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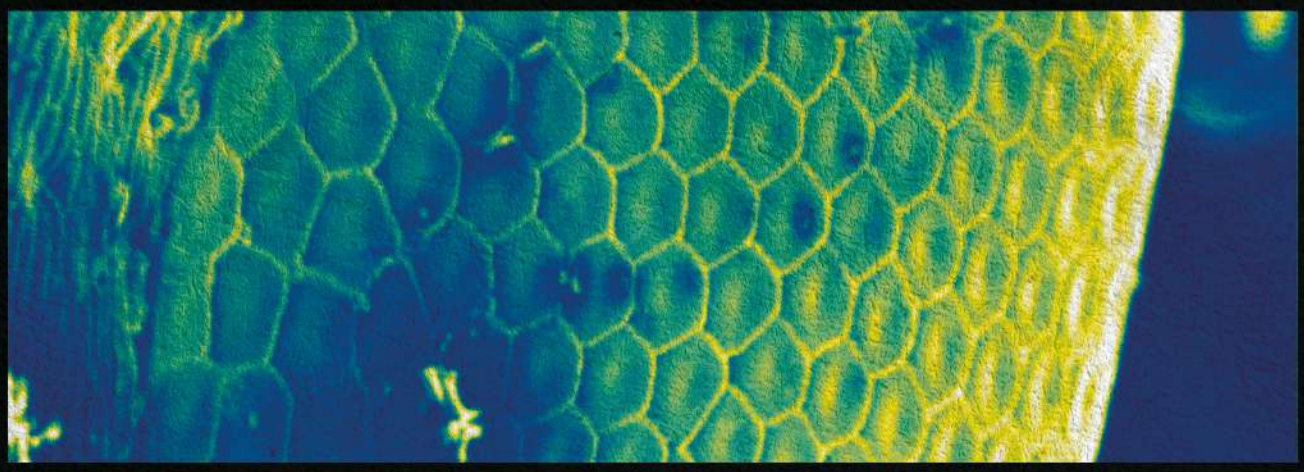

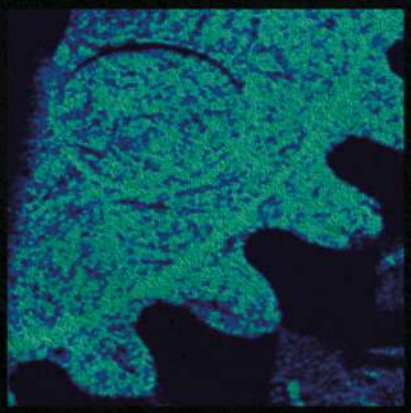

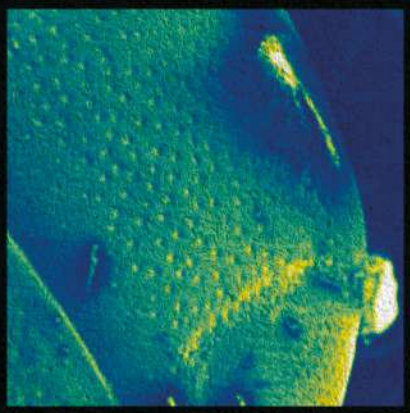

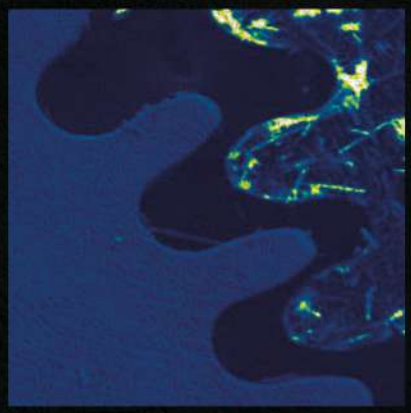

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AUTOR DO LIVRO

Hey! I am Renato Damásio!

You can call me Re!

The images found in this collection are examples of the knowledge and skills I have developed through the Scanning Electron Microscopy course taken in the fall of 2024 and spring 2025.

PhD and researcher

Currently, I am a PhD student at SUNY College of Environmental Science and Forestry. I received Master's and License degree in Forestry Sciences from Lisbon University- Portugal. In 2015, I completed my master's degree in forestry sciences in 2015 from the Federal University of Viçosa and graduated with Forestry Engineering degree from the Federal University of Viçosa in 2013. I have contributed to journal publications and patents through collaborative work. I have participated as a Scientific Initiation Fellow in many projects. I have worked as a specialist research scientist at the company Klabin S/A for 7 years in the R&D sector. I have an expertise in the following areas: Agricultural Sciences, Engineering Sciences and Technologies, Materials Engineering with an emphasis in Pulp, Paper and Wood, Coatings, Films, Cosmetics, Biomedicine, and Nanotechnology.

Enjoy this personal and private book! It is free! As my science donation for you!

All the images acquired in Baker laboratory at State University of New York College of Environmental Science and Forestry.
Thanks to **List, Richard PhD and Professor** at the same University.

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report

SUMMARY

SEM

INSTRUMENT BASICS: SECONDARY ELECTRON IMAGING

ABSTRACT

Size of different materials and structures are important to understanding how some properties and tissues are organized in their own biological or non-biological disposition. The purpose of this laboratory is to learn the basic operation of the microscope and how to acquire a secondary electron image. In this report SEM technique will be explored throughout JEOL JSM-IT100 InTouchScope™ available at Baker laboratory at ESF-SUNY. Small Au-coated beetle already placed in an SEM stub from the laboratory collection was selected for this first imaging experiment and evaluation. 3 images of the sample in 3 different regions were acquired named as image 1 (I1), image 2 (I2) and image 3 (I3). Sample preparation and placement at the sample holder and stage are divided in 4 steps as following: 1. Sample at stup after coating; 2. Sample at sample holder; 3. Sample stage preparation and 4. Sample stage placement and equipment start-up. Vaccum (0 Pa); accelerating voltage (10 kV); probe current (50); work distance(10 mm) at 180x 600x and 2500x magnification was applied as parameters to acquire the images. The image acquisition represents a key step to obtain pictures that real represent the structure that we are evaluating in the equipment. In this case the main parameters that expressed and influence the image quality was accelerating voltage at 10 kV, work distance at 10 mm and probe current at 50. However, one of the most strategies to explore and navigate into the structure was the magnification assumed in each picture.

1 INTRODUCTION

Size of different materials and structures are important to understanding how some properties and tissues are organized in their own biological or non-biological disposition.

Scanning electron microscope (SEM) is an important research and production tool extensively used in many phases of industry throughout the world. The popularity of the instrument results from the need to inspect and obtain information about samples with ever-decreasing structure. The SEM provides higher resolution analysis and inspection than that afforded by current techniques using the optical microscope[1].

