

Lab Report 2 - Electron Microscopy

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Abstract

In this work, we aim to explore the use of a Scanning Electron Microscope (SEM) and a Transmission Electron Microscope (TEM) to analyze samples at the nanometer scale. Using SEM imaging, topography and compositional differences were examined through the use of secondary electron, backscattered electron, and characteristic X-rays. TEM imaging was then used to observe gold nano islands at high resolutions using STEM-HAADF imaging, elemental mapping, and electron diffraction patterns to determine structural properties. Together, these techniques demonstrate how SEM and TEM provide complementary information about a sample at the nanoscale.

1 Background

Compared to Atomic Force Microscopy (AFM), the SEM and TEM share many more similarities in procedure and parameters. Below is a background analysis of the systems, their features, and parameters.

1.1 Source and Lenses

1.1.1 Thermionic and field emission sources

Both SEM and TEM begin by generating an electron beam used to probe the sample. The purpose of the source is to produce a stable beam with sufficient brightness and stability such that high-resolution images can be obtained. Two common types of electron sources are thermionic emission sources and field emission sources. In a thermionic emission source, electrons are produced by heating a filament until they gain enough energy to overcome the material's work function. Thermionic emitters produce a beam with lower brightness and a larger energy spread compared to field emission sources. Because the filament operates at high temperature, these sources require high vacuum conditions to prevent contamination and extend filament lifetime. Field emission sources instead rely on the quantum tunneling of electrons from a sharp metallic tip under a strong electric field. These sources produce beams with much higher brightness and improved spatial coherence, which allows for better image resolution. A commonly used source in modern electron microscopes is the Schottky field emission source, which combines thermal heating with field emission to improve beam stability while maintaining high intensity. Compared to thermionic emitters, field emission sources typically require ultra-high vacuum conditions but produce significantly sharper beams.

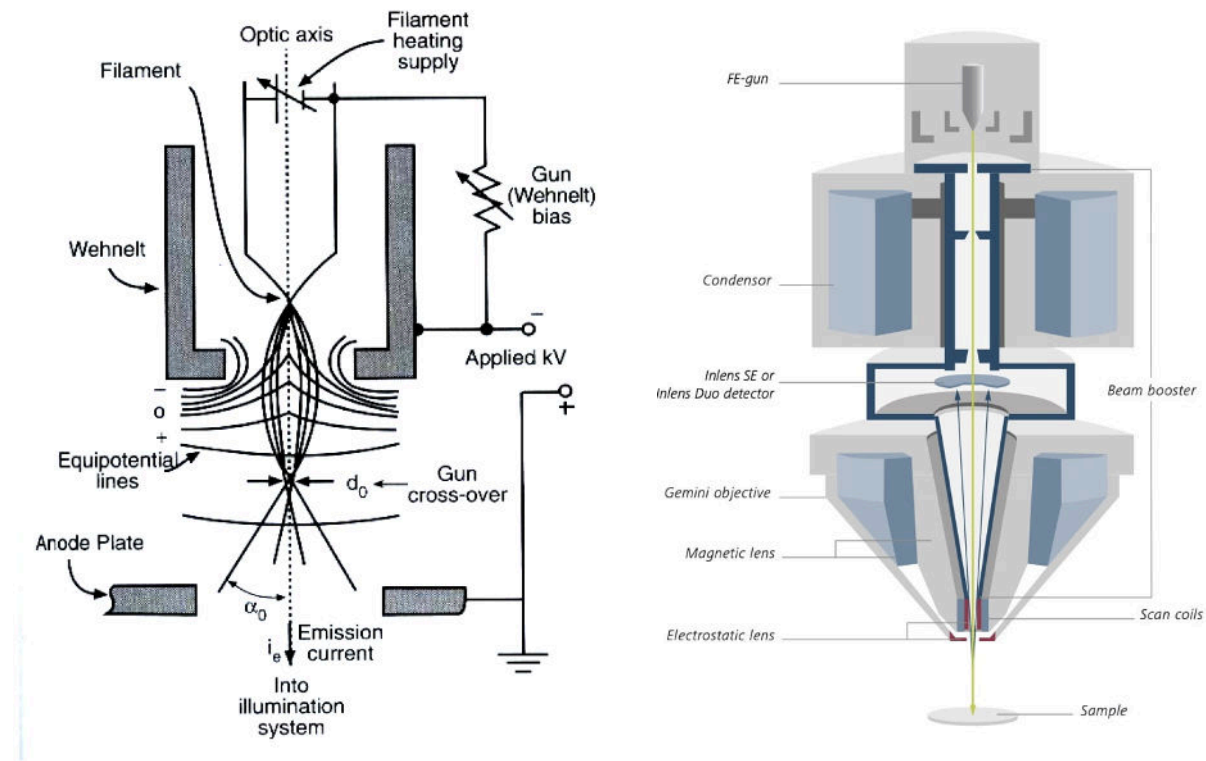


Figure 1: Thermionic source and field emission source.

1.1.2 Electron Lenses

After leaving the electron source, the beam passes through a series of electron lenses that control its shape and direction before it reaches the sample. Unlike optical microscopes, which use glass lenses, electron microscopes rely primarily on electromagnetic lenses to focus the beam. These lenses operate by passing current through a coil to generate a magnetic field that changes the trajectory of the electrons. This produces both a radial and a longitudinal force which cause the electrons traveling off-axis to move in curved paths toward a certain focal point. As one adjusts the lens current, the focal length of the lens changes as well allowing one to manipulate the magnification and beam convergence. However, such lenses are not perfect and introduce many types of aberrations that can limit the resolution of the image. One important limitation is spherical aberration, which occurs when electrons farther from the optical axis focus at different positions than those near the axis. Another is chromatic aberration, which results from differences in electron energy within the beam. Further, electron lenses are affected by astigmatism. This is where electrons traveling along perpendicular directions focus at different points. This results in stretching of the image

thereby reducing the sharpness and therefore must be corrected as one scans a sample. Because electrons behave as waves, the resolution of an electron microscope is also limited by diffraction, meaning that even with ideal lens conditions there is still a fundamental limit to image resolution. All of these various parameters play a role in the clarity and accuracy of one's scans; it is a matter of properly parameterizing and correcting for one to obtain successful images.

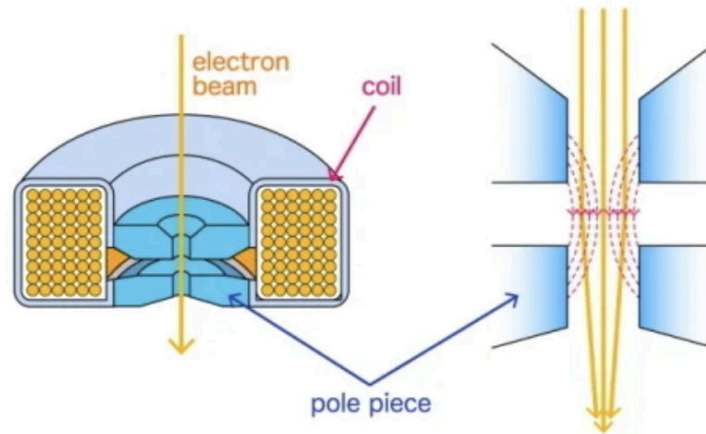


Figure 2: Electromagnetic lens

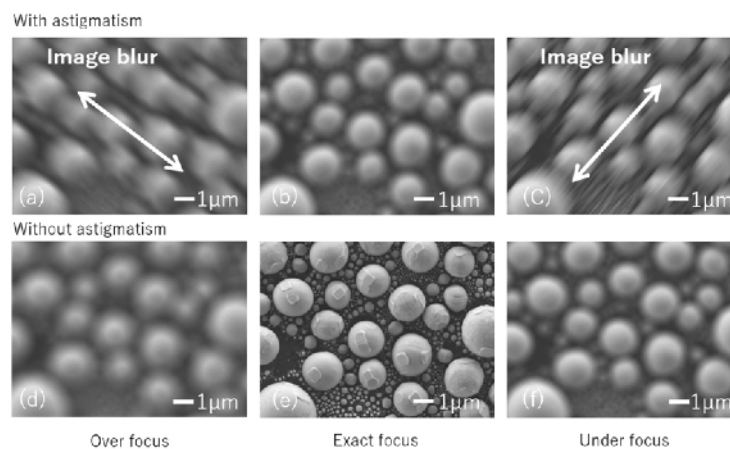


Figure 3: Astigmatism in SEM

1.2 Scanning Electron Microscope

SEM is a very versatile technique that uses a focused electron beam to acquire precise, high-resolution images that can be used to evaluate various aspects of a given sample. An ordinary

optical microscope does not obtain the resolution necessary to obtain such clear images on the nanoscale. With a SEM, the entire scanning area is traced using a scanning coil that allows the electron beam to probe the sample. By doing so, the electrons may interact with the sample to produce various signals. More specifically, in this lab we focus on the use of secondary and back scattered electrons, along with characteristic x-rays. For the electron beam to probe the sample, the sample must be of a conductive material or have a conductive coating. It is best to use a conductive sample since no sputtering is required, but on a good sample with a strong conductive coating, one can still acquire very clear images with the SEM. In this lab all samples were sputtered with gold. Materials such as gold and platinum are great coating options as they are very conductive and won't oxidize.

