Note that changing the detector brightness and contrast is entirely independent from the electron beam settings. There are a variety of noise reduction modes available - mostly consisting of averaging many images together, or scanning lines multiple times before moving to the next. Putting everything together, the electrons being detected (and therefore resulting image) will depend on the detector type, detector placement, detector settings, beam energy, beam current, and electron-sample interaction.

4.4 SEM Sample Preparation

Correct sample preparation is key to obtaining high resolution images. Also, ensuring that your sample preparation minimizes potential chamber contamination is crucial keeping the microscope operating to specification. In this section we will give some tips on how to correctly prepare samples for high resolution imaging. We encourage users to bring their own supplies for proper sample preparation. There is a limited stock of communal items in the SEM labs including stubs and carbon tape.

4.4.1 Handling and loading samples

Important Always wear gloves when preparing and manipulating samples and sample holders. This is to prevent contamination of the SEM and your sample.

- Any samples or parts that are going to be put inside the SEM must be cleaned with IPA.
- Only grab the sides on the sample holder with SEM tweezers as shown in Fig. 4.2 a).
- Place pin securely into stage hole.
- If applicable use the hex key for the stage position the sample is placed in. Clockwise rotation tightens and vice versa for loosening screw. Only use $\frac{1}{4}$ turns in each direction. Notify staff for missing screws.
- Never load samples of vastly different heights and shown in Fig. 4.2 b)

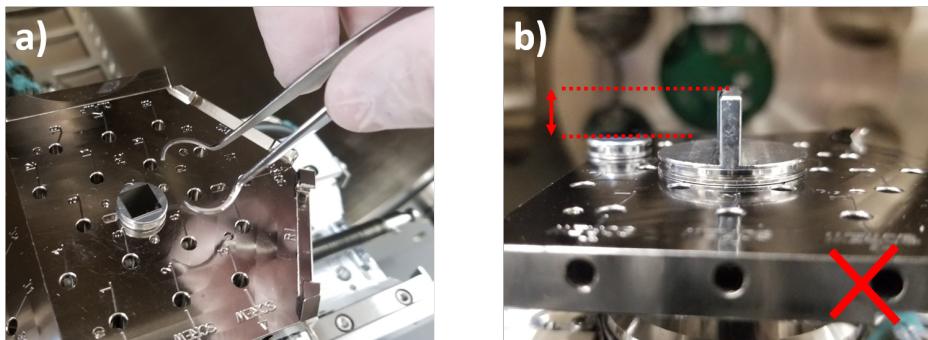


Figure 4.2: Sample loading and handling. a) Always use gloves when preparing samples. Load samples with SEM tweezers. b) Never load samples of vastly different heights.

4.4.2 Cleaning sample stubs

Important Always use clean the sample mounts to maintain vacuum quality and to avoid sample cross-contamination. Acetone residues must never enter the vacuum chamber.

1. If possible, peel off old carbon tape.
2. In fume hood, use Acetone and IPA to remove adhesive residue. Ensure solid solvent waste is placed in the appropriate waste container.
3. Always end with an IPA scrub since acetone can damage vacuum seals.
4. Use nitrogen gun to dry

4.4.3 Common sample preparation

Here we outline common and quick preparation techniques for various sample types. There are other types of adhesive that you can source (e.g. silver paint, carbon glue, etc). However we focus on

methods using carbon tape since it is quick and simple. Depending on your sample, it may not be the ideal method. Consult literature for advice regarding your specific samples.

Silicon chips

1. Apply small strip of carbon tape (double sided) to preferred stub. Do not over-use carbon tape since it is a source of system contamination. See Fig 4.3 for ideal silicon sample preparation.
2. Tap carbon tape with gloved finger reduce stickiness.
3. Place the sample on the stub and check it is secure by carefully flipping upside down over your hand.

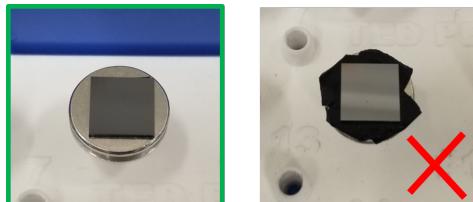


Figure 4.3: Ideal silicon sample preparation. Do not overuse carbon tape as shown on right.

Tip After imaging, the silicon chip may be strongly adhered to the stub. Do not attempt to pry or leverage the sample from the stub since you may break the sample. Instead, push or torque the sample from the edge directing the force with tweezers in a plane parallel to the chip surface. See Fig. 4.4.

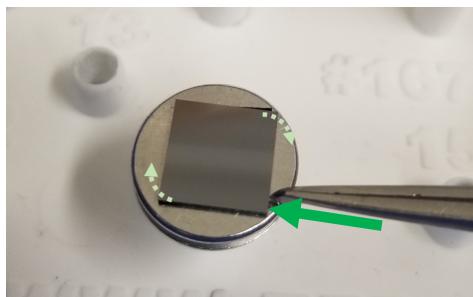


Figure 4.4: To remove a silicon (or other fragile chip) push on the edge with tweezers to twist it.

Powder

Important All powder samples pose significant risk to SEM systems since they have the tendency to accumulate static charge (regardless of the powder composition) owing to the particles' small radii of curvature. Incorrectly prepared powders can destroy the electron optics and heavily contaminate the system. An ideally prepared sample is shown in Fig. 4.5.

1. Make sure to prep samples on a new clean wipe to catch loose powder.
2. Always use fresh carbon tape. Old tape is not adhesive enough.
3. Dispense as little powder as possible. Thin, single layers of powder image better.
4. Dust excess powder over the trash can.
5. Use nitrogen gun in fume hood to remove any additional loose particles.

6. Swipe prepared sample over magnet to remove loose magnetic particles. This also ensures no magnetic particles come from cross-contamination if your samples aren't magnetic.
7. After imaging, if you are using Nano3 stubs - clean them off thoroughly.

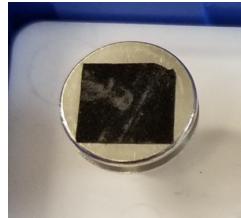


Figure 4.5: Ideal powder sample preparation

Non-conductive samples

Non-conductive samples can generally be imaged with the appropriate beam settings. However, whether a sample will show charging artifacts or be difficult to image is a function of sample size, composition, structure (e.g. layers), geometry (e.g. surface area), and more. For example, clumps of metallic nanoparticles can misbehave in an SEM due to the large amount of interfaces between touching particles that can potentially trap charge. Therefore, sometimes it is necessary to take additional measures like sputter coating a thin layer of metal. This thin layer of metal can draw away static charges that would otherwise occur and even increase imaging contrast. Sputter coating is not a magic fix as samples still need to be prepared properly. The coating (which is typically < 10 nm in thickness) provides an electrical path for the electrons to follow and therefore must be connected to the electrical ground of the sample stage.

1. Prepare samples as normal on a stub
2. Place in coater of choice. (e.g. Nano3 has the Emitech K575X specifically for SEM sample preparation)
3. For thick chips, wafers, and glass place etc. place a piece of conductive tape that connects the top surface of the metal to the stub to ground it (see Fig. 4.6).

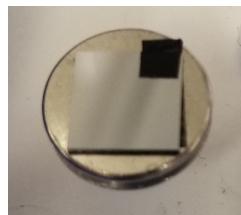


Figure 4.6: Ideal preparation of coated sample

Biological and wet specimens

Biological specimens pose many difficulties for SEM imaging. First, SEM takes place inside a vacuum, meaning in general samples cannot be hydrated. Second, they are often non-conductive and will show charging artifacts. Third, they can be fragile and therefore could be damaged by certain beam conditions. Fourth, due to low Z numbers they can often present poor imaging contrast. Below are a list of suggestions to get started preparing biological and other wet samples.