The purpose of this laboratory is to learn the basic operation of the microscope and how to acquire a secondary electron image (SEI). You will learn how to manipulate the electron beam to produce high quality images. The electron beam scans across the sample surface causing secondary electrons to be emitted. Probe current (PC) is a measure of the size of the electron beam. In this exercise you will demonstrate the effect of probe current on image quality. This will be accomplished by producing three micrographs of the same sample using two different probe currents (PC) and then assessing the differences between the two images.

In this report SEM technique will be explored throughout JEOL JSM-IT100 InTouchScope™ available at Baker laboratory at ESF-SUNY.

2 METHODS

2.1 SAMPLE

A small Au-coated beetle already placed in an SEM stub from the laboratory collection was selected for this first imaging experiment and evaluation. 3 images of the sample in 3 different regions were acquired named as image 1 (I1), image 2 (I2) and image 3 (I3).

2.2 SEM

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY.

2.3 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to capture all the images (I1, I2 and I3) in this lab report using the JEOL JSM-IT100. The microscope conditions were the same for I1, I2 and I3 with slight variations and 3 conditions of magnification.

Table 1 – SEM working parameters and their working values defined for the images 1-3

SEM* parameters	Image 1	Image 2	Image 3
Vacuum (V) (Pa)	0	0	0
Accelerating Voltage (AV) (kV)	10	10	10
Probe Current (PB)	50	50	50
Working Distance (WD) (mm)	10	10	10
Magnification (M) (x)	180	600	2500
Objective aperture (OA) (X,Y), stage tilt (T), rotation (R) and z (Z).	X -1.989	X -2.070	X -2.070
	Y -6.101	Y -5.905	Y -5.905
	T 0.000	T 0.000	T 0.000
	Z 11.000	Z 11.000	Z 11.000
	R 0.000	R 0.000	R 0.000

*SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS. V = Vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation and Z =z.

2.4 MICROSCOPE MANAGEMENT STEPS

Acquire three images of the same object. With assistance from the instructor follow the steps described below:

1. Select a sample from the prepared samples developed for this course.
2. Secure the sample into the sample holder.
3. Look at the stage Z control (working distance or height) on the front of the chamber, and
4. make sure it is at 20 mm. This is to ensure that the stage does not hit and damage the
5. backscatter detector. Z should never be less than 10 mm.
6. Open the InTouch software. Select CHANGE icon to open the sample chamber and insert the sample.
7. Follow the software instructions to insert the sample. When prompted by the software, measure sample height, adjust stage height (Z), and take a picture of the stage.
8. The software will then ask you to select the appropriate Observation Conditions for your sample. With instructor assistance, select the conditions.
9. When prompted, close the chamber door, and evacuate the chamber.
10. The software will automatically provide the Observation Conditions for the sample type that you selected: accelerating voltage, and Probe Current and detector.
11. With assistance adjust kV to 10, magnification to 500, focus and auto-adjust the brightness and contrast (ABC)
12. Use the stage navigation system (SNS button), move the stage to place your sample under the electron beam.
13. Focus image and adjust ABC as needed.
14. Select objective aperture 2 (on column)
15. Center the objective aperture:

a) Press “wobbler” icon.
b) Adjust knobs on aperture holder (on column) until movement is minimized in both the X and Y directions.

16. For the first image, set the probe current (P.C) to 30.
17. To focus, increase magnification to higher than desired, and focus.
18. Optimize focus, brightness, and contrast.

19. Adjust astigmatism.
20. Return to desired magnification.
21. To save images to your folder, and label your image, go to the Conditions button; instructor will assist.
22. Collect the image (Photo scan) and save to your folder.
23. With assistance, turn off the beam (OBSERVE off), and vent the chamber, or use the CHANGE button to vent the chamber.
24. Before opening the chamber, return Z to 20 mm. 26. Remove sample and evacuate the chamber.

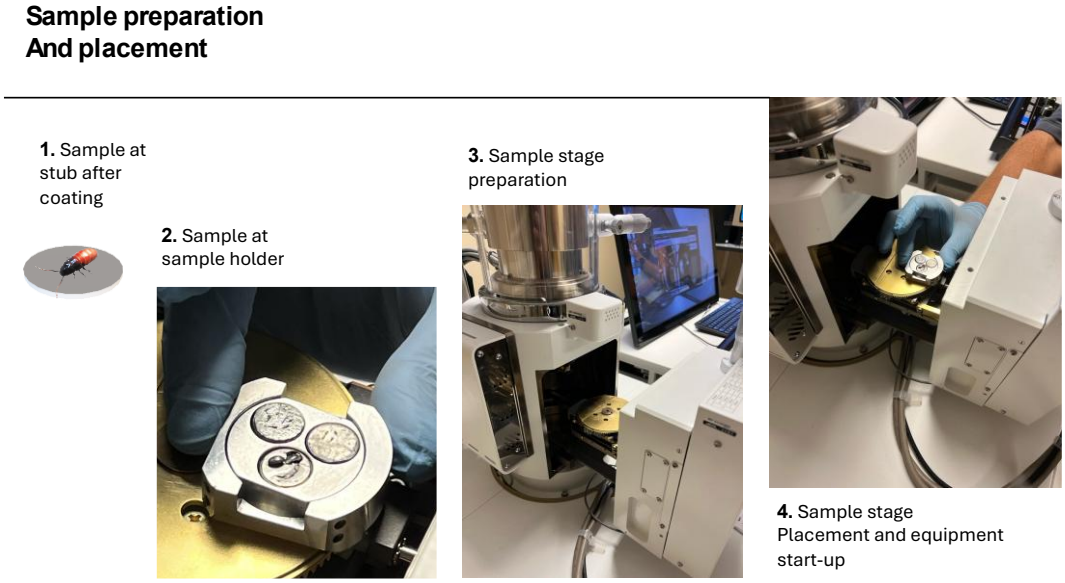
3 RESULTS

3.1 SAMPLE PREPARATION

Exist many techniques for sample preparation when comes to discuss microimaging evaluation. At this laboratory practices the main techniques to prepare our samples will be explored during the semester. However, to learning from the SEM how to place and manage the equipment, samples already prepared from the laboratory collection was used in this report. The sample selected from the collection was a small Au-coated beetle.

Fig. 1 shows the preparation steps and placement at the SEM equipment during this first training and evaluation.

Figure 1 – Sample preparation and placement at the sample holder and stage at SEM model JEOL JSM-IT100 InTouchScope™. Steps of placement are divided in 4 in this report as following: 1. Sample at stup after coating; 2. Sample at sample holder; 3. Sample stage preparation and 4. Sample stage placement and equipment start-up.



3.2 SEM IMAGES

SEM model JEOL JSM-IT100 InTouchScope™ is capable to achieve max accelerating voltage of 20 kV and magnificalton of 30.000 from the specification.

Fig. 2-4 bellow shows the acquired images of the head and eyes region from the small beetle. The imagining acquiring start from fig. 1 to fig. 4. Fig 1 the magnification was 180 x and increased to 2500 x in the fig. 4.