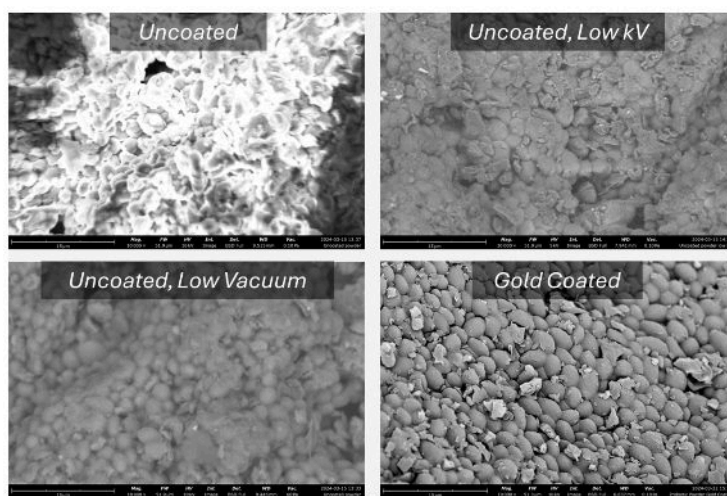


Figure 4: Example of importance of conductive coating

The signals picked up from the SEM system are a result of many parameters, most importantly the capacity to which the electrons interact with the sample and how deep into the sample a given electron can penetrate. This is most notably determined by the electron velocity and atomic number of the electron; the higher the energy of the electron, the further it will penetrate the sample. Similarly, smaller atomic weight allows for deeper penetration of the material.

The resolution of the image is proportional to the accelerating voltage, and further, the signal received is highly dependent of the electron velocity. As mentioned by Dr. O'Neil, the resolution of the image can decrease if spot size is too large the areas measured can overlap in the image. There are many parameterizations that can be done to improve this, but it is most important to keep in mind the scan-noise ratio as you conduct your scans. Astigmatism can occur during scanning in both the downward and diagonal directions. A notable approach is to always start at a low magnitude and move toward higher magnitudes; by repeating,

conducting correct line integration, and increasing the aperture one can drastically improve results. Further, there are five imaging settings on the SEM; 1-4 refer to scan speeds, and more specifically, rank from worst to best scan-noise ratio. The fifth setting is for frame integration. Frame integration is used to go over the same scanning area many times at a fast rate for improved image clarity; this is a very useful tool but will not work if there is drift occurring. Another way to improve resolution is to reduce the working distance between the lens' and sample; changing the working distance will not effect the magnification of the image, but it will reduce the depth of the field. All these techniques are important pieces of the recipe for better focus and stronger results.

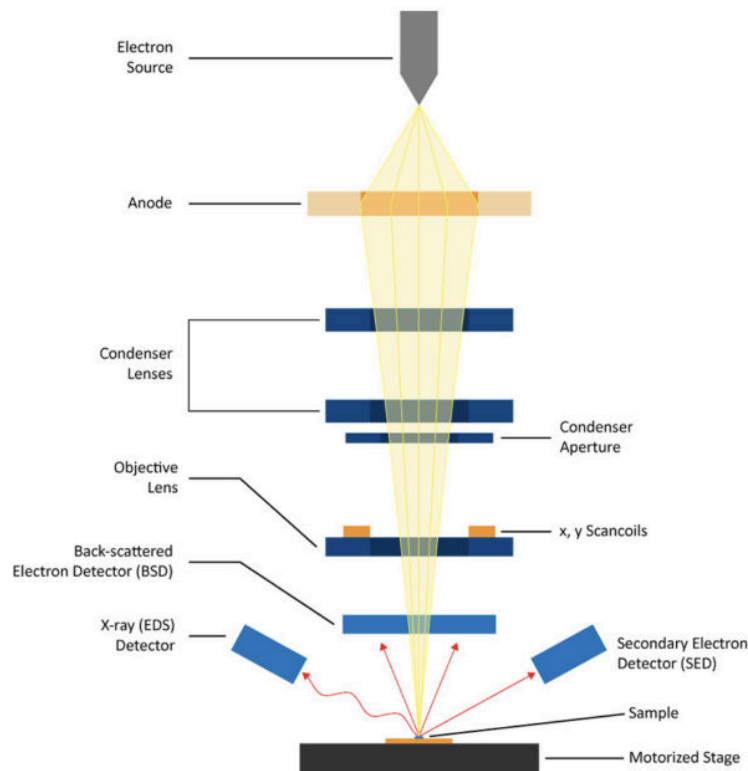


Figure 5: SEM Diagram with Detectors

1.3 SEM Detectors

1.3.1 SE2 Detectors

The first of the detectors in SEM is used to measure secondary electrons and is key in obtaining high resolution images of a given sample. Secondary electrons generate when an incident primary electron beam undergoes an inelastic scattering interaction with the electrons in the sample. As a result, very low energy secondary electrons emit from the sample surface and are detected by SE2 detector. The SE2 signal is highly sensitive to these

low energy electrons that come out of the surface of the sample, making it a very effective method for obtaining surface topography. The detector consists of a photomultiplier tube, light guide, scintillator, and Faraday cage. Due to the low energy of the electrons, when a positive bias is applied to the Faraday cage, these electrons are attracted and move toward the detector. This causes the said electrons to hit the scintillator which then converts the electrons to photons, passing through the light guide and photomultiplier tube, and creates the amplified signal used to form the resultant image.

The InLens detector, as mentioned earlier, is located inside the lens assembly; this allows it to collect electrons along the optical axis of the microscope column. The differing position of this detector provides a different view point to the SE2 detector. While the SE2 detector is a powerful tool, it is flawed and can create a shadow effect; this is due to the angle at which the detector is to the secondary electrons coming from the sample surface. The InLens detector can produce an improved image of the surface, and further, can work quite effectively when operating at a short working distance forming very high resolution surface image.

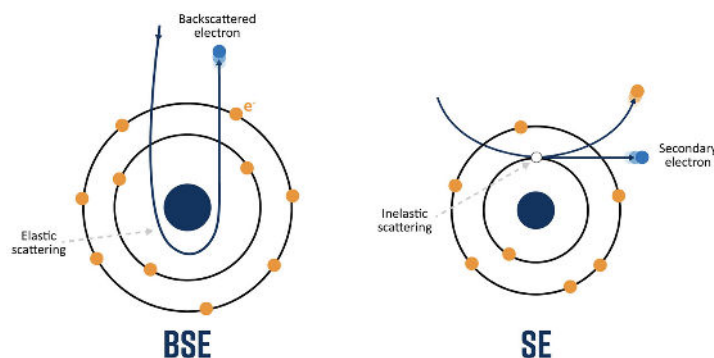


Figure 6: Secondary electrons vs Backscattering electrons at atomic level.

1.3.2 Backscattering Detector

The next detector used in SEM forms signals from the backscattered electrons to obtain information about both the composition and topography of the sample. Backscattered electrons are primary beam electrons that undergo elastic scattering interactions within the sample which are then redirected back toward the detector. Contrary to the secondary electrons, these electrons hold a relatively high energy and can therefore escape from much deeper regions within the sample (as opposed to just the surface).

Unlike secondary electrons, the signal intensity of backscattered electrons is strongly dependent on the atomic number of the electrons in the sample. As the atomic number increases, the probability of elastic scattering increases, resulting in a stronger signal. As a result, regions with heavier elements appear brighter in backscattering images. As seen later, this is very useful in determining the composition of a given sample.

These detectors are commonly arranged as multiple solid state detector segments positioned near the sample. Signals from the various segments are combined to generate images of different contrast. By selecting which detector segments contribute positively or negatively to the signal, different surface features can be formed for a given image.

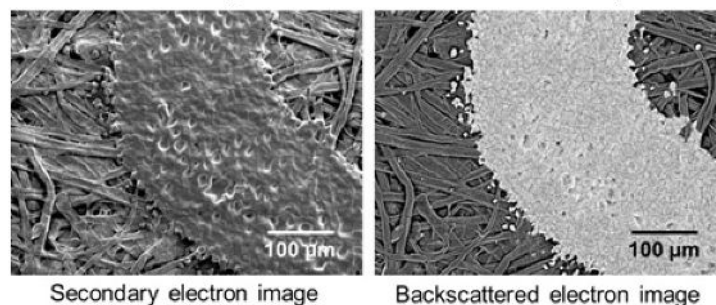


Figure 7: Secondary electrons vs Backscattering electrons at imaging level.