Important Samples must be completely dry before putting them in the vacuum system of an SEM.

- Ensure samples are dry.
 - Leaving samples overnight in a fume hood or under an incandescent bulb can often accommodate this.
 - For delicate samples, a more careful drying process can be completed using a critical point dryer (e.g. Tousimis AutoSamdri 815 A and B).
- Sputter coating (as above) on dried samples can improve imaging quality.
- FEI Quanta 250 FEG has low vacuum mode (for charge mitigation) and environmental (ESEM) mode for hydrated sample imaging.
- Consult literature for advice regarding your specific samples - in particular chemical fixation and staining.



Figure 4.7: One example of an ideally prepared biological sample (dried leaf, sputter coated).

4.4.4 Cross-sectional preparation

For the best cross-sectional imaging, generally the sample needs to be at a very high angle (e.g. $> 70^\circ$). This is a larger angle than most SEM stages can accommodate. Therefore, loading the sample pre-tilted is the easiest way to accommodate cross-sectional imaging. This section outlines some notes on cross-sectional imaging techniques.

Important Be extra cautious during cross-sectional imaging with relation to sample-pole piece distances and tilting. This is because you are generally loading a much higher sample (e.g. 90° mount).

- Prepare samples as above for silicon except on a 45° or 90° mount (see Fig. 4.8). 45° mounts are useful if you want a shallowly tilted image and a full cross-section in the same imaging session (since you can tilt the stage a further 45° to obtain a 90° image).
- Mount sample close to the top edge of the stub.
- If coating was required, place a small amount of conductive tape to connect the top surface to the mount as above for non-conductive samples.
- Orient the stub toward the ETD detector to allow maximum for signal as shown in Fig. 4.9. This is even more important for cross-sectional EDX. The EDX detectors are also on the same side of the chamber as the ETD.
- Take extra care when tilting.
- For best elemental contrast between e.g. silicon and metal layers, use a high keV and a backscatter electron detector (e.g. T1).

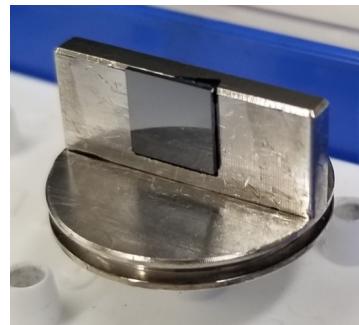


Figure 4.8: Ideal silicon cross-section preparation on 90° mount

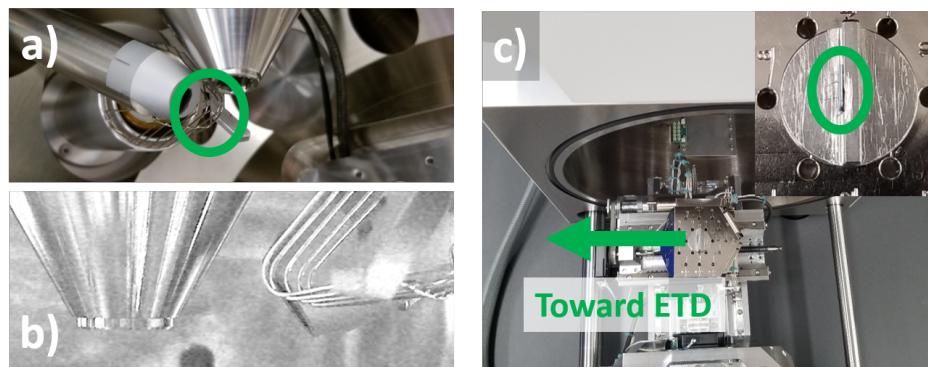


Figure 4.9: Sample loading for cross-sections. a) Looking inside the chamber, the ETD detector is on the left. The ETD always has a small wire cage around it. b) View from chamber CCD looking from the camera at the back of the chamber. c) Load cross-sectional samples facing toward the left of the chamber.

4.5 Software and Server

PC and software credentials:

- Username: user
- Password: nano3zeiss

If there is a problem with the user interface/software, feel free to close and then reopen the software. Close the software and the ‘EM Server’. Then click on the *Smart SEM* Software: . Wait until the server is online (indicated by the full green bar) and then log into the software using the above credentials when prompted.

4.6 Standard Operating Procedure

1. Log into your FOM user account, activate Zeiss Sigma 500 from the calendar.
2. Begin an entry in the paper log book.
3. Click **Vent** button: .
4. Sample Preparation
 - Never touch samples/holders with bare hands. Always use gloves.
 - Do not load samples of vastly different heights (beyond $\sim 1 - 2$ mm).
 - Powders: use as little as possible on fresh carbon tape. Dust off excess over trash can and use nitrogen gun.
5. Load your samples onto the sample stage. All positions require using a hex key to secure the stub. Only lightly tighten the set screws in the sample holder.
6. Close chamber door and click **Pump** button . Hold chamber door closed until pump engages.
7. Wait for high vacuum, indicated by green check mark on VAC indicator: |Vac: | (lower right). Usually < 10 minutes.
8. Whilst waiting for vacuum you can position the sample. Be sure to navigate to your sample (**Double Click** on cartoon) first before proceeding to the next step.
9. Using the small **Z Joystick**, move sample stage to ~ 8 mm working distance (WD). Use the green horizontal ‘STEM’ line as a guide for the WD.

Important Always view the chamber when adjusting Z to prevent crashing stage into pole piece.

To switch back to chamber view from the electron image click the TV button: .

10. Turn on beam.
 - In the ‘Gun’ tab in the ‘SEM Controls’ panel on the left.
 - Set accelerating voltage. A good starting point is 3 – 5 keV.
 - Finally, turn on the beam in one of these ways:
 - Change *Beam State = EHT Off* to *= EHT On* in the ‘Gun’ tab.
 - Click **EHT** button:  (lower right) and select **EHT On**.

Important Do not set EHT above 25 keV directly. You must ramp from 25 keV at 1 keV every 30 s to a maximum of 30 keV. For example, if your desired EHT is 27 keV, first set to 25 keV, wait 30 seconds, then set to 26 keV, wait 30 more seconds, and finally to 27 keV.

Tip For energies < 3 KeV there will be distortion at the edge of the image, this is normal. When you magnify the distortion will disappear.

11. Select detector with the **SE/InLens** button:  **Left Click** (SE2), or **Right Click** (InLens). Both the SE2 detector (ETD) and the InLens are primarily secondary electron detectors.
12. Navigate roughly to sample area to get a low magnification image.
 - Ensure magnification is as low as possible using the **Magnification** dial.
 - Adjust **Brightness** and **Contrast** dials as necessary. To activate automatic brightness and contrast navigate to Detectors > Signal Adjust > Auto BC and set to BC until levels balance out. Remember to turn off.
 - Ensure appropriate aperture is selected in the ‘Apertures’ tab of ‘SEM Control’ panel. Default: 30 µm for imaging.
 - Navigate with: large **X-Y Joystick**, **Double Click** on sample holder diagram, or **ctrl+tab** to activate green crosshair  and **Left Click** to set center point.
 - Fast scan speeds are best for navigation. **Left Click** the **Scan1** button  to select scan speed ‘2’.
 - Check image resolution in the ‘Scanning’ Tab. Default: 1024 × 768.
 - Ensure scan rotation is set to 0° or off. This setting is in the ‘Rotate/Tilt’ panel on the left. Ensure the Scan Rot box is unchecked: **Scan Rot**.
 - Set scan parameters as desired in ‘Scanning’ tab of the ‘SEM Control’ panel. Recommended Noise Reduction Mode: Line Average with $N = 3$.
 - Adjust **Focus** dial to obtain image of sample surface.
13. Navigate to area of interest on the sample.
 - Begin increasing to higher and higher magnification.
 - Adjust focus as necessary.
 - Continue magnifying area of interest as desired.
 - Eventually focusing alone will not improve image sharpness/quality.