Key parameters for the imaging quality were keep constant for the imagining acquisition as accelerating voltage at 10 kV, work distance at 10 mm and probe current at 50. The strategy of imaging acquisition was magnification increasing instead change set-up conditions to obtain the images as showed below.

Figure 2 – Image 1 (I1) Beetle head and eyes region. Acquired with SEM model JEOL JSM-IT100 InTouch Scope™. V (0 Pa); AV (10 kV); PB (50); WD (10 mm); M (180x); OA (X -1.989 and Y -6.101); T (0.00); R (11.000) and Z (0.00) at 100 µm. The beetle head are composed for an intersection with the body. In the front part, where the head is located, one of the eyes are in the image and will be evaluate in details in the next images.

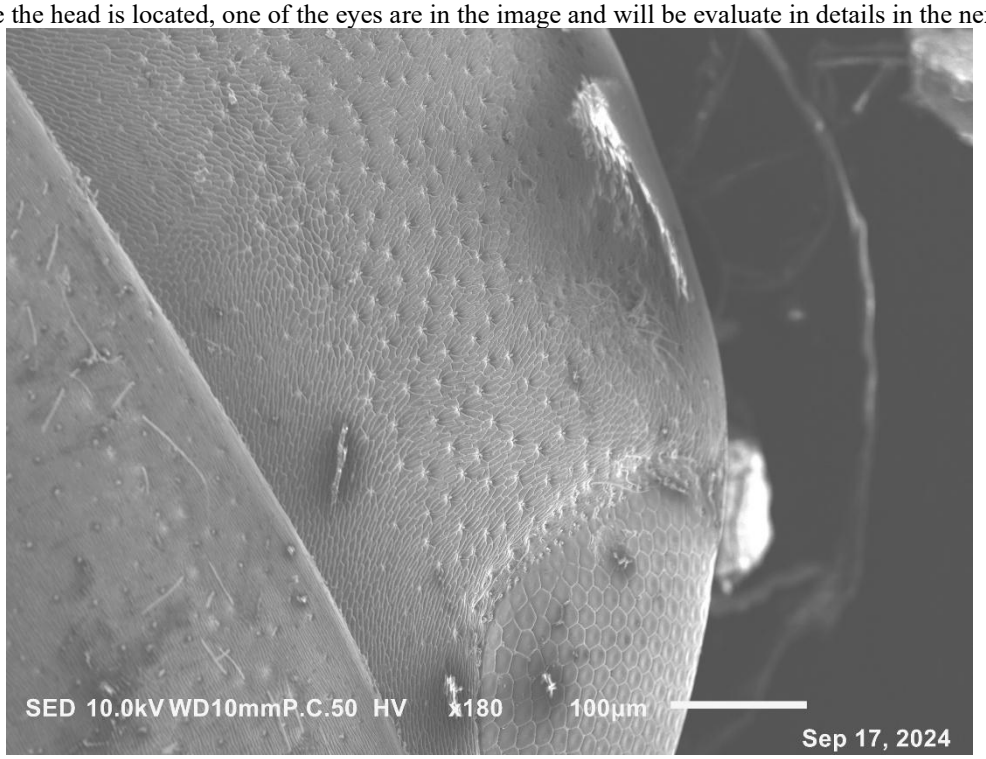


Figure 3 – Image 2 (I2) Beetle eyes region. Acquired with SEM model JEOL JSM-IT100 InTouchScope™. V (0 Pa); AV (10 kV); PB (50); WD (10 mm); M (600x); OA (X -2.070 and Y -5.905); T (0.00); R (11.000) and Z (0.00) at 20 µm. In this image the eye section and their intercsection with the beetle head can be visualized. The eyes regions are anatomicaly different that the head and the image acquired show the tissue diference between this two areas.

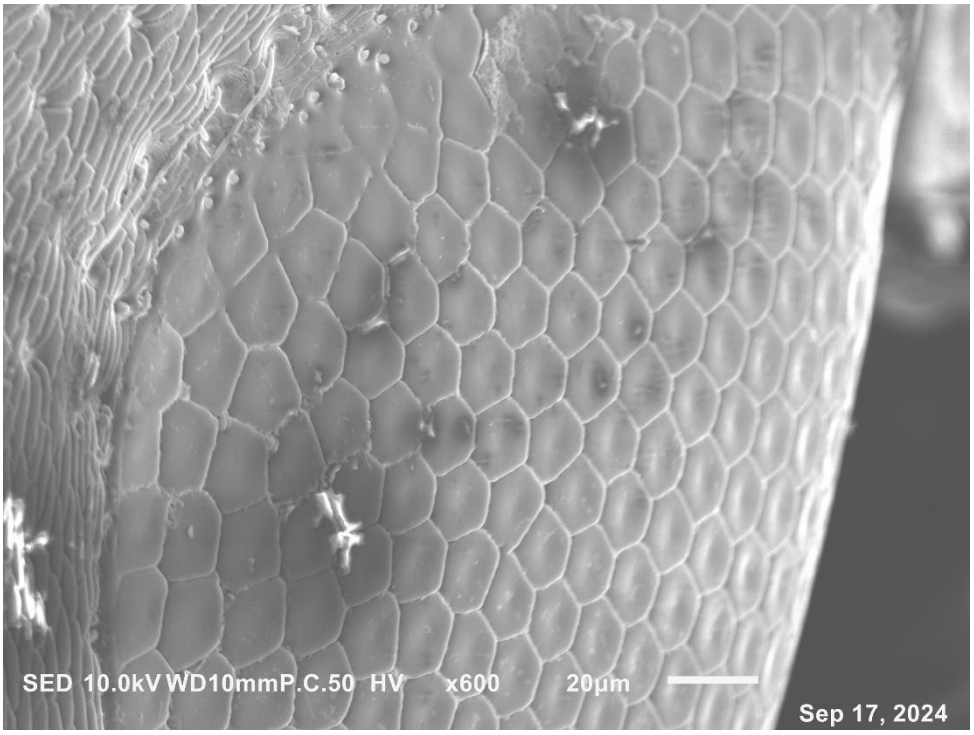
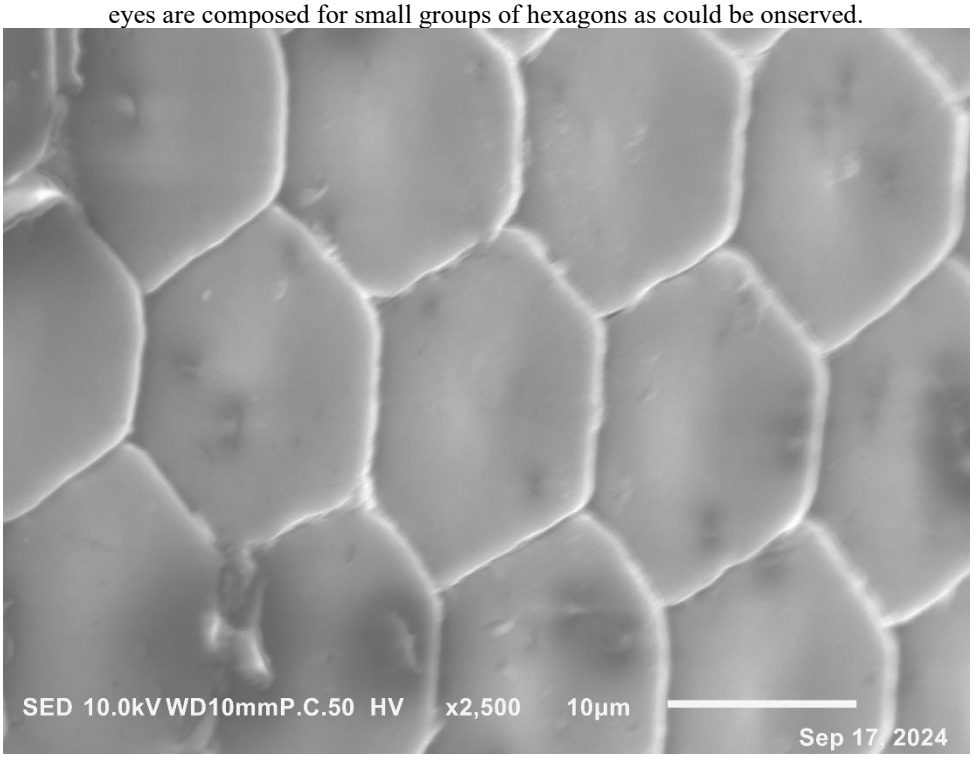