1.3.3 Energy Dispersive Spectrometer

The final detector used in SEM is the Energy Dispersive Spectrometer (EDS). The EDS measured characteristic x-rays produced when the incident electron beam interacts with atoms in the sample. These x-rays are formed when primary electrons from the beam undergo inelastic collisions with inner orbital electrons in the sample; this causes electrons from higher energy orbitals to fall into vacancies caused by the collisions. The x-rays with energies characteristic to a specific atom are emitted and can then be used to determine the elemental composition of a sample.

Because the energy difference between electron orbitals is unique for a given element, the detected x-ray energies can be used to identify the elemental composition of the sample. There is of course some error to this system, but unlike secondary electrons, the characteristic x-rays generate deeper within the sample due to the previously mentioned higher energies, allowing for subsurface compositional information to be acquired.

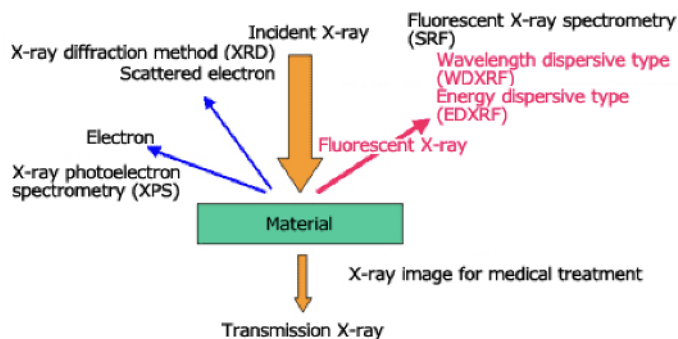


Figure 8: Characteristic X-rays in SEM.

1.4 Transmission Electron Microscope

While TEM and SEM have many similar specifications and parameters, the TEM operates as very similarly to an optical instrument. TEM uses electromagnetic lenses to form an image from the electrons transmitted through the sample. More specifically, TEM images are produced using electrons that pass through the sample, allowing information about the internal structures to be observed. As a result, it is required that the sample be ultra thin so that it is electron transparent and permits transmission for imaging.

TEM systems operate at significantly higher accelerating voltages than SEM. This allows electrons to penetrate the sample and thereby achieves a much higher resolution. Increasing accelerating voltage increases electron momentum and reduced De Broglie wavelengths of the electrons. These shorter wavelengths produce the improved image resolution.

In addition to direct imaging, TEM can also be used to obtain crystallographic information through electron diffraction. Because electrons exhibit a wave-like behavior, when interacting with atomic structures in the sample, the electrons diffract. These diffraction patterns formed in the TEM provide information regarding the spatial frequencies in the sample, and are therefore a very useful tool when characterizing lattice structures. For polycrystalline samples, diffraction most commonly appears as rings that correspond to sets of lattice planes with different orientations; these rings can be used to determine inter-planar spacing within the sample. As a result, TEM is useful in providing both high resolution imaging of internal sample structures and diffraction based characterization of crystal structures. Such fine resolution is what makes TEM stand out in comparison to SEM; its ability to resolve such fine structural features is a very valuable tool when observing materials on the nanoscale.

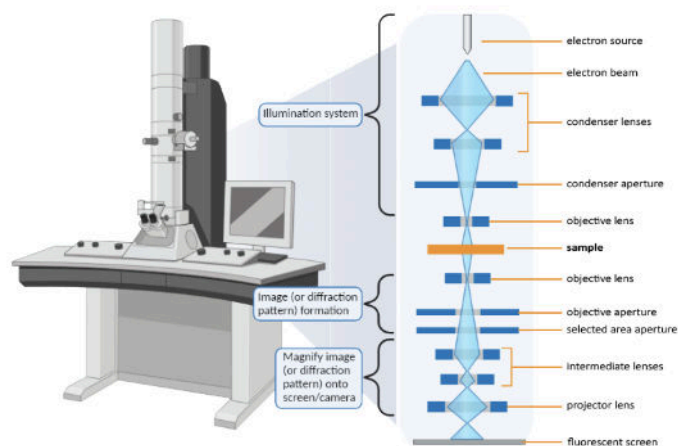


Figure 9: TEM Schematic

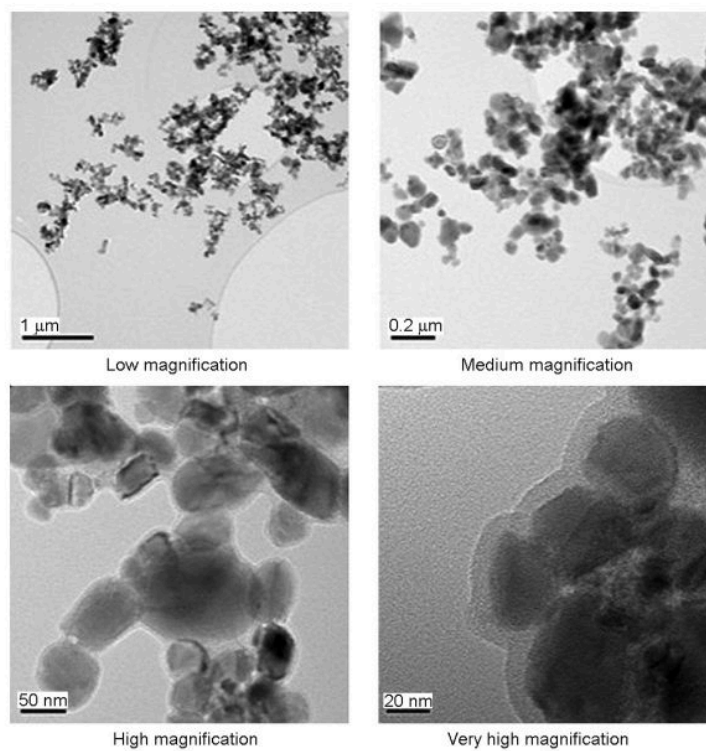


Figure 10: Effect of Magnification in TEM

2 Procedure

All imaging was conducted in Wilmot Building. The SEM used in this lab was the Zeiss Auriga Scanning Electron Microscope/Focused Ion Beam Tool. The TEM used in this lab was the FEI Tecnai F20 G2 Scanning Transmission Electron Microscope (S)TEM. All imaging was conducted by Dr. Sean O'Neil.

2.1 SEM Procedure

1. If the sample loading ante-chamber, located on the right side of the SEM, has not already been vented, vent the chamber before inserting the sample.
2. Attach the sample to the loading rod by threading it securely onto the sample holder mount.
3. Close the chamber door and pump down the ante-chamber until the required vacuum interlock condition is reached.
4. Use the loading arm to transfer the sample into the main chamber. Once the stage becomes visible in the chamber view, disconnect the loading rod and retract the arm.
5. Move the sample stage to position the sample beneath the objective lens.
6. Open the isolation valve and enable the electron beam under high-voltage operation.
7. Adjust the accelerating voltage to an appropriate value for the sample and imaging mode.
8. Translate the stage to locate a region of interest on the sample.
9. Adjust working distance, focus, and astigmatism to optimize image sharpness.
10. Adjust magnification and repeat focusing and stigmatization until satisfactory image quality is achieved. Save images as needed.
11. For topographic contrast using the backscattered electron detector, adjust detector segment polarity to emphasize surface features.
12. For compositional analysis, collect characteristic x-ray spectra using the EDS software acquisition tools.

2.2 TEM Procedure

1. Mount the sample onto the TEM sample holder and verify proper alignment and secure placement.
2. Confirm acceptable vacuum conditions using the column vacuum status display before inserting the holder.
3. Insert the holder into the loading ante-chamber and allow the system turbo pump to evacuate the chamber.
4. After the ante-chamber reaches operating vacuum, insert the holder fully into the microscope column.
5. Using the phosphor viewing screen, translate the stage to locate the desired sample region.
6. Adjust the z-position until the image remains stationary during stage tilt, indicating that the sample lies at the correct height.
7. Correct astigmatism using the condenser lens stigmators.
8. Adjust magnification and objective focus to refine image clarity.
9. Locate a feature of interest, reducing magnification if necessary to aid navigation and increasing magnification for detailed observation once the region is found.
10. Switch imaging from the phosphor screen to the CCD camera for digital acquisition.
11. Continue refining focus and sample positioning as required.
12. Insert the Fourier transform controls to further optimize focus and astigmatism when necessary.
13. Adjust focus carefully to balance image contrast and spatial resolution depending on imaging requirements.
14. Capture digital images once optimal imaging conditions are achieved.
15. To obtain diffraction information, switch from imaging mode to diffraction mode.
16. Select the appropriate diffraction aperture.
17. Insert the beam stop to block the transmitted central beam and observe the resulting diffraction pattern, which may appear as rings for polycrystalline samples.
18. Return to CCD imaging mode to record diffraction data.