Tip By pressing **Reduced** button (above the magnification dial) you can toggle between full scan and reduced area scan. This is useful for beam optimization because for a given scan rate, the image refreshes much faster and maintains the resolution of the full frame.

14. Improve image by optimizing electron beam. Optimizing the beam is the key step to obtaining the best SEM images. The goal is to improve the image for a higher magnification than the intended image capture. Most of your time may be spent performing this step. **Any time you change acceleration voltage or aperture you will have to optimize the electron beam again.**
 - Obtain best focus possible by adjusting the **Focus** dial.
 - **Adjust stigmation:** using **Stigmator X** and **Stigmator Y** control dials until focus only blurs symmetrically and doesn’t stretch image.
 - Zoom to higher magnification and repeat as desired.
 - **Align aperture:** *Only adjust aperture alignment at high magnification*
 - Turn on focus wobble to aid in aperture alignment.
 - Use **Aperture X** and **Aperture Y** control dials to adjust. Good alignment is when focus wobble doesn’t translate the image. Remember to turn off focus wobble when

complete. Note that the X and Y directions often don't correlate with left/right and up/down on the screen and may change with acceleration voltage.

- Always end on focus adjustment.

Tip Use scan rotation to aid in the aperture alignment. First determine the angle that the image translation makes with respect to the horizontal when adjusting the Aperture X dial. Then turn on scan rotation to counteract this angle offset. After you have made the adjustments remember to turn off scan rotation.

15. Zoom out to desired image capture magnification. You can set a specific magnification by typing it into the magnification field on the bottom of the image pane.
16. Adjust scanning settings to reduce noise and obtain highest quality image possible. See Section 4.7.2 for more advanced recommendations.
 - **Right Click**  for default capture scan speed '7'.
 - Alternatively select the desired speed from the 'Speeds' button in the 'Scanning' tab.
 - **Click Freeze**  to pause image after the current frame completes scanning.
17. Save images.
 - **Right Click on Save button**  to select parameters
 - Change directory to desired folder within your Username and Group on *D: drive*
 - Type filename ending in underscore. Select a number of digits to add to the end of each filename. Image number will automatically iterate each time you save.
 - Check 'Annotations' and 'Color merge' to ensure the details of the beam (i.e. the box at the bottom with acceleration voltage, detector type, magnification, and so on) and any annotations or measurements are saved along with your image.
 - In the settings Tab, ensure file 'image' settings are set to 'Grey' to allow the data to be loaded back into the SEM software for measurements.
 - **Left Click on Save button**  for each capture you want to save.
18. You can continue examining different regions of your sample and collect as many images as necessary.
 - Unfreeze image by pressing **Freeze** button.
 - Switch back to faster scan mode for navigation (**Left Click** 'Scan1' button).
 - Repeat step 14 as necessary.
 - **Left Click on Save button** to save the image to the previously set directory.
19. When complete, return system to starting configuration.
 - Use TV mode to ensure sample and stage is clear of pole piece.
 - Lower sample stage.
 - Low magnification.
 - 30 μm aperture.
 - 0° tilt.
 - Ensure scan rotation is set to 0° or off. This setting is in the 'Rotate/Tilt' panel on the left. Ensure the Scan Rot box is unchecked: **Scan Rot**.
20. Turn off EHT beam..
21. Click on **Vent** Button: .
22. Unload samples. Only lightly loosen the set screws in the sample holder.

23. Hold chamber door closed and click **Pump** button:  Ensure pump engages - the chamber must be maintained at high vacuum between users.
24. Finish paper logbook entry and log out of tool with FOM. Remember to note down the emission current: **Ext I Monitor = 211.0 μA** (in ‘Gun’ tab of ‘SEM Control’).
25. Collect images/data from data PC. Note the *D: drive* is not permanent storage and data may be removed. Collect all important files ASAP.

4.7 High Resolution Imaging on the Zeiss Sigma 500

Section 4.6 details the standard procedure for imaging samples. However, these settings are not necessarily the optimum for all sample types. When the beam parameters and system are configured correctly for your samples, the resolution of the machine can reach ~ 1 nm. This section details empirically how to optimize imaging for your samples on the Zeiss Sigma 500.

4.7.1 General Recommendations

1. **Focus, Stigmation, Lens Alignment:** Any time you change the Working Distance (WD) or Beam energy (keV), etc. the Focus, stigmation, and lens alignment must be re-tuned. For the highest quality images it is essential that you set the focus, stigmation, and lens alignment at a higher level of magnification than the intended image capture magnification. For example, if you wish to take a $100k\times$ magnification image, adjust the parameters at $150 - 200k\times$ magnification. Once the parameters are set at the higher magnification, zoom out and do not adjust them again before taking the image. Fig. 4.10 shows an example of the focus and stigmation procedure.
2. **Beam Energy / Voltage (keV):** Even when the beam parameters are perfect, the limiting factor is often how the beam interacts with your specific sample which is dictated by beam voltage. The recommended starting voltage (3 – 5 keV) may not be ideal for your specific sample type. Try < 3 keV and > 5 keV to qualitatively test whether your samples are imaged better at higher or lower voltages. Remember you will have to adjust focus, stigmation and lens alignment every time you change voltage. Low voltage imaging (< 3 keV) is discussed in Section 4.7.2.

Important Do not set EHT above 25 keV directly. You must ramp from 25 keV at 1 keV every 30 s to a maximum of 30 keV.

3. **Detector Selection:** The Zeiss Sigma has two easily swappable detectors (SE2/Inlens). For high resolution work the Inlens is recommended (but try both).
 - SE2: This is an ETD detector which sees both secondary and backscatter electrons. Typically images that are collected with this detector look more ‘3D’ because it is at an angle with respect to the electron gun.
 - Inlens: This is a secondary electron detector situated in the pole piece, meaning images collected with this detector look ‘flat’. More importantly since this detector is sensitive to secondary (low energy) electrons it is better suited to resolve small features, surface details, and contaminants on the sample surface. For best results, use this detector at a smaller working distance as discussed in item 4.
 - Backscatter (BSE): This is an insertable backscatter electron detector that is good for elemental (Z number) contrast. Additional training required.
4. **Working Distance :** Although we start imaging at the green line (WD ~ 8 mm), the smaller the WD the higher the resolution of the captured image. Therefore, in general when trying to resolve

the smallest features move the stage Z higher. An accurate reading of the WD is obtained on the data bar when the sample is in focus. You obtain a better signal-to-noise ratio when using the Inlens detector in conjunction with a smaller WD. Never move closer than WD= 2 mm.

Important Always view the chamber when adjusting Z to prevent crashing stage into pole piece. Never move closer than WD= 2 mm.