Figure 4 – Image 3 (I3) Detailed beetle eyes region. Acquired with SEM model JEOL JSM-IT100 InTouchScope™. V (0 Pa); AV (10 kV); PB (50); WD (10 mm); M (2500x); OA (X -2.070 and Y -5.905); T (0.00); R (11.000) and Z (0.00) at 10 µm. In this picture was possible observe the eyes region in detail. The eyes are composed for small groups of hexagons as could be onserved.



4 CONCLUSION

The image acquisition represents a key step to obtain pictures that real represent the structure that we are evaluating in the equipment. In this case the main parameters that expressed and influence the image quality was accelerating voltage at 10 kV, work distance at 10 mm and probe current at 50. However, one of the most strategies to explore and navigate into the structure was the magnification assumed in each picture as (180x; 600x and 2500x).

5 REFERENCE

(1) Postek, M. (1997), The Scanning Electron Microscope, CRC Press, Inc., New York, NY (Accessed September 21, 2024)

6 SELF-ASSESSMENT

6.1 PERSONAL SKILLS

This laboratory practice reinforces my ability to evaluate micro and nanoscale materials form biological and non-biological sources. This opportunity in use a sample from the laboratory collection allows us to accelerating your training capabilities to management the SEM. The next images I will acquire in near future I am confidently to end in a great result from my evaluations.

6.2 IMAGE QUALITY

From this report and discussions during the imaging acquisition in the class, the most important parameter to improve our image quality was a small group of work properties inherent to SEM used. The main parameters that expressed and influence the image quality was accelerating voltage, work distance, probe current and magnification to explore the structure details.

IMAGING WITH COATED AND UNCOATED SAMPLES

ABSTRACT

Electron beam has many different interactions with materials, and these interactions will affect the image acquisition according to the SEM equipment settings and sample properties. One strategy to improve image quality and sample interaction with the electron beam is the sputter coating on the top of the sample surface. Uncoated and Au-Pd coated Tannin powder samples were placed in a stub covered with a carbon adhesive tape. Sputter coating preparation steps using a Denton Desk V sputter coater was designed to prepare SEM samples. Considering the multiple samples coated at same time the set-up conditions were Argon atmosphere of 10 psi at 30 mÅ in the interval of 60-90 s was the condition applied for this sputter coating. A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. In the pictures (I1 to I3) key variables was evaluated during the imaging acquisition as different accelerating voltage (1 to 10 kV) and magnification (1200 to 5500 x) of coated (C) and uncoated (U) SEM images. Tannin granules from the sample powder with 50-70 μm was identified and characterized using the strategies discussed in this report. Image acquisition represents a key step to obtain pictures that real represent the structure that we are evaluating in the equipment. 3 key parameters could be defined with higher influence in imaging quality: surface treatment with Au-Pd coating, acceleration voltage and magnification to explore the surface and imaging information from the tannin granules observed.

1 INTRODUCTION

Electrons are the source of the beams in the electron microscopy, they are part of a coordinate ray generated via a tungsten filament at the electrum gum in the SEM (Scanning electron microscopy). The beam has many different interactions with the sample placed in the stage of the equipment, and these interactions will affect the image acquisition according to the equipment settings and sample properties.

Samples properties changing accordingly their composition and preparation and will influence the image quality.

One strategy to improve image quality and sample interaction with the electron beam is the sputter coating on the top of the sample surface using a Denton Desk V sputter coater designed to preparation of SEM samples.

The samples are composed for granules of tannin powder in their dry format. Tannin is a naturally occurring water soluble polyphenol compound resistant to biodegradation. present in vascular plants with molecular weight ranging from 500 to 3000 kDa. Tannins are characterized, by their ability to form strong complex with different minerals and macromolecules such as protein, cellulose, starch, and among other[3].

In this report SEM technique will be explored throughout the evaluation of coated and non-coated samples with JEOL JSM-IT100 InTouchScope™ available at Baker laboratory at ESF-SUNY. The lab report will assess the effects of golden-palladium (Au-Pd) coating on image quality and demonstrate the instrument's ability to imaging non-conductive specimens.