3 SEM Results

3.1 Asbestos Sample

The first sample imaged on the SEM was asbestos plated with gold. The sample was made more than a few years ago. As a result, it is not the cleanest, most prime sample, but still produced strong results for the purposes of this lab. Three different images with various magnifications were taken, followed by two images taken after frame integration was preformed.

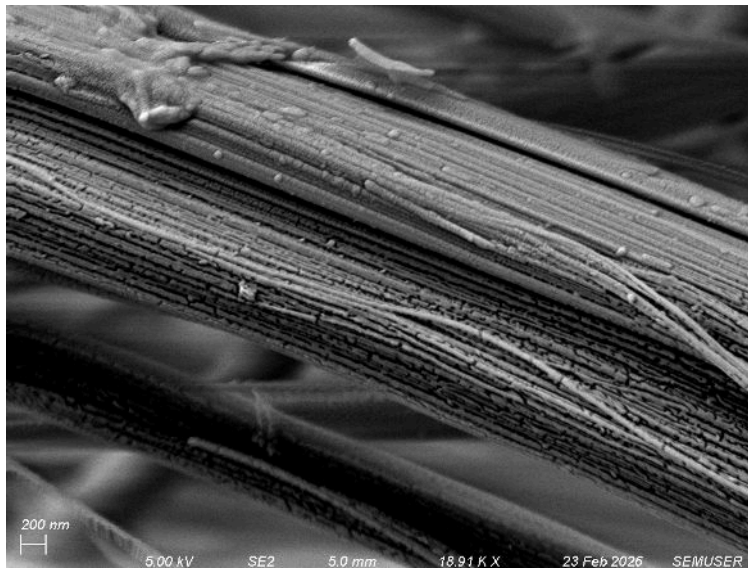


Figure 11: SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 5.0mm, and a magnification of 18910X.



Figure 12: SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 5.0mm, and a magnification of 5000X.

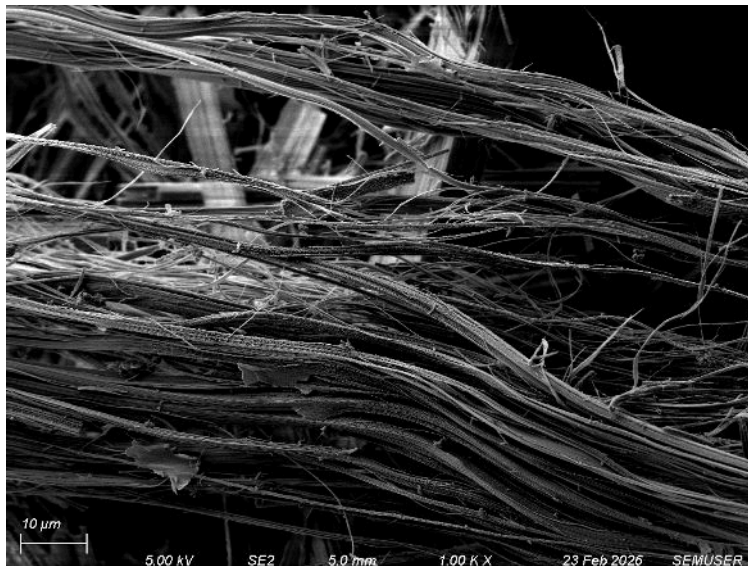


Figure 13: SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 5.0mm, and a magnification of 1000X.

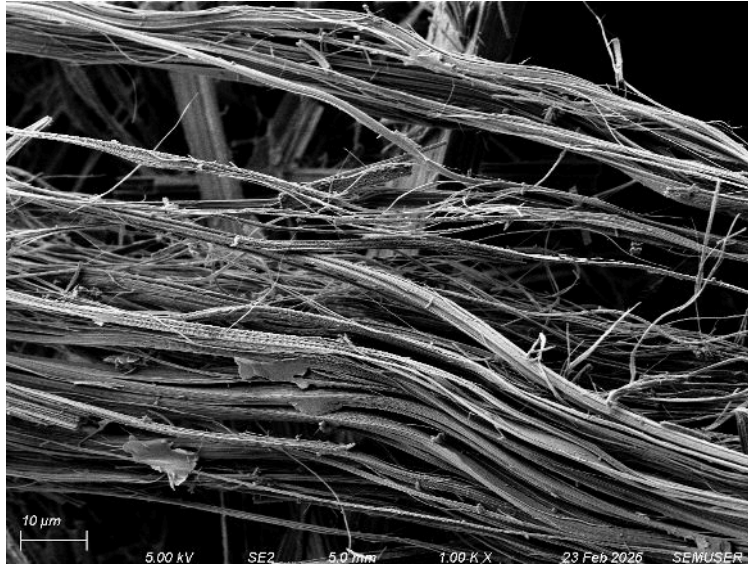


Figure 14: Frame-integrated SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 5.0mm, and a magnification of 1000X.



Figure 15: Frame-integrated SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 5.0mm, and a magnification of 100X.

From imaging, it is visible the SE2 detector emphasizes surface topography, allowing individual fibers and bundles to be clearly distinguished along with their alignment across the sample surface. At higher magnifications, strong shadowing effects caused by the interaction geometry between the electron beam and sample become visible; this produces the contrast variations that highlight height differences between overlapping fibers seen in the images. This shadow effect is a result of secondary electron emission from steep topographic features

in the sample.

3.2 Peacock Feather Sample

The following SEM images show the surface morphology of a peacock feather with gold coating sample examined at various magnifications using both the SE2 and InLens. The gold coating was applied prior to imaging to improve conductivity and reduce charging effects, thereby allowing for clearer observation of the sample. Lower magnification images highlight the overall arrangement of the feather structure, while higher magnification images reveal surface texture and nanoscale details. Figure 19 shows a contamination square formed during prolonged electron beam exposure, demonstrating beam-induced effects that can occur during SEM imaging. Together, these images illustrate how detector choice and magnification influence the level of structural detail visible in coated biological samples.

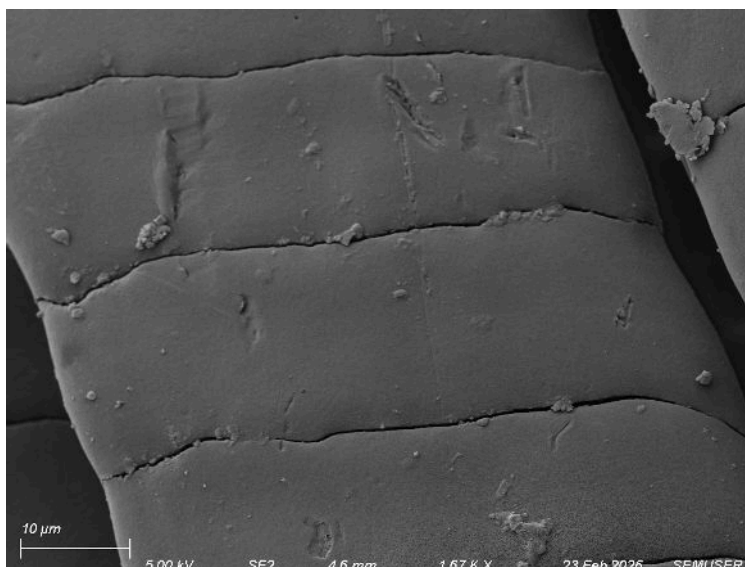


Figure 16: SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 1670X.

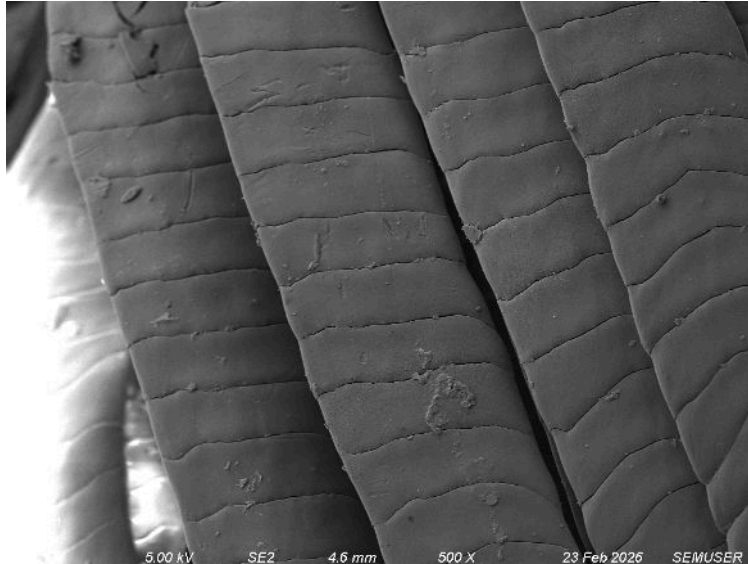


Figure 17: SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 500X.