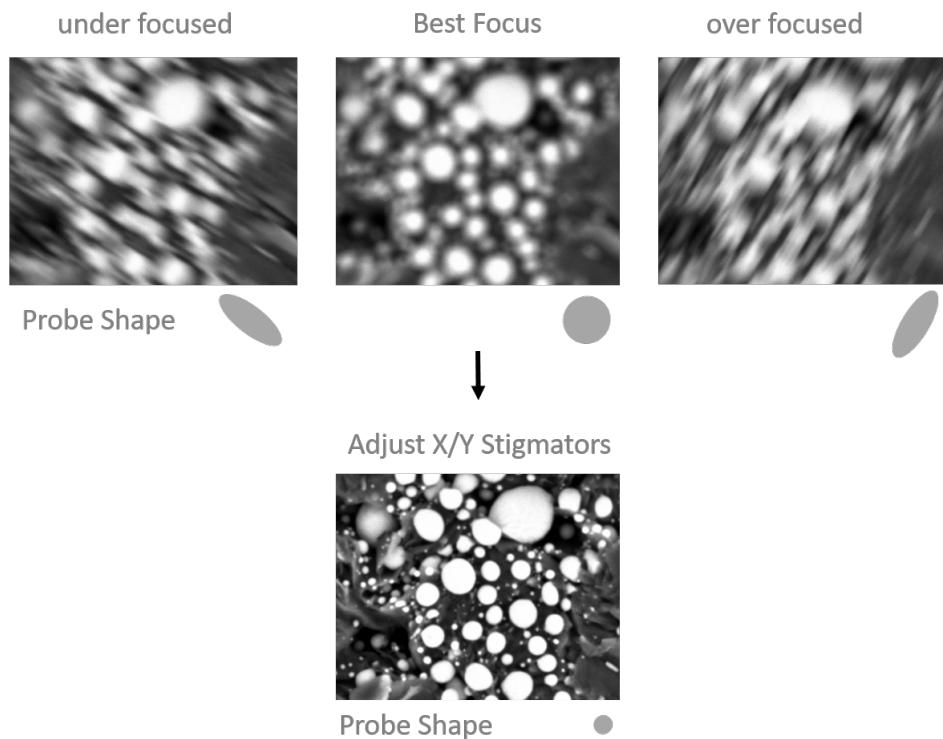


Figure 4.10: Example of finding the best focus and then performing X and Y stigmation to form the best image. The grey shapes show a schematic of the e-beam probe on the sample surface.

4.7.2 Advanced Recommendations

1. **Low Voltage Imaging:** The Zeiss can support imaging at < 1 keV. This can be advantageous for particular sample types and achieving very high resolution. There are a few important considerations when attempting to image with very low voltages.
 - (a) For energies < 3 KeV there will be distortion at the edge of the image at low magnification, this is normal. When you magnify the distortion will disappear.
 - (b) You cannot focus beyond a working distance of 7.9 mm. Therefore you must raise stage Z when using low voltages.
 - (c) For best detection of low voltages in conjunction with reduced working distance use the Inlens detector (discussed in Section 4.7.1).
2. **Scan Settings :** It is best to scan with the longest dwell time (scan speed number) possible assuming your sample doesn't show ill effects such as charging. It can be advantageous to increase the line averaging in addition to increasing scan speed number. For example the default

Noise Reduction Mode: Line Average with $N = 3$ could be increased to $N = 10$. The down side is that the image will take longer to capture, therefore there is a balance between obtaining a high resolution, low noise image and optimizing capture time. Please remember to change the scan settings back to the default when complete.

3. **Aperture:** The standard imaging aperture is $30 \mu\text{m}$. This gives the best balance between a high signal to noise ratio and resolution. Resolution can be increased at the expense of signal by decreasing the aperture size. Aperture sizes lower than $30 \mu\text{m}$ can give better resolution, but to compensate for the increased noise you will have to adjust the scan settings to collect more signal (as discussed in item 2). Please remember to change the aperture back to the default when complete.

4.8 STEM - Additional Training/Authorization Required

1. **Vent** system as usual
2. Sample Preparation
 - (a) Using gloves, locate STEM holder and screwdriver from drawer
 - (b) Load TEM grids onto holder. Be gentle with the fasteners
3. Remove normal sample holder from stage, and replace with the STEM holder. Ensure the holder is seated correctly on the stage.
4. **Pump** system as usual
5. Select *STEM 12x v2* sample holder from the list in the software as shown in Fig. 4.11

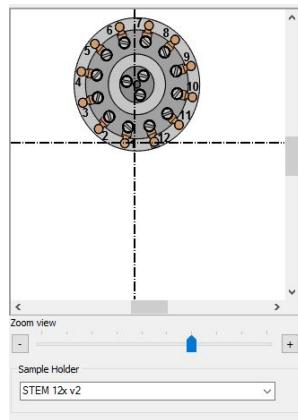


Figure 4.11: Selecting the *STEM 12x v2* holder. Remember to change back to *Carousel 9x9* at the end of your session

6. Navigate to your sample by **Clicking** the position on the stage point list and then **Goto** as shown in Fig. 4.12. \$STEM 1 corresponds to position 1 in the STEM holder.
7. Turn on EHT and set to 25 keV.
8. Ramp up beam in 1 keV increments every 30 s to a maximum of 30 keV
9. Select SE2 detector by **Left Clicking** button:
10. Without moving the stage, confirm that the TEM grid is roughly centered in the image. For example, if you moved to \$STEM 1 position, that grid should be in the center of the field of view. If it is not, perform *Stage/Stage Initialize* to home the stage and try again. If this still doesn't fix

the centering, Vent the system and re-seat the sample holder on the stage.

Important Do not set EHT above 25 keV directly. You must ramp from 25 keV at 1 keV every 30 s to a maximum of 30 keV.

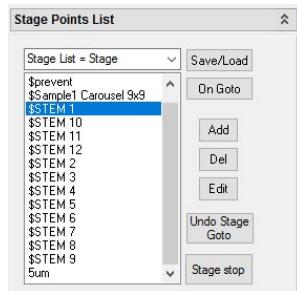


Figure 4.12: Stage Point List on the left hand side of the software interface

Important Only use the \$ positions from the stage point list for navigation. Never navigate using the sample holder cartoon (by double clicking). You risk crashing the stage into the STEM detector and damaging it.

11. Ensure sample stage is at a \$ position e.g. \$STEM 1.
12. **Left Click:** to bring up STEM Control Window as shown in Fig. 4.13.
13. Click **Insert**. A message will pop up asking if it is ok to move the stage to the safe position. Click ok. The pneumatic actuator will automatically insert the STEM detector.
14. Select the SE2 detector, this time to confirm STEM detector alignment without the presence of the sample. Be sure to focus on the detector first. The center circle of the detector should be centered in the FOV. You can use *View/Crosshairs* to aid in alignment. Should any adjustments be made, use the X and Y micrometers of the detector to re-center the detector and shown Fig. 4.14. Never touch the Z alignment.
15. Once you have confirmed the alignment of the detector you can move the sample stage back to a \$ position e.g. \$STEM 1.
16. **Left Click:** and select *aSTEM4A* to active STEM detector view.
17. Use the STEM Control window (Fig. 4.13) to activate/deactivate/invert (White/Grey/Black) by **Left Clicking** the detector regions (S1, S2, S3, S4, S5).
18. Segment modes:
 - S1: Bright Field Imaging (BF)
 - S2-3: Dark Field Imaging (DF)
 - S4-5: High Angle Dark Field imaging (HADF)
19. In addition to the normal brightness and contrast settings, you can select the detector gain settings in the STEM control window. *Medium* is a good starting point.
20. Capture and save images as normal
21. When complete return to the chamber view by **Clicking** .
22. **Click Retract** on the STEM control window to remove the detector.
23. **Vent** and replace original sample holder.