2 METHODS

2.1 TANNIN SAMPLES

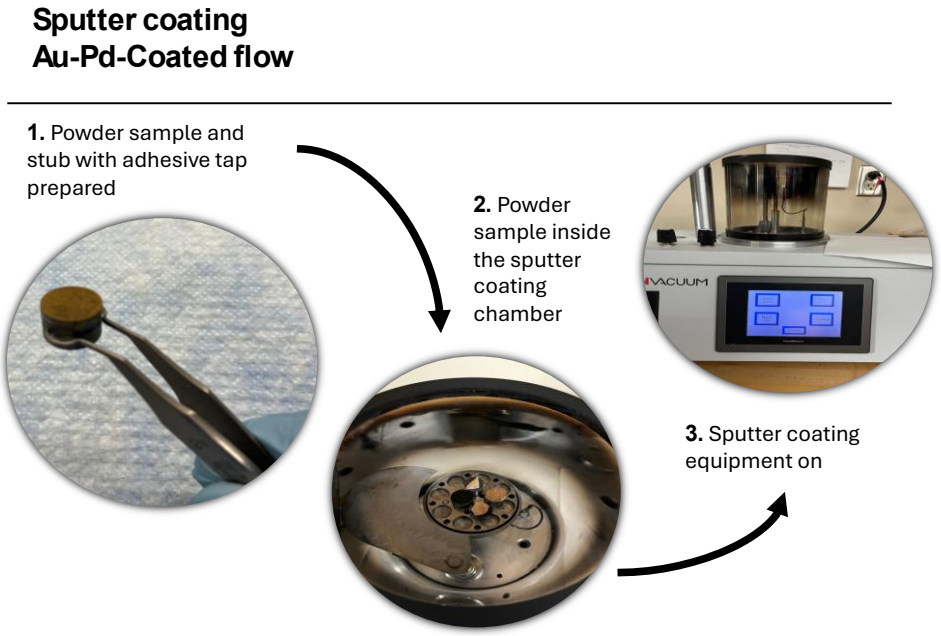
Fig. 1 shows uncoated and Au-Pd coated Tannin powder placed in a stub covered with a carbon adhesive tape. SEM images of each sample uncoated (U) and Au-Pd coated (C) named as images U (I1) and image C (I2 and I3).

Figure 1 – General scheme of sample preparation at the stub for SEM images. Uncoated and Au-Pd-Coated tannin powder samples, starting from step 1 to 4 in the sample preparation laboratory.

2.2 AU-PD SPUTTER-COATER

Fig. 2 shows the sputter coating preparation steps using an Denton Desk V sputter coater designed to preparation of SEM samples. The equipment has a 2" cathode and is supplied by DC power. SPUT6 is furnished with a gold target. The operation of this system is controlled thru a 6 inch touch screen[2]. Considering the multiple samples coated at same time the set-up conditions were Argon atmosphere of 10 psi at 30 mÅ in the interval of 60-90 s was the condition applied for this sputter coating.

Figure 2 – Denton Desk V sputter coating Au-Pd for tannin samples for SEM images was used an Argon atmosphere of 10 psi at 30 mÅ in the interval of 60-90 s. Au-Pd-Coated tannin powder samples, starting from step 1 to 3 in the sample preparation laboratory.



2.3 SEM

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY.

2.4 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to capture all the images (I1, I2 and I3) in this lab report using the JEOL JSM-IT100. The microscope conditions were the same for I1, I2 and I3 with slight variations and 2 conditions of magnification.

Table 1 – SEM working parameters and their working values defined for the images 1-3. SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS

SEM parameters	I1	I2	I3
Vacuum	MODE HV	MODE HV	MODE HV
Accelerating Voltage (kV)	1	10	10
Au_Pd coating	U	C	C
Probe Current	50	50	50

Working Distance (mm)	11	10	10
Magnification (x)	1200	1200	5500
Objective aperture (X,Y), stage tilt (T and R) and z (Z) coordinates	X -4.778	X 10.660	X 10.690
	Y 5.168	Y 2.606	Y 2.625
	T 0.000	T 0.000	T 0.000
	Z 10.000	Z 10.000	Z 10.000
	R 0.000	R 0.000	R 0.000

V = Vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation, Z =z, Au_Pd coating = Gold-Palladium coating at sample surface, C = coated sample and U = uncoated sample.

2.5 MICROSCOPE MANAGEMENT STEPS

The image acquisition will be conducted with an instructor following the steps bellow:

1. The natural/organic specimen to be imaged needs to be dry.
2. Sample is then divided into two similar sections.
3. With assistance, use carbon tape to affix both samples to an aluminum stub.
4. Use one sample for sputter coating in step 23.
5. The other sample will be imaged as follows.
6. With assistance, secure the samples into the sample holder.
7. With assistance, insert the sample into the SEM stage.
8. Evacuate the chamber.
9. Identify and state the function of each component and control on the instrument.
10. With assistance set the working distance to 10 mm and Z-focus the instrument
11. Manipulate the stage to place your sample under the electron beam at a working distance of 10.
12. Set accelerating voltage to 10 kEV
13. With assistance center aperture, optimize filament saturation, and gun alignment
14. Focus image at low magnification.
15. Focus image at a magnification higher than 500X
16. Adjust Brightness and Contrast
17. Adjust probe current
18. Optimize stigma, focus, brightness, and contrast

19. Capture images of sample.
20. Repeat 14 to 19 two more times at different magnifications.
21. Vent Chamber
22. Remove samples
23. With assistance, following published instructions, use the Denton Sputter coater to coat samples.
24. Independently secure second sample into the holder.
25. Independently insert the samples into the SEM stage.
26. Have Instructor check your work
27. Evacuate the chamber.
28. Identify and state the function of each component and control on the instrument.
29. Independently optimize instrument.
30. Adjust Brightness and Contrast
31. Set the probe current to optimize image
32. Perfect image quality
33. Capture image at the same magnification as step 19.
34. Repeat 14 to 19 two more times at different magnifications.
35. Under supervision desaturate filament, turn off HT, and vent the chamber.
36. Remove sample from Stage and evacuate the chamber
37. Follow lab procedures for securing SEM and cleanup work area.
38. Have Instructor check your work.

3 RESULTS

3.1 SEM COATED (C) AND UNCOATED (U) SAMPLES

SEM model JEOL JSM-IT100 InTouchScope™ is capable to achieve max accelerating voltage of 20 kV and magnificalton of 30.000 from the specification. In the following pictures (I1 to I3) some important variables was evaluated during the imaging acquisition as different accelerating voltage once we will observe coated (C) and uncoated (U) SEM images.

Fig. 3 shows the Image 1 (I1) bellow, and refers to an uncoated tannin powder sample applying 1 kV of acceleration voltage at 1200 times of magnification. It was possible evaluate the complete tannin granulle from the original powder sample.

Figure 3 – Image 1 (I1) Uncoated granules of tannin powder. Tannin powder was attached to the carbon tape in the top of the stub for SEM imaging model JEOL JSM-IT100 InTouchScope™. Small granules of tannin according the image diameter 50-70 µm (Scale bar-10 µm). 1 µm = $1x10^{-4}$ cm according to International System of Units (SI). Accelerating voltage = 1kV; Probe current = 50; Working distance = 11 mm at 1,200 x of magnification.