Figure 18: SEM image collected using the SE2 detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 25X.

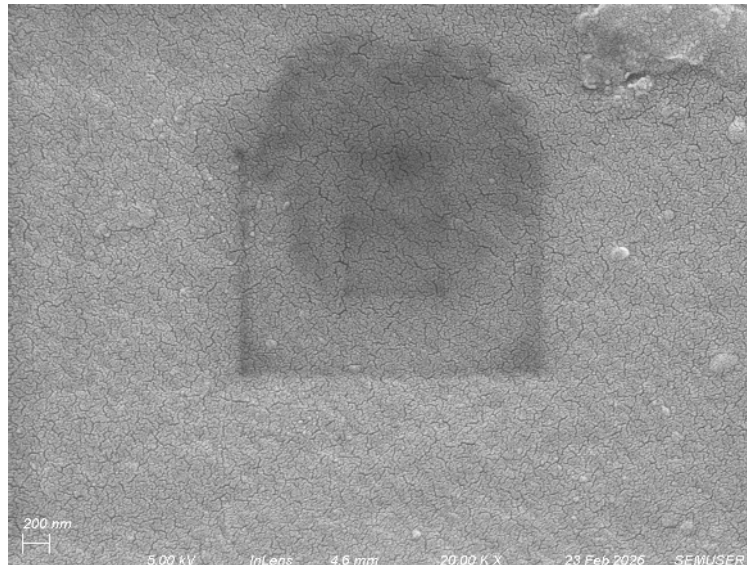


Figure 19: SEM image showing a contamination square formed after scanning, collected using the InLens detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 1670X.

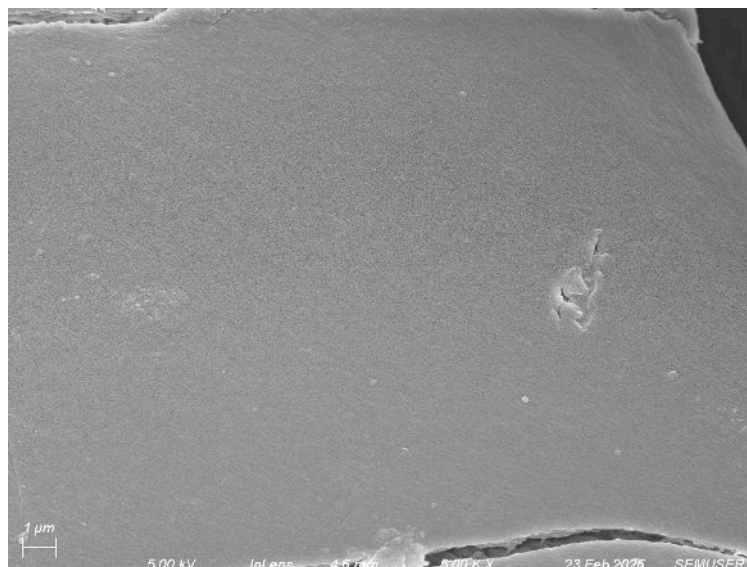


Figure 20: SEM image collected using the InLens detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 25X.

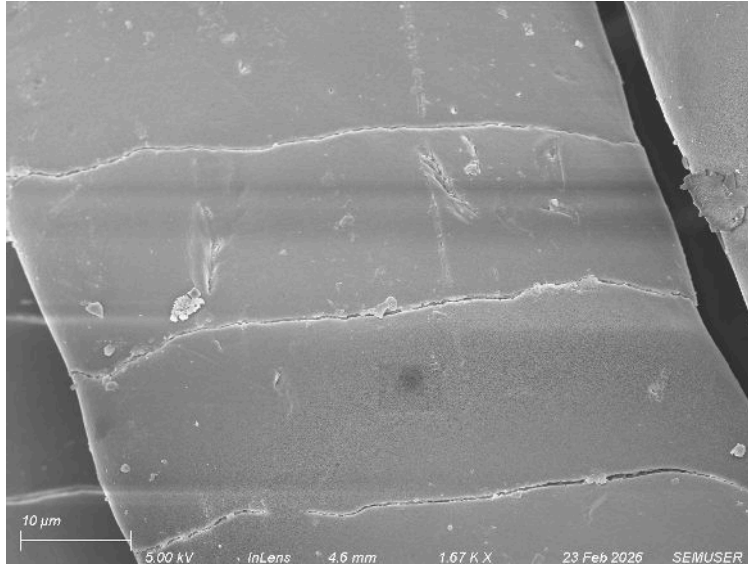


Figure 21: SEM image collected using the InLens detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 155210X.

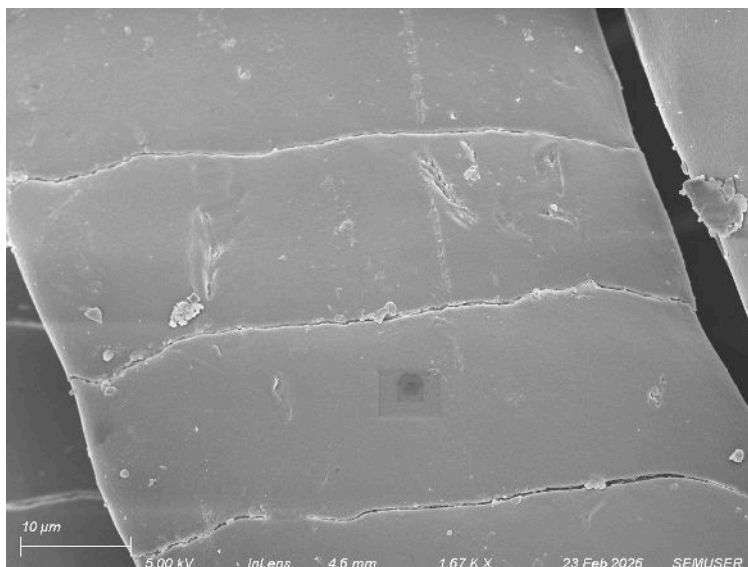


Figure 22: SEM image collected using the InLens detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 20000X.

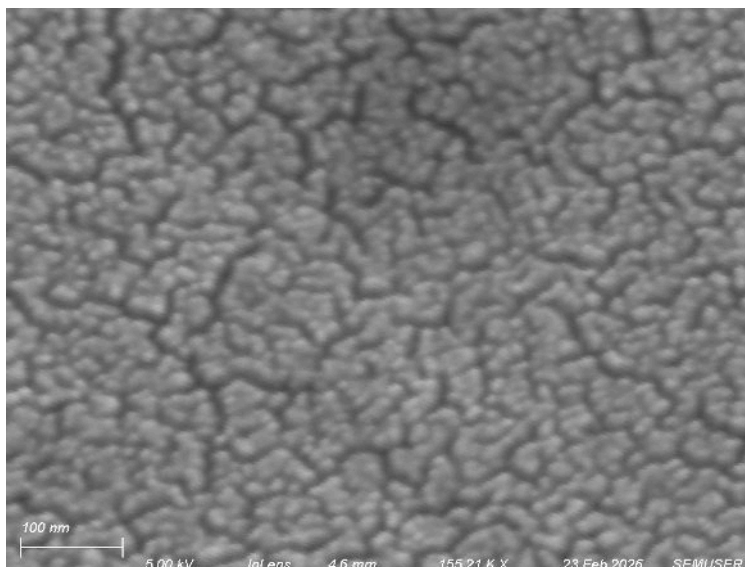


Figure 23: SEM image of the Au sputter coating using the InLens detector at an accelerating voltage of 5kV, a working distance of 4.6mm, and a magnification of 5000X.

Just as with the asbestos sample, the SE2 image reveals fine surface topography, including variations in texture and nanoscale features across the sample surface. With this sample we also had the opportunity to observe the effects of SEM scanning on a sample; more specifically, the visible contamination square is seen in Figure 19, representing the area we scanned for the previous images.

3.3 Geological Sample

The following images show a geological sample examined using both the SE2 and Backscattering detectors, along with EDS analysis for elemental characterization. The SE2 image was used to observe surface morphology, while the backscattering images emphasize compositional contrast between different mineral phases through differences in brightness. Higher magnification imaging through the use of the backscattering detectors was used to examine finer phase boundaries and localized regions of interest in greater detail. EDS mapping, the EDS spectrum, and the quantitative composition table were then used to identify the elemental distribution and relative abundance of the elements present in the sample.