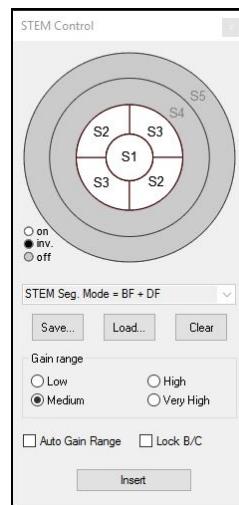


Figure 4.13: STEM Control window where you can insert/retract the detector, control detector modes, and gain settings

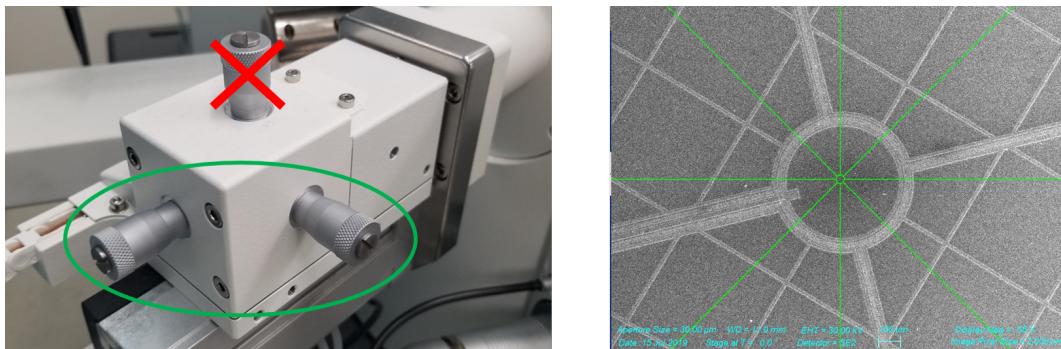


Figure 4.14: Left: Showing X and Y STEM positioning dials that you may adjust. Do not adjust Z. Right: Showing correct alignment of the STEM detector - SE2 view of the STEM detector with cross-hairs

24. **Pump** chamber
25. Change the cartoon navigation holder back to the original *Carousel 9x9* sample holder in the software

4.9 How to image samples that charge

If your research involves non-conductive samples, imaging with the SEM (electrons) can be problematic because the charges can get stuck in the sample and interfere with the incoming beam resulting in images that can show glowing spots, streaking, drifting, and warping. One example is shown in Fig. 4.15. In fact, even if your samples are conductive this can still be an issue. In this section we discuss the main reasons that charging happens, and what you can do to prevent it in your final image.

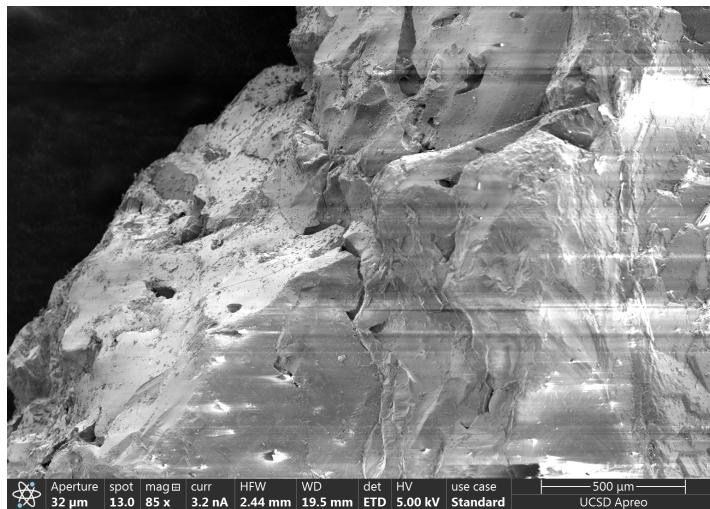


Figure 4.15: One example of charging artifacts. Shown in the image is ‘streaking’ and glowing spots.

Why does charging happen?

The electrons used for imaging are the ones that are scattered from your sample and reach the detectors. The remaining electrons from the beam or induced from the beam-sample interaction must be driven away from the area of interest and reach electrical ground to not cause any imaging issues. If electrons remain in the region of interest they may deflect the incoming beam resulting in the aforementioned charging artifacts. The chamber and sample stage in the SEM are grounded. Ensuring the electrons can get from your sample to ground as fast as possible will minimize the effects of charging in your images or prevent it entirely.

Sample type

Whether your sample is conductive or insulating may *not* be the determining factor in deciding if your sample is at risk to charging. To understand this, consider that the following examples:

- Non-conductive sample: If your sample has a large surface area (e.g. a glass slide), you may be able to image the surface without charging since the electrons have space to spread out. Conversely you could have a small fragment of the same glass slide you were able to image, and now it may show charging simply because the charges cannot spread out as much.
- Conductive sample: When imaging a bulk gold surface, you typically shouldn't see any charging. However, when imaging gold nanoparticles you may see effects of charging. Even though the particles are conductive, the electrons still have to make their way to electrical ground. In clumps of nanoparticles there are only very small areas where the particle touch each other and the sample stage. Therefore the total effective conductivity of the sample may be very low.

In short, sample geometry is an important consideration in understanding why your samples may charge in some forms and not others.

Sample contamination

Sample contamination and residues from handling (e.g. oil and fingerprints) or processing (e.g. photoresist) may limit your ability to obtain optimal images. Always ensure your sample is as clean as possible. Always handle samples with gloves / tweezers.

Sample preparation

Tip Ensure your sample is securely connected (mechanically and electrically) to the stub and stage. Additionally, if you have lots of charging issues consider sputter coating the sample with metal.

The more resistance and capacitance between the sample and stub/stage the more you may see charging artifacts in your image. Therefore, a poorly mounted metal specimen may still charge and metal on an insulating substrate is at an even higher risk to charge. It is important that there is a good connection between the sample and the stub with carbon tape. Carbon tape is not the only way to mount samples, but often it is used because it is quick and easy. If you are having problems with a conductive sample, the first step to troubleshoot is to check that it is mounted securely.

Even though you could potentially image non-conductive samples successfully (e.g. the surface of a glass slide) there will be instances where it will still be extremely challenging even after following all the advice in this section. In those cases it is best to have a sacrificial sample that you can coat with a thin layer of metal. The purpose of the thin layer of metal is to allow the electrons to be mobile and reach ground. Often you will also have to put an additional connection with carbon tape from the top to the stub (as shown in Fig 4.16, since the metal coating process cannot physically conformally coat high aspect ratio features (like the edge of a silicon chip). This is also the reason why you still may have charging problems on non-conductive particles (μm sized) that were coated in metal (since the metal coating may not completely connect the surface of the particle to the stub). In those instances, other sample preparations may be required such as partially embedding the particles in conductive paste (silver paint).



Figure 4.16: Coated sample with an additional piece of carbon tape electrically connecting the top surface of the sample to the stub (ground).

Beam voltage (keV)

Tip Try higher and lower voltages to determine which works best on your sample. For example if you normally image with 5 keV then try 1 and 10 keV or anywhere inbetween for a trial and error approach.