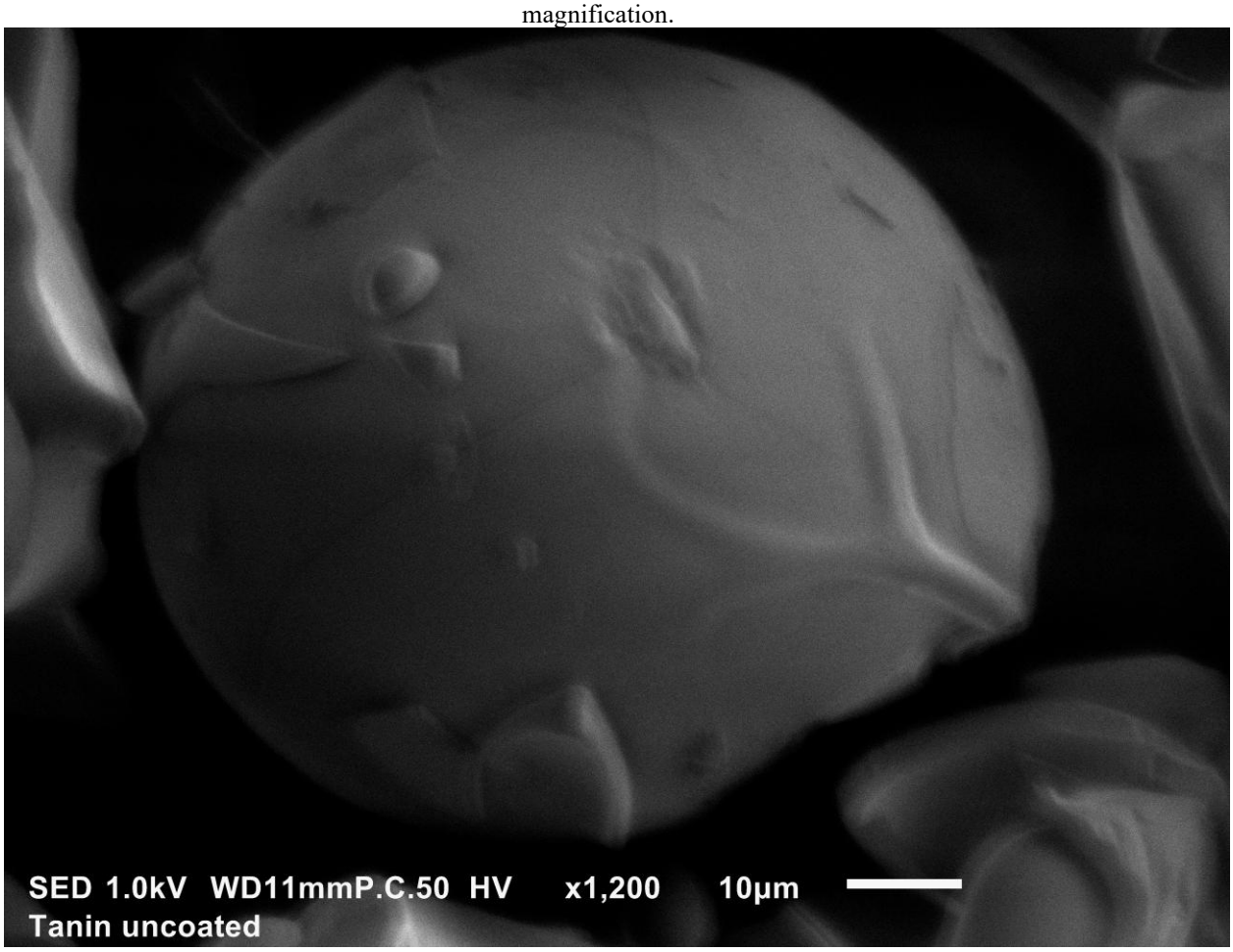


Fig. 4 bellow shows the Image 2 (I2) and refers to a coated tannin powder sample applying 10 kV of acceleration voltage at 1200 times of magnification. I2 showed a improved image considering key parameters as focus, brigtheness and contrast during the image acquisition, as an main outocome to improve the imaging quality as we are seeing in I2. The better quality of this image are strongly correlated with the strategy of sample coating treatment with Au-Pd in a controled atmosphere. Another grannulle of tannin from the powder was identified in I2 as we can observe in the center of the image.

Figure 4 – Image 2 (I2) Coated granules of tannin powder. Tannin powder was attached to the carbon tape in the top of the stub and submitted into a sputter Au-Pd coating at Argon atmosphere. SEM imaging was made with JEOL model JSM-IT100 InTouchScope™. Small granules of tannin according the image diameter 50-70 µm (Scale bar-10 µm). 1 µm = $1x10^{-4}$ cm according to International System of Units (SI). Accelerating voltage = 10 kV; Probe current = 50; Working distance = 10 mm at 1,200 x of magnification.