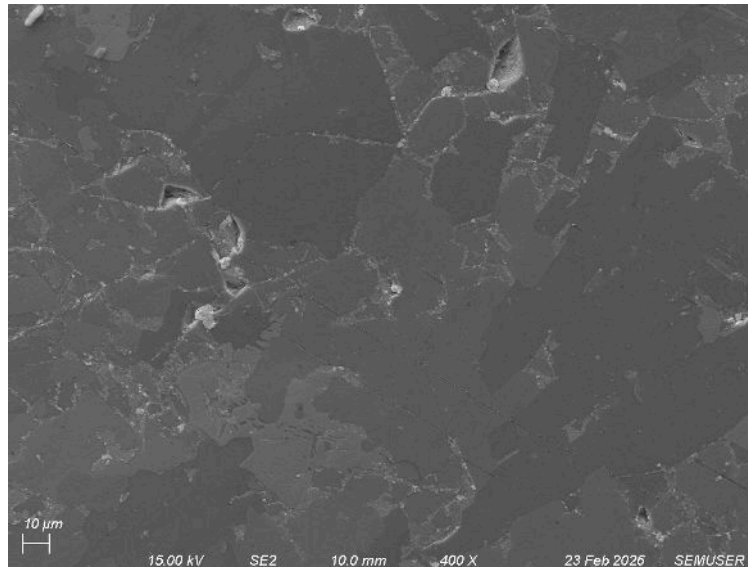


Figure 24: SEM image collected using the SE2 detector at an accelerating voltage of 15kV, a working distance of 10.0mm, and a magnification of 400X.

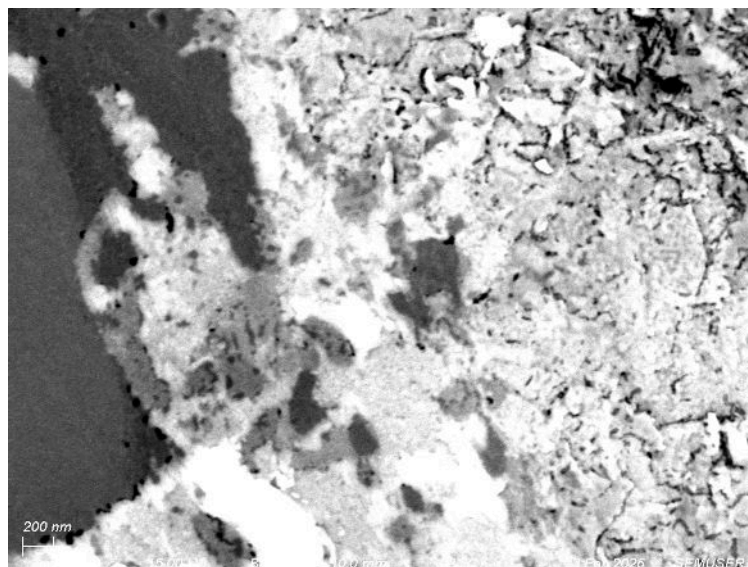


Figure 25: SEM image collected using the BSD detector at an accelerating voltage of 15kV, a working distance of 10.0mm, and a magnification of 400X.

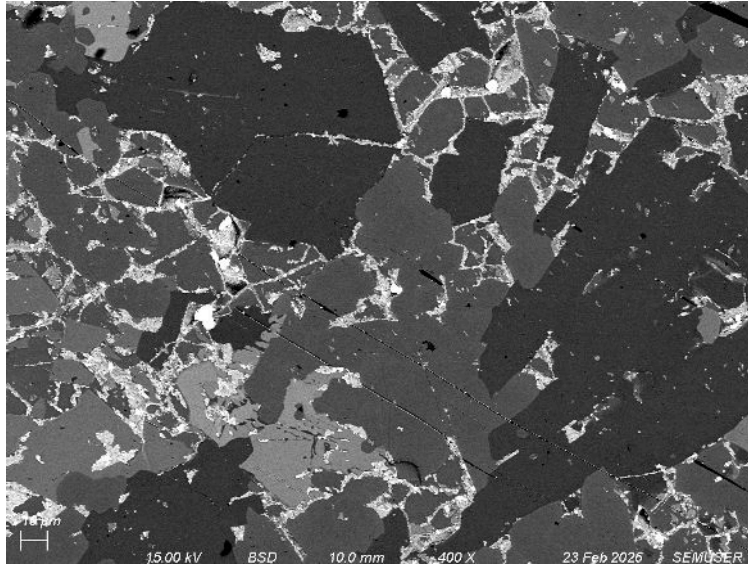


Figure 26: SEM image collected using the BSD detector at an accelerating voltage of 15kV, a working distance of 10.0mm, and a magnification of 3300X.

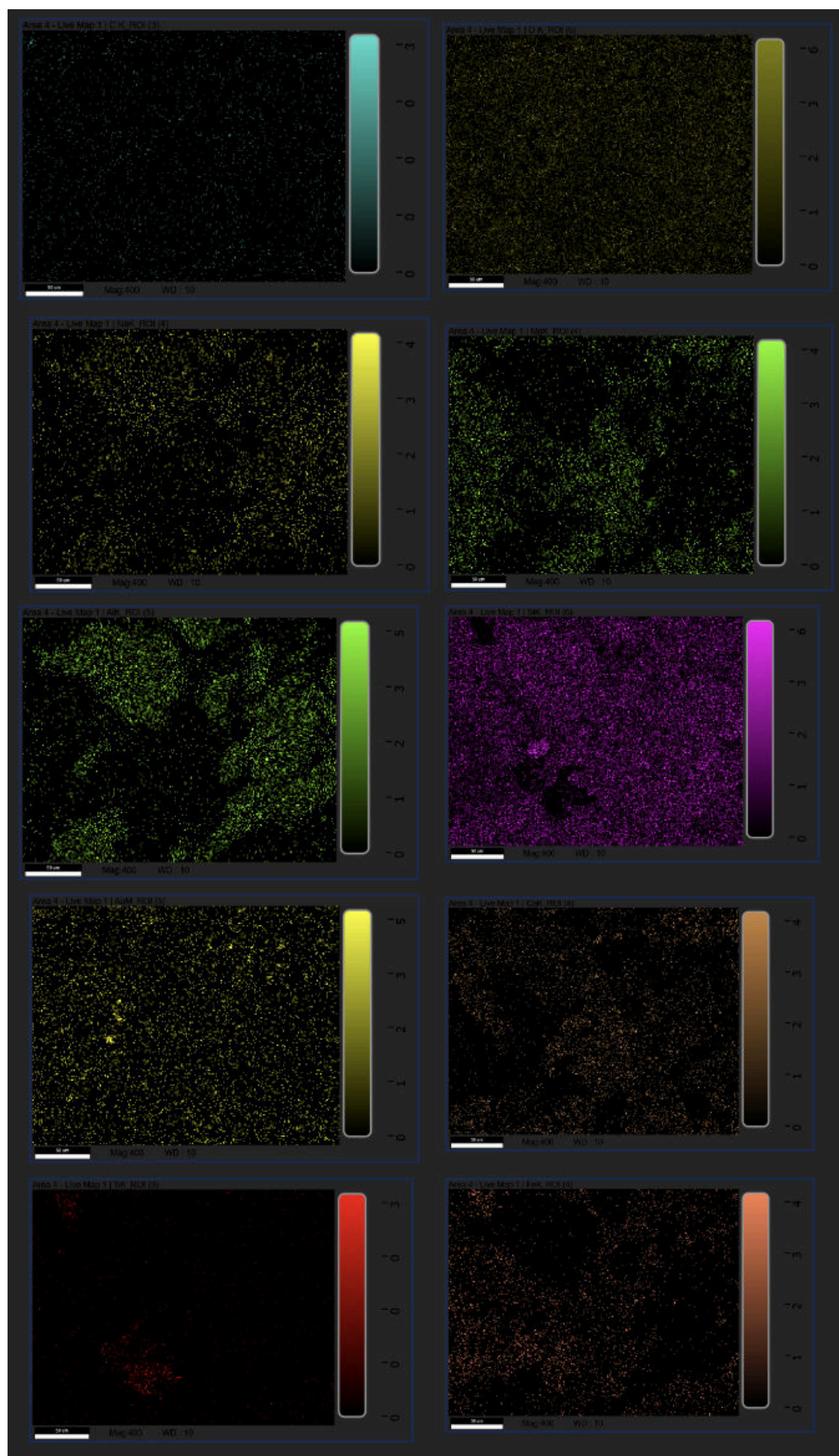


Figure 27: EDS elemental mapping, showing the spatial distribution of multiple elements across the selected region of interest.