You may have heard that using lower voltage ($\sim 1 \text{ keV}$) to image non-conductive samples is better. However, the choice of voltage is a more nuanced consideration than simply ‘going lower’ and depends

on the structure of your sample. Low voltage generally will give you better surface sensitivity and therefore potentially allow you to see more details / high resolution of your surface. However with reference to charging, the lower voltage also means the beam has less penetrating power to go deep into your sample. As an example, in the case where you have a thick oxide layer on your sample (maybe 1 μm) using low voltage may mean the electrons mainly get stuck in the oxide layer and contribute more to charging. Whereas using a higher voltage on this sample will mean many of the electrons will penetrate deeper and go to ground - not getting stuck in the oxide layer and therefore potentially not contributing to charging. As another example, a porous or scaffold structure may show the same problems. Lower voltages may allow the electrons to get stuck in the structure and contribute to charging. An intermediate or higher voltage will allow the beam to interact with the sample, but not embed charges thereby minimizing the charging effect.

Beam current or aperture

Tip Lower the current as much as possible (or aperture/spot on some systems).

Charging problems become more pronounced at higher currents. This is because there are more charges to interact with the sample to potentially get stuck, and more charges in the beam that can be repelled from those charges. Additionally, how well your sample is grounded may, in part, be affected by the total current incident on the sample. Your sample may not be connected as securely as possible so that the sample-stub connection behaves like a capacitor. This may be okay at some levels of current, but at high currents (or even normal operating currents) the sample may misbehave. Therefore a simple technique is to lower the beam current as much as possible. In some systems this is as simple as directly changing the current. In other systems you may have to decrease the aperture or spot (lower aperture or spot sizes will limit the beam size and therefore current). Note that this has the side effect of lowering the total imaging signal as well (will decrease signal to noise ratio in your images). So use your judgement when lowering or adjusting current.

Charging can be dynamic

Tip If charging is subtle like only slight drifting - observe an area for an extended period of time (few minutes) to see if the drifting subsides.

For some samples and preparations charging may be dynamic. To say this another way, sometimes the charging issues (like drifting) can reach an equilibrium if you view a particular region for a long enough time. Physically this is when the total charges hitting the sample from the incoming beam reaches a balanced steady state with the electrons that are leaving the area through reaching ground. The reason why it takes some time, is analogous to charging a capacitor. There is no way to force this condition, it is entirely luck-based or sample specific.

Choice of Detector

Tip Use a backscatter electron detector (e.g. T1) rather than a secondary electron detector (e.g. T2 or InLens).

Even if charging is physically occurring, some detectors are blind to it. Backscatter electron detectors are most sensitive to high energy electrons. These electrons are very fast and are therefore less affected by sample charging (less interaction time). Conversely, secondary electron detectors are most sensitive to low energy (slow) electrons, and therefore show the effects of charging more dramatically. Try different detectors to see how the sample looks. The standard (ETD) detector can also be configured to filter away the low energy electrons.

Image Acquisition settings

Tip Use fast dwell times and frame integration to capture an image rather than slow dwell times. Additionally you could lower the capture resolution and/or magnification.

Sometimes during live navigation an image will look perfect, but when you scan slower charging artifacts suddenly appear. Also, sometimes the image will look great at a certain level of magnification and then at a higher level of magnification begins to charge. Both of these effects are due to the total integrated current per area. To minimize charging, it is critical to minimize the total integrated current (or total charge calculated as current \times time). This is, in part, why lowering the current in general will help. However, there is much more we can do in the software about controlling how the beam scans the sample to account for this.

The main scan parameters when capturing an image in an SEM are: digital resolution, dwell time (scan speed), and integration/averaging (full frame or line). The current in the beam only defines the magnitude of the current incident per pixel. Whereas the scan settings describe how many pixels are being scanned per area (i.e. the frame area) and how long the beam is on each pixel.

- Digital Resolution: defines how the area is binned. A typical digital resolution on the SEM may be 1536×1024 . This means a total of 1572864 pixels are being scanned with the beam. Selecting half the resolution would be 768×512 resulting in a total of 393216 being scanned. Halving the resolution therefore quarters the number of pixels being scanned and therefore the total integrated current for the image will also be quartered. The same result can be achieved at 1536×1024 resolution by halving the microscope magnification (i.e. doubling the field of view or viewing $4 \times$ area).
- Dwell time or Scan speed: defines how long the beam spends on each pixel. A typical dwell time for a live view is 300 ns per pixel. Whereas to get a high signal image you may wish to scan as long as 10 μ s per pixel. In combination with the digital resolution, this will define the total integrated current per frame or the total charge that interacts with the sample area per image. Lowering the acquisition dwell time will result in scanning much faster, minimizing the integrated current and perhaps avoid charging. However, this has the downside of capturing an image with much higher noise. This can be compensated for by averaging/integrating.
- Line integration / average: defines how many times each line is scanned in the image before moving onto the next. Generally, when you have charging issues you want to avoid scanning the same area for a long time. Therefore you want to make sure this setting is off or set to 1.
- Frame integration / average: defines how many frames will be added together to form the entire image. Having a > 1 frame integration with low dwell times is a great way to minimize charging by scanning the regions as quickly as possible with as little integrated current per frame. Signal is gathered over the course of many frames meaning that any charge in a particular region has time to dissipate before getting scanned again (in direct contrast to the function of line integration). Note that the more frames you average the more likely some small misalignments can occur when the software stacks the images together. This can result in an image that has poorer spatial resolution than a single frame.

4.10 Introduction to Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy (EDX/EDS) is a microanalysis technique that sorts out the x-ray emissions from elements in a sample based on their energy. Since atoms have distinct energy levels, the x-ray spectra shows discrete peaks which can be attributed to particular elements. EDX has proven to be a very useful tool in the research and development field of many science/engineering disciplines when trying to observe chemical composition of your sample. EDX in an SEM is an *experiment*. Therefore it's important to understand the underlying physical processes in order to connect how the SEM beam parameters will impact your data.

X-ray emission

The electron beam of the SEM acts as the excitation source to liberate bound electrons in the sample material. The electronic vacancies in the atoms allow higher energy electrons to drop down into the lower energy level thereby emitting a photon of energy. These photons are typically in the keV energy range and are therefore classed as X-rays.

The beam penetrates the sample surface and interacts over a volume that may be many μm^3 in size depending on the beam energy (keV) and material properties. This region is known as the **interaction volume**. X-rays are generated deep within this interaction volume. For this reason EDX is not strictly a thin film analysis technique. Materials of interest $< 1 \mu\text{m}$ in thickness may pose difficulties in gathering enough signal. Chiefly, the choice of **beam energy (keV)** depends on the elements you suspect are present within your sample, as your goal is to use the electron beam to excite the electronic transitions of the material. You can check the EDX software, paper posters/references in the lab, or online resources to determine transition energies of the elements. One rule of thumb is to use a beam energy 5 – 10 keV higher than the transition to ensure you can excite it. For the optimum beam energy you can refer to *ionization cross-section calculations* but generally this isn't necessary for most experiments.

The naming conventions of the X-rays pertain to the interatomic levels that the electron transitions from and to. The most inner atomic shell ($n = 1$) is called *K*, the next shell ($n = 2$) is *L*, and the next shell ($n = 3$) is *M*. These three shells are the most typical shells you will encounter when performing EDX. A transition from $L \rightarrow K$ is called the $K\alpha$ whereas $M \rightarrow K$ is called the $K\beta$ etc. The $K\alpha$ is a highly distinct transition due to its high energy and probability of occurring (especially in light elements). If possible, you will want to excite this transition for positive element identification. Transitions between other shells like *M* and *L* lead to families of spectral lines which can overlap and be difficult to interpret if your sample contains many elements. However, these lines may be the only accessible transitions in heavy elements since the maximum beam energy of the SEM is 30 keV.