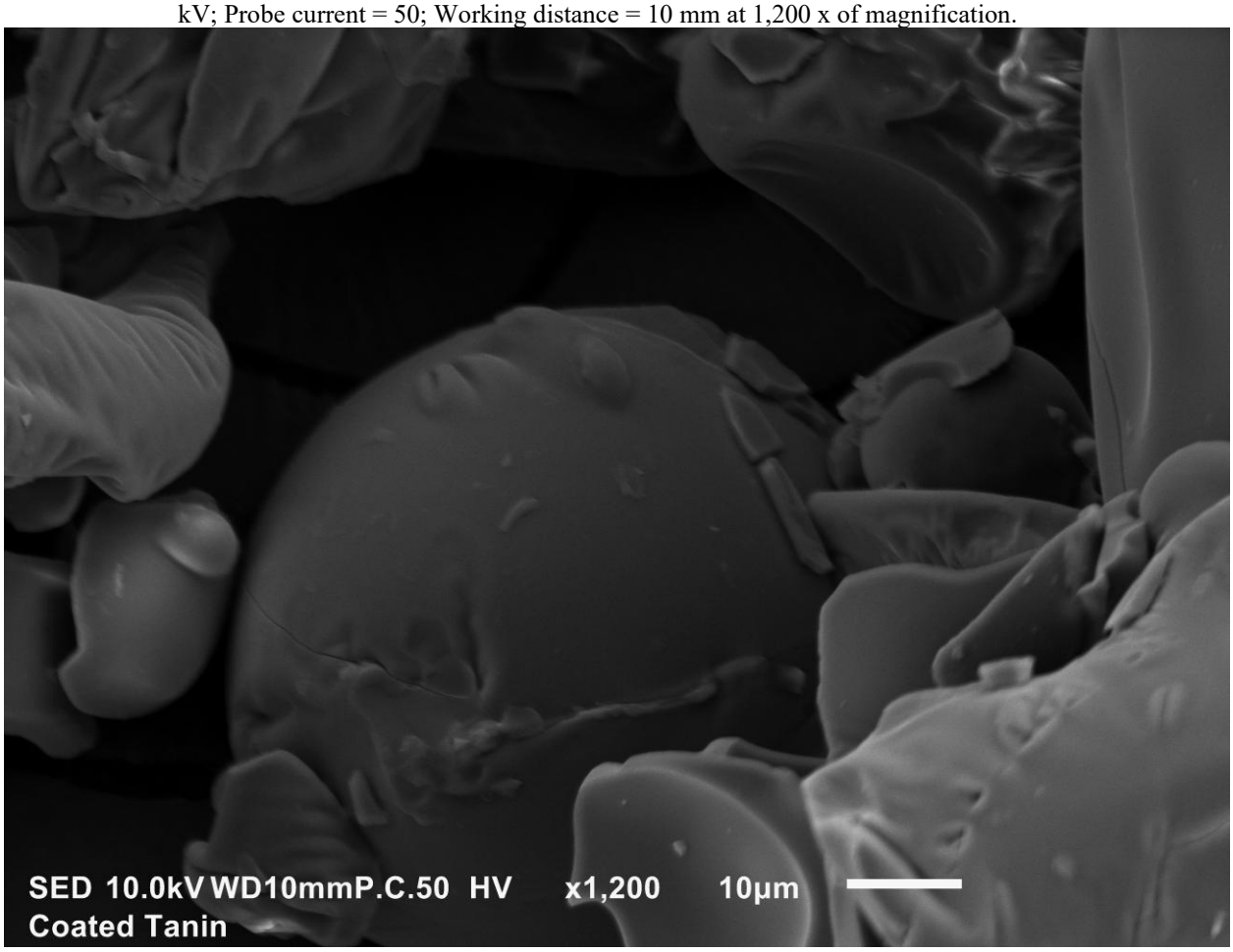
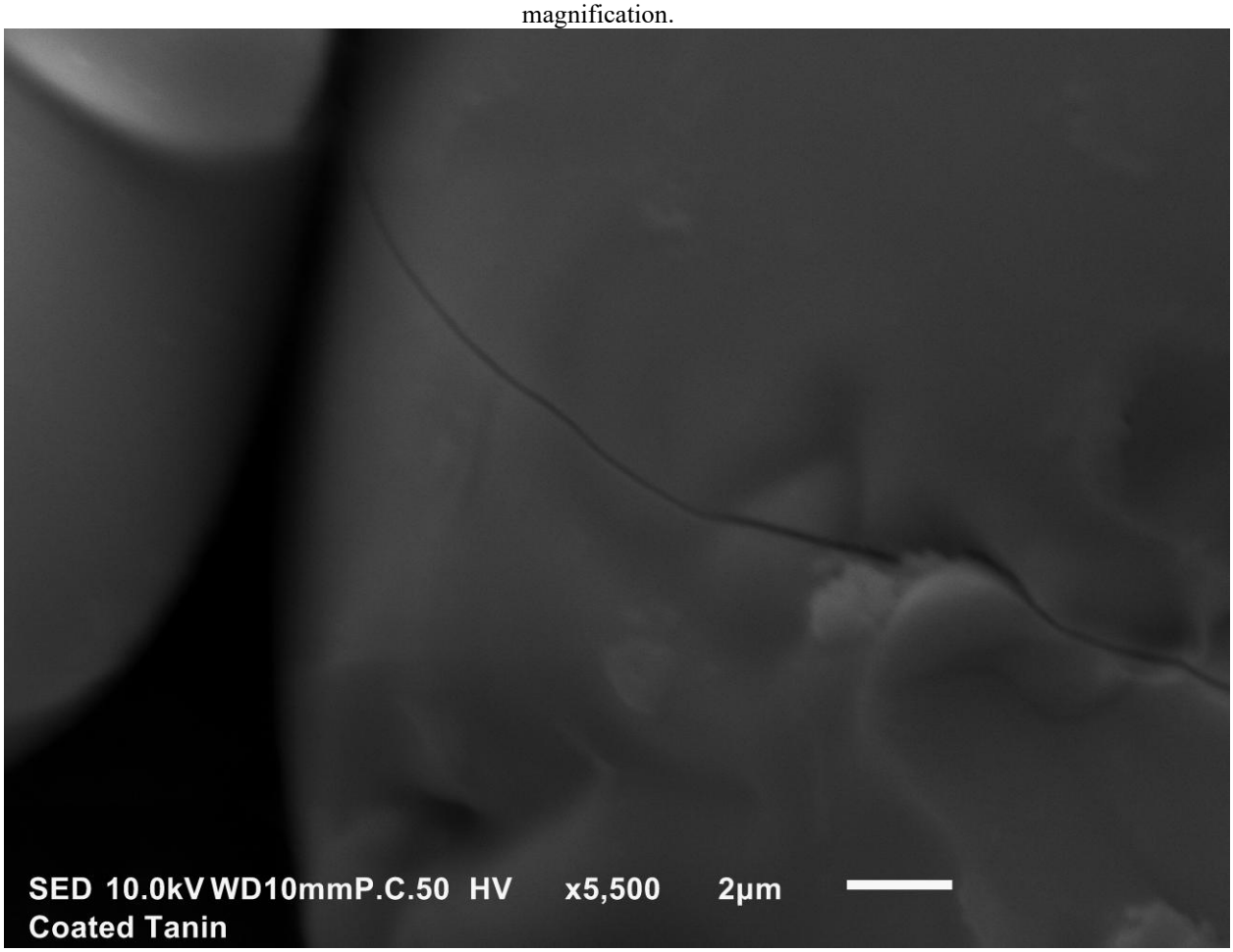


Fig. 5 bellow shows the Image 3 (I3) and refers to a coated tannin powder sample applying 10 kV of acceleration voltage at 5500 times of magnification. I3 is an detailed surface evaluation from the I2. A higher magnification was applied in the same grannule of I2 focusing in the craked surface. I3 shows a cracked tannin grannule surface. The increase in magnification is also noted by the changes in the scale bar as from I2 (10 µm) to I3 (2 µm).

Figure 5 – Image 3 (I3) Coated granules of tannin powder detailed of cracked surface. Tannin powder was attached to the carbon tape in the top of the stub and submitted into a sputter Au-Pd coating at Argon atmosphere. SEM imaging was made with JEOL model JSM-IT100 InTouchScope™. Small granules of tannin according the image diameter 50-70 µm (Scale bar-10 µm). 2 µm = $2x10^{-4}$ cm according to International System of Units (SI). Accelerating voltage = 10 kV; Probe current = 50; Working distance = 10 mm at 5,500 x of magnification.



4 DISCUSSION

Imaging acquisition are the main step to obtain high quality pictures from SEM sample evaluations. However, a critical and sometimes detailed is called sample preparation and their steps and techniques involved according to the material will observe in the SEM equipment.

As an important strategy to improve the quality of samples many metal coatings are used to treat the surface of sample according to their availability and interaction. The metal coatings when applied with the correct thickness had the capacity to improve the interaction with the sample surface and beam, resulting in a better surface evaluation considering the short electrons wavelength.

From the 3 images acquired in this lab practices, 3 key parameters could be defined with higher influence in imaging quality: surface treatment with Au-Pd coating, acceleration voltage and magnification.

Tannin granules from the sample powder with 50-70 μm was characterized using the strategies discussed in this report.