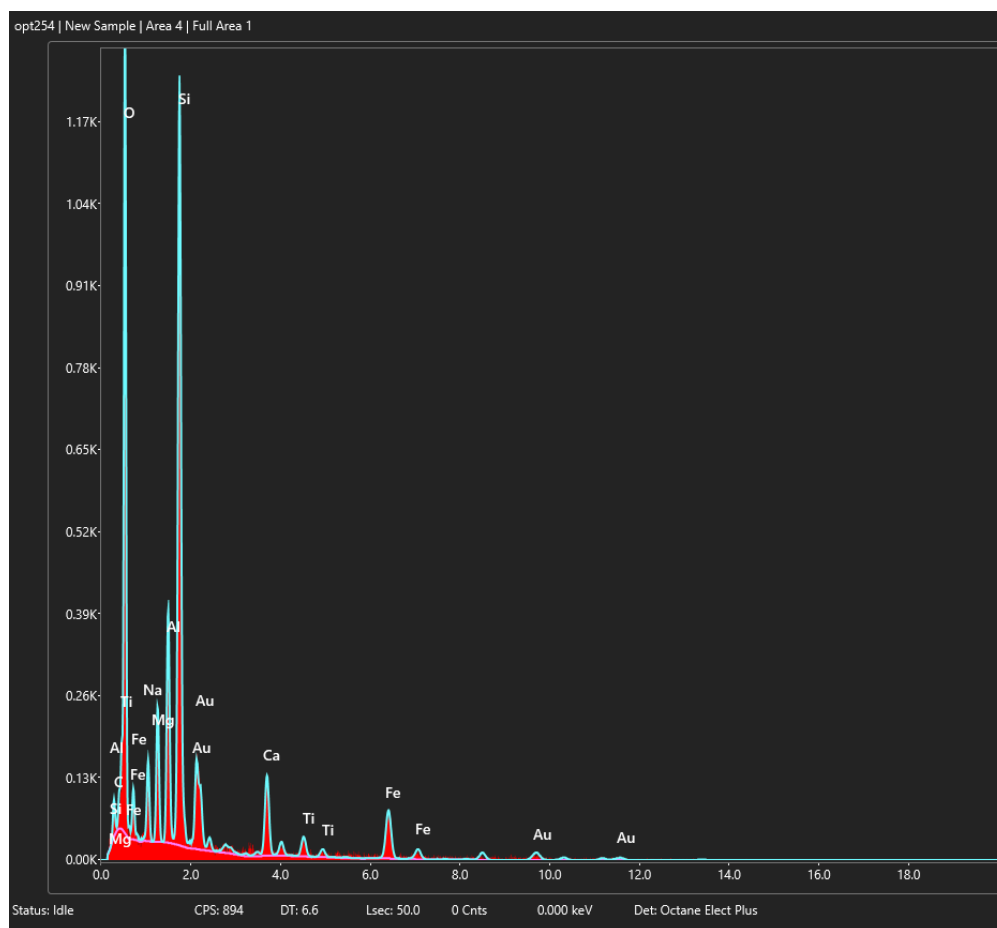


Figure 28: EDS composition graph collected from the selected region of interest, showing peaks corresponding to the detected elemental composition.

Element	Weight %	Atomic %	Error %	Net Int.	R	A	F
C K	6.47	12.22	15.62	8.31	0.8737	0.0778	1.0000
O K	36.22	51.38	9.65	163.60	0.8873	0.2203	1.0000
Na K	3.03	2.99	10.12	18.36	0.9031	0.3756	1.0037
Mg K	3.26	3.04	8.34	30.76	0.9080	0.5036	1.0057
Al K	6.41	5.39	6.56	66.80	0.9127	0.6093	1.0078
Si K	20.26	16.37	5.11	222.21	0.9171	0.6784	1.0046
Element	Weight %	Atomic %	Error %	Net Int.	R	A	F
Ti K	1.29	0.61	12.19	5.71	0.9480	0.9339	1.0394
Fe K	9.49	3.86	5.80	21.96	0.9629	0.9762	1.0619

Figure 29: EDS results for the geological sample, listing the measured elemental weight percentages and atomic percentages.

With the geological sample, the topography and composition were examined using both the SE2 and Backscattering detectors. This was done at 15kV with a working distance of 10.0mm. The SE2 image highlights surface topography, allowing features such as grain

boundaries, fractures, and pits within the sample to be observed clearly. In contrast, the backscattering images emphasize the effect of atomic number in imaging, where brighter regions correspond to areas containing elements with higher average atomic number and darker regions correspond to lighter phases.

The EDS spectrum and elemental maps confirm the presence of major elements including O, Si, Al, Fe, Mg, Na, and Ca. This is consistent with what would be expected for a geological sample such as this one. Gold peaks detected in the spectrum are attributed to the Au sputter coating, rather than representing sample composition itself. Overall, with both the EDS data and other images, we were able to clearly observe the variance in the sample's composition and topological features.

4 TEM Results

4.1 Gold Nano Islands Sample

Gold Nano Islands were used as the sample for the TEM lab. This sample had a copper support grid which is used to hold the sample. Images were taken using the Ceta Camera and STEM-HAADF. The Ceta camera is used to capture field images and diffraction patterns through the recording transmitted electrons on a highly sensitive detector, which allows for structural information from the islands to be observed clearly in imaging. STEM-HAADF imaging is used to obtain high-contrast images of the islands through height contrast, where image intensity increases with the atomic number being measured. With this method, heavier elements such as Au appear significantly brighter than the surrounding support film, making the nano islands quite distinguishable to the substrate.

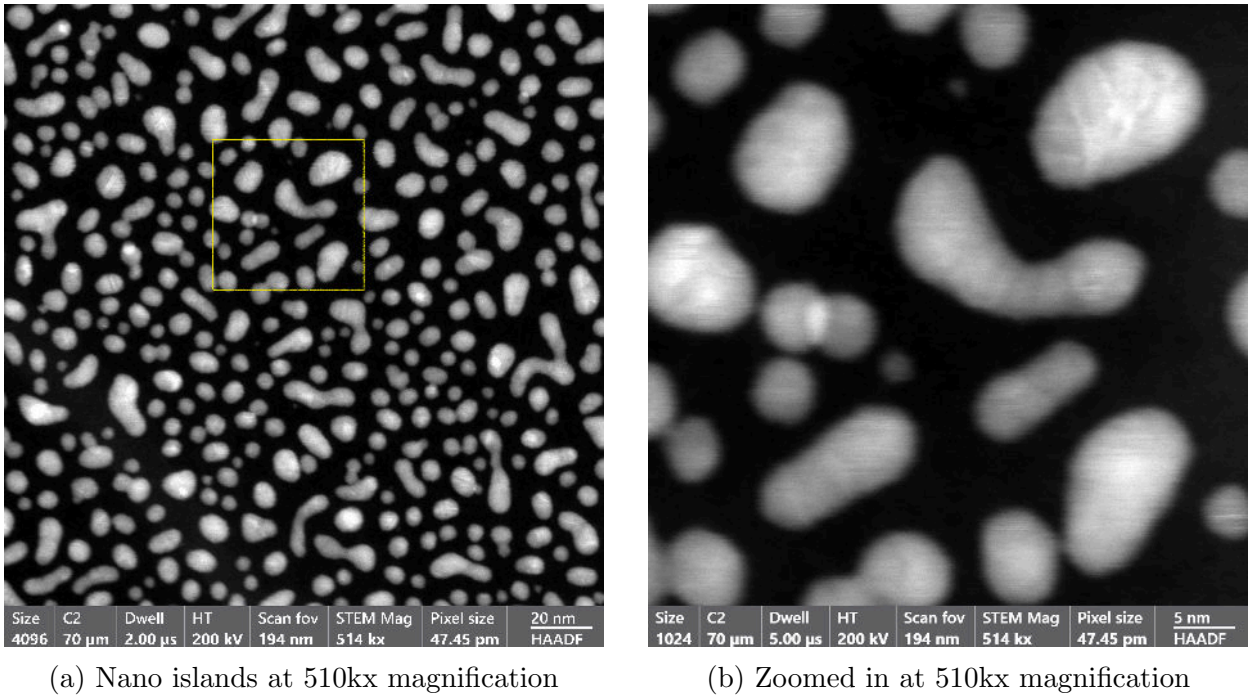


Figure 30: STEM-HAADF images of nano islands.