An important note is that decelerating electrons also emit X-rays (Bremsstrahlung). These X-rays form a continuous background signal (noise) in your experiments. Even if you do not excite specific x-ray transitions you will still generally see counts from Bremsstrahlung. This is why careful selection of the beam energy is important, you want the majority of the counts to come from transitions.

Signal processing

Exciting the electronic transitions is only one half of the experiment. The other is the signal processing that results in the x-ray spectra. To ensure the x-ray detector collects enough signal you will typically need to adjust the SEM parameters to allow a higher current to be incident on the sample by increasing one or all of the following (depending on the SEM): **spot**, **aperture**, and/or **current**. This increased current (i.e. more electrons) on the sample correlates to an increased emission of X-rays that can be collected by the detector measured in **counts per second (cps)**. This standard operating procedure will have a guide of how many counts is ideal - typically in the thousands (kcps). In principle, experiments

can be run at any cps but with a low number the signal to noise would be so low that you wouldn't be able to see a meaningful spectra. As noted above, although increasing the beam energy (keV) may show an increased number of counts on the software, those counts are not necessarily useful as they may just be from the background Bremsstrahlung. Conversely, there is an upper limit of the throughput of the signal processing system known as **dead time**. In other words, the system can only handle a certain amount of counts before the detector saturates and information is lost. Typically this is in the > 40% dead time range but the software will warn you when the dead time is too high by having this parameter flash on the software.

Resultant spectra

Generally the resulting spectra will show many peaks which will be either identified by the software automatically or manually from user input. Fig 4.17 shows an example spectrum. You are able to select the region over which to perform the experiment by using the SEM to navigate to the specific region and magnification of interest. In this way, the spectrum will be collected over a relatively large area. The X-ray counts could therefore come from any region in the sample in view. You can also be selective and choose a smaller region or point to designate an area of interest more specifically.

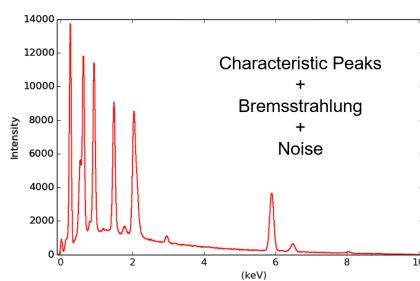


Figure 4.17: Example spectrum showing discrete peaks from specific electron transitions within the atoms, a large Bremsstrahlung background signal, and other noise components (e.g. electronic noise in the detection system).

Mapping

If you want to get spatially descriptive information, then there is also a mapping option. The output of mapping is not a single spectrum, but multiple images of x-ray counts for each element able to be detected. The maps identify what regions of your sample has higher concentrations of elements. Note: Just like regular EDX, mapping is an experiment and there are some more considerations you need to be aware of.

As the sample is getting scanned, the x-ray counts are sorted and binned per pixel. This means instead of all the counts getting added to a single spectrum, the signal is spread out amongst the map. In essence, the counts per pixel is extremely important for high quality maps that show good contrast between regions. This is controlled by the **digital or capture resolution**. Selecting a low capture resolution will increase counts per pixel and increase contrast of the map significantly. Choosing a high capture resolution will spread your counts into a much larger number of bins (pixels) often making a very poor map unless you wait an extremely long time for signal to accumulate. An example of this difference is shown in Fig. 4.18. It is generally desired to have a high resulting spatial resolution of the map, but this seems in conflict with the previous discussion. The achievable resolution of the map is dependant on the SEM beam parameters - at worst this is roughly the size of the interaction

volume ($\sim \mu\text{m}$) and at best this is $\sim\text{nm}$. The capture resolution of the map does not determine the spatial resolution achieved (i.e. if you are viewing a highly magnified area of the sample, choosing a high capture resolution may not be of benefit as you may be over-sampling). To achieve the best maps you want to balance the magnification of the SEM and the digital capture resolution of the EDX map. Higher quality maps are achieved with lower capture resolution on a highly magnified area since this is how you will achieve maximum contrast in the maps.

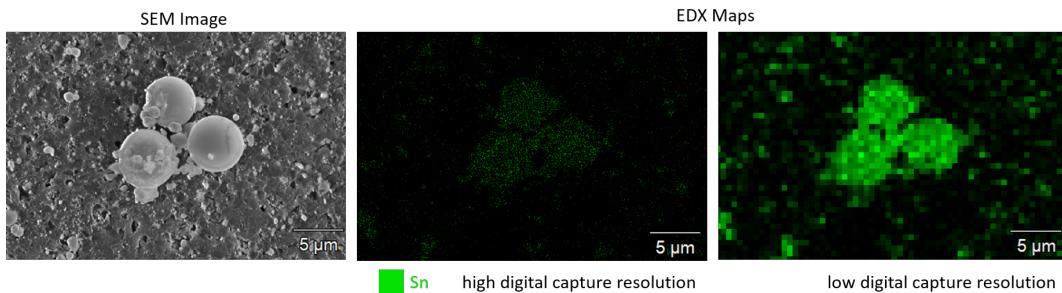


Figure 4.18: Choosing a lower digital capture resolution results in a better map due to higher contrast (more counts per pixel)

Just like imaging, the **shadowing** effect can occur in EDX maps as well since the X-ray detector is also at an angle. If you have a sample with high aspect ratio features, highly topographical surfaces, or large features the X-rays can be blocked from ever reaching the detector (shadowing). This can give the false impression that there is not a specific element present in certain regions of the sample. It is important that you understand where the X-ray detector is with reference to your sample. Fig. 4.19 shows an example of shadowing occurring for a gold bond. The bond is partially blocked by a large silicon chip - therefore in some regions the X-rays cannot reach the detector. This can be remedied by rotating the sample to face the detector with minimal obstructions.

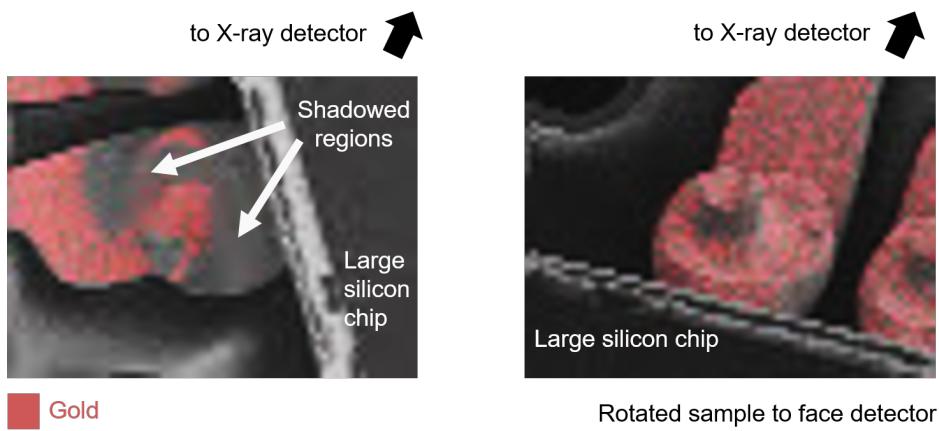


Figure 4.19: Two example X-ray maps of gold. Left: Regions appear to be missing gold because the large structure occludes the X-rays from reaching the detector. Right: Rotated sample physically to face detector so that there is minimal shadowing.

Experimental limitations, tips, and suggestions

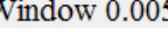
- The quality of the SEM image won't affect the spectra strongly, however for best signal you should still try to focus and stigmatize accordingly.
- EDX cannot detect elements with $A < 5$.
- EDX is not a thin film analysis technique. Samples < 100 nm thick may be impossible to detect, and < 1000 nm thick may be challenging.
- If possible excite the $K\alpha$ for positive element identification. Even better if you can identify more than one peak per element.
- Use a beam energy $\sim 5 - 10$ keV higher than the transition you wish to excite.
- Use max beam energy (30 keV) if you have very thick or bulk samples.
- Use lower energies if you are interested in EDX of your sample surface.
- For higher mapping resolution use lower keV when possible.
- Balance the digital mapping resolution with SEM magnification. Lower digital resolutions will allow your map to have high contrast.
- The x-ray detector is mounted at an angle, so shadowing in the x-ray map can occur. Rotate the sample physically to compensate or take multiple maps at different angles.

4.11 EDX - Iridium Ultra Software

1. Complete steps 1 through 14 in Section 4.6 to find area of interest.
2. Ensure sample stage is at ~ 8 mm working distance (WD). Use the green horizontal ‘STEM’ line as a guide for the WD.
3. Open ‘Iridium Ultra’ EDX Software on second monitor: .
4. Select desired beam energy (KeV) for your analysis in SEM software.

Important Do not set EHT above 25 keV directly. You must ramp from 25 keV at 1 keV every 30 s to a maximum of 30 keV. For example, if your desired EHT is 27 keV, first set to 25 keV, wait 30 seconds, then set to 26 keV, wait 30 more seconds, and finally to 27 keV.

Tip First, for the best element identification it is recommended to use a beam energy of 5 – 10 KeV higher than the $k\alpha$ energy. Second, for larger elements you can use the $L\alpha$ and $M\alpha$ lines as well, but note that there is a higher chance for element misidentification using these lines since many elements have overlapping or closely spaced low energy electron transitions. Third, higher beam energy means larger electron interaction volumes. In other words, the higher the energy the deeper electrons will probe into the bulk of your sample. Use lower energies if you are interested in EDX of your sample surface.

5. With the beam scanning your sample in focus, increase aperture size in the ‘Apertures’ tab to achieve acceptable x-ray counts per second (cps). You can select between 45, 60, and 120 μm . A stored rate between 5 and 15 kcps is a good number for general applications, e.g. .
6. Finely improve beam imaging conditions: Focus, stigmation, and direct adjustments (step 14 in Section 4.6). Note that your image may not be as crisp because the high counts required for EDX means you are using apertures and currents not optimized for imaging.
7. Ensure viewer button is selected to show interactive help panel on the left: .
8. Select desired EDX data collection mode from the following:
 -  **Work with Spectra:** X-rays from entire frame with no spatial discrimination.
 - Click ‘Review Spectra Properties’ to open new data acquisition window and adjust settings.
 - To gather data click:  ‘Acquire’ or ‘Acquire a spectrum’ from the help panel.
 - To adjust settings prior to data acquisition click ‘Review Spectra Properties’: 
 -  **Work with X-ray Maps:** X-ray mapping.
 - Click ‘Review Map Properties’ to open new data acquisition window and adjust settings.
 - To gather data click:  ‘Acquire’ or ‘Acquire Map’ from the help panel.
 - To adjust settings prior to data acquisition click ‘Review map properties’: 
9. Data Exporting
 - To export the raw data click export: . Most options will be image formats. Select .emsA (.emsA files are readable by ‘notepad’ software). You will notice there is only one column, this is the counts (y-axis) at each energy. You will have to reconstruct the energy scale (x-axis) from the header of the .emsA file. Specifically, the number of data points e.g. #NPOINTS: 4096 and energy step (in eV) e.g. #XPERCHAN: 10. The spectra data range is shown below the spectrum: . This means the x-axis starts at 5

- eV increases in 10 eV increments up to 40.955 keV (i.e. 4096 data points).
- You can also choose to save your data as an image.
10. When complete, close the EDX software and return system to starting configuration following steps 19 to 25 in Section 4.6. Ensure the aperture is set back to 30 μm (default).

4.12 Hardware

Everything you need to mount and store samples is available from Ted Pella, Inc.

- **Sample Storage** (http://www.tedpella.com/storage-boxes-bags_html/sem-mount-storage-boxes.htm) e.g.
 - 16709 (PELCO 18 SEM Pin Mount Storage holder)
 - 16795 (PELCO X-TREME 10 SEM Mount Box)
- **Stubs/Mounts** (http://www.tedpella.com/SEM_html/SEMpinmount.htm) e.g.
 - 16111 (Standard SEM Pin Stub Mount, Ø12.7mm)
 - 16119 (Large SEM Pin Stub Mount, Ø18mm)
 - 16104-9 (Low profile 45° / 90° SEM Mount)
 - 16354 (Double 90° SEM Mount Ø25mm)
- **Tweezers** (http://www.tedpella.com/SEMmisc_html/SEMgripper.htm#pin_stub) e.g.
 - 1664 (SEM Pin Stub Mount Gripper Tweezers)
- **Conductive tape** (https://www.tedpella.com/semmisc_html/semadhes.htm) e.g.
 - 16073 (Copper Tape, 6.3 mm wide)
 - 16073 (Carbon Tape, 8 mm wide)
- **TEM**
 - Lift-out grids e.g 460-203
(http://www.tedpella.com/grids_html/4510half.htm)
 - Lift-out grid holder e.g. 15465
(http://www.tedpella.com/SEMmod_html/SEMspecl.htm)
 - Grid storage (recommended) e.g. 139-50
(https://www.tedpella.com/storage-boxes-bags_html/membrane-boxes.htm)

4.13 Zeiss Sigma - One sheet

4.13.1 PC and Software

SmartSEM software credentials:

- Username: user
- Password: nano3zeiss

If there is a problem with the user interface/software, feel free to close and then reopen the software. Close the software and the ‘EM Server’. Then click on the *Smart SEM* Software: . Wait until the server is online (indicated by the full green bar) and then log into the software using the above credentials when prompted. If you cannot close the server, restart the PC.

Windows Account credentials:

- Username: ZeissUser
- Password: nano3zeiss

4.13.2 Sample Preparation

- Never touch samples/holders with bare hands. Always use gloves.
- Do not load samples of vastly different heights (beyond ~ 1 – 2 mm).
- Powders: use as little as possible on fresh carbon tape. Dust off excess over trash can.

4.13.3 Default Settings

- Low magnification.
- 3 – 5 keV.
- 30 μm aperture.
- 0° tilt.
- Noise Reduction Mode: Line Average with $N = 3$ in ‘Scanning’ tab of the ‘SEM Control’ panel.
- Ensure scan rotation is set to 0° or off. This setting is in the ‘Rotate/Tilt’ panel on the left. Ensure the Scan Rot box is unchecked: **Scan Rot**.

Important Do not set EHT above 25 keV directly. You must ramp from 25 keV at 1 keV every 30 s to a maximum of 30 keV.

4.13.4 Shutdown

- Return system to default condition and carefully lower stage away from pole piece
- Collect data from the data PC immediately. Nano3 is not responsible for your data
- Never connect USB sticks or other devices to the Zeiss PC
- Pump system after retrieving samples
- Complete paper log book entry
- Log out of FOM - your reservations are your responsibility