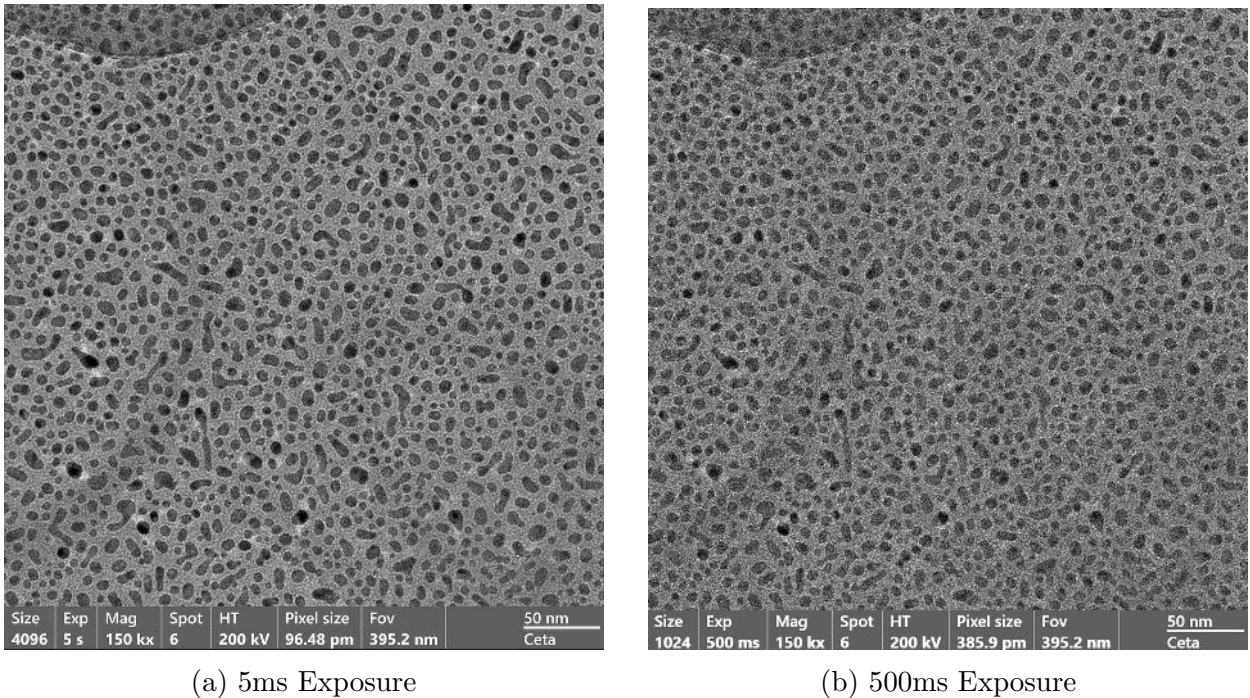


Figure 31: Nano islands at 150kx magnification comparing short and long exposure times.

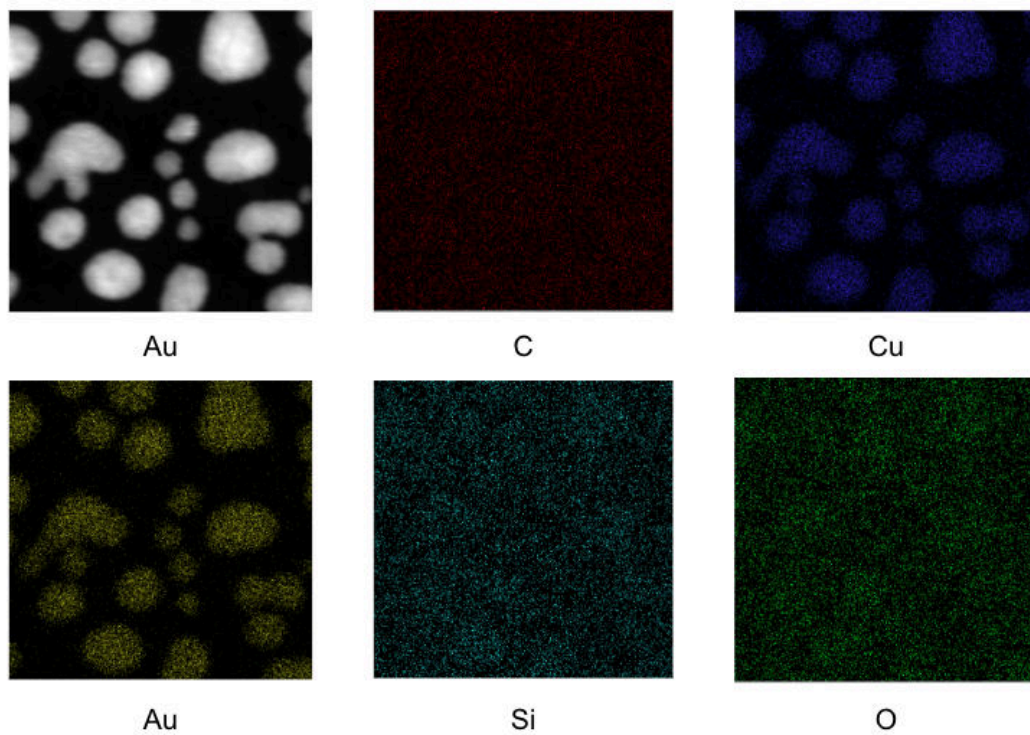


Figure 32: EDS maps with corresponding Au, C, Cu, Si, and O distributions.

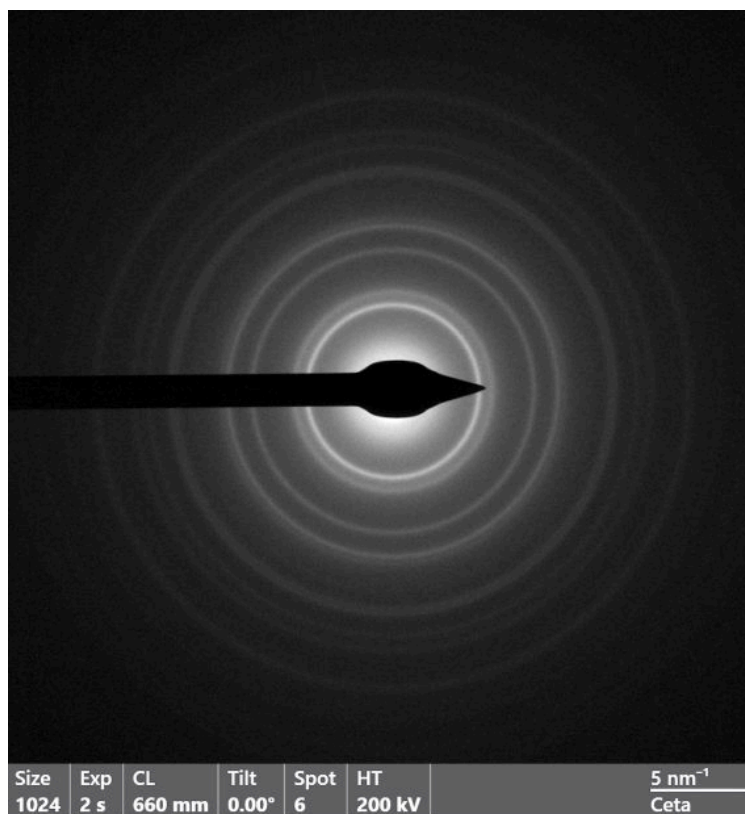


Figure 33: Diffraction image of nano islands.

Figures 30 through 33 show TEM characterization of the gold nano islands with various imaging conditions. STEM-HAADF images in Figure 30 reveal the distribution of the islands at the nanoscale with varying sizes and shapes at 510kx magnification. Figure 31 compares images acquired at 150kx using 5 ms and 500 ms exposure times; this demonstrated that the signal-to-noise ratio is improved at longer exposure. Elemental maps in Figure 32 confirm that the nano islands correspond to Au, as expected, while C, Cu, Si, and O signals originate from the sample and grid. The diffraction pattern shown in Figure 33 exhibits concentric rings, as explained and demonstrated in lab by Dr. O'Neil.

5 Conclusion

The data analysis above follows our previous understanding of SEM and TEM as high precision imaging techniques that provide various types of structural and compositional information about a given sample. From the SEM images, it was clear that secondary electrons were most effective in highlighting surface topography, allowing features such as asbestos fibers' and peacock feathers' surface textures to be imaged clearly at multiple magnifications. In contrast, the backscattering detector emphasized compositional differences within the geological sample by producing brightness variations that corresponded to atomic numbers. Further confirmation of elemental composition was obtained using EDS mapping and spectra graphs. This showed that the distributions were consistent with the expected elements present in the sample as well as the applied gold coating present on every sample. The TEM images of the gold nano islands provided higher resolution structural information than the SEM images and allowed features at the nanoscale to be observed directly. Observing how changes in exposure effect imaging, and further, identifying the importance of fine tuning parameters to improve the signal-noise ratio. Further, STEM-HAADF imaging clearly displayed the nano islands through by contrasting atomic numbers. Elemental graphing and color maps identified all the present elements in the nano island sample (including the copper grid) and confirmed the high presence of gold within the islands. We even had the opportunity to observe how the diffraction pattern exhibited concentric rings which let us further understand the nano islands beyond our previous knowledge. Overall, the results demonstrate that SEM is most useful for rapid surface imaging and compositional contrast across larger sample regions, while TEM provides higher resolution imaging and diffraction-based structural characterization at the nanoscale. Together, these techniques provide a strong understanding of both surface topography and internal structure than either method alone. These, along with Atomic Force Microscope, play an important role in nanoscale imaging and analysis.

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