5 CONCLUSION

The image acquisition represents a key step to obtain pictures that real represent the structure that we are evaluating in the equipment. However, the sample preparation and treatment were a critical factor in this laboratory practices evaluation. 3 key parameters could be defined with higher influence in imaging quality: surface treatment with Au-Pd coating, acceleration voltage and magnification to explore the surface and imaging information from the tannin granules observed.

6 REFERENCE

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7 SELF-ASSESSMENT

7.1 PERSONAL SKILLS

This laboratory practice reinforces my ability to evaluate micro and nanoscale materials form biological and non-biological sources. One important addition from the first laboratory practices was the time dedicated to understanding how important the sample preparation step is. The importance of a great sample preparation was the key discussion that showed from the acquired images as a result from this new learning.

7.2 IMAGE QUALITY

Some of key factors that affects the final image at SEM is the sample preparation at the bigging of the process to get an image from SEM microscopy. The classification of different materials from their composition and conductive/dielectric properties is essential to define the best protocol of sample preparation before acquiring images at the SEM. Materials that are non-conductive or biological were recommended to be coated using this protocol and will result in a better image quality acquisition and observation at SEM. For some cases as powders, even if the material are conductor, the coating could be not effective due to the particle size of the sample, however each sample need to be discussed separately.

DEPTH OF FIELD (DOF)

ABSTRACT

DOF or the focal depth is the range of distances for which the specimen (object) is imaged with an acceptable sharpness on the image plane. In this report SEM technique will be explored throughout the evaluation of depth of field using TEM grids samples with JEOL JSM-IT100 InTouchScope™. TEM grid is a metal-wire used to observe samples at TEM and to evaluate DOF strategies was adhered to a carbon tape in the top of the stub for SEM. 3 conditions of aperture (AP1; AP2; AP3) was tested to achieve the DOF changing the WD (Work distance) in 3 different conditions as 15, 21 and 26 mm respectively. Each of WD condition was tested in combination with the aperture conditions. 3 conditions of aperture (AP1; AP2; AP3) was tested to achieve the DOF changing the WD (Work distance) in the images A to I. Images and their parameters, AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification are described A to I. A – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP1. B – AV: 20kV; PB: 50; WD: 21mm; M: 200x; AP1. C – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP1. D – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP2. E – AV: 20kV; PB: 50; WD: 21 mm; M: 200x; AP2. F – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP2. G – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP3. H – AV: 20kV; PB: 50; WD: 21 mm; M: 200x; AP3. I – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP3. DOF increase as we increase the WD and as we are keeping the M constant no decreases in DOF due magnification was observed. Comparing the set of images A, D and G we will observe at same M, PB and WD how the increase in AP (AP1 to AP3) influence in the quality of the imaging. This are the most important change in DOF capture in the set of images from the characterized samples. One of the key conclusions of this practices is that changes in WD versus AP affect DOF differently. AP changes affect more DOF than WD variations.

1 INTRODUCTION

Scanning electron microscope (SEM) enables imaging of micronano scale. It is an analytical tool widely used in the material, earth and life sciences[2]. Changing some parameters during the imaging acquisition is one of the strategies to reach image quality using electron microscopy.

DOF or the focal depth is the range of distances for which the specimen (object) is imaged with an acceptable sharpness on the image plane[1]. DOF is one strategy to even if not ideal work distance and aperture, define parameters according to the SEM aperture to control the image quality. DOF is larger as the opening (aperture) angle of the incident probe is smaller but is smaller as the magnification of the image is higher[1].

The lack of DOF becomes more serious in various applications. A possible way for increasing DOF without degradation of image resolution is to reconstruct a deeper DOF image by using multiple images obtained at different focal positions of the beam[3].

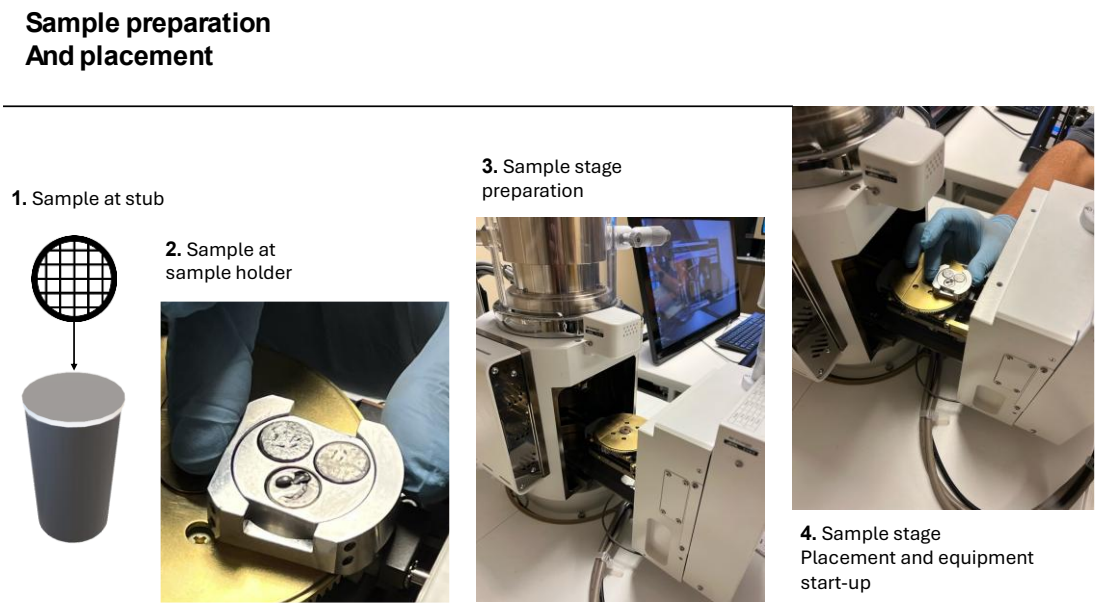
In this report SEM technique will be explored throughout the evaluation of depth of field using TEM grids samples with JEOL JSM-IT100 InTouchScope™ available at Baker laboratory at ESF-SUNY. The lab report will assess the effects of DOF strategy on image quality and demonstrate the instrument's ability to imaging applying different configurations.

2 METHODS

2.1 SAMPLE PREPARATION

TEM grid was chosen and placed at the stub for DOF evaluations. Fig. 1 shows the preparation steps and placement at the SEM equipment during this laboratory practices.

Figure 1 – Sample preparation and placement at the sample holder and stage at SEM model JEOL JSM-IT100 InTouchScope™. Steps of placement are divided in 4 in this report as following: 1. Sample at stup; 2. Sample at sample holder; 3. Sample stage preparation and 4. Sample stage placement and equipment start-up.



2.2 SEM

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY.

2.3 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to capture all the images from aperture 1 to 3 (Images A to I) in this lab report using the JEOL JSM-IT100. The microscope conditions changed work distance (WD) and aperture, as know 15, 21 and 26 mm and apertures 1, to and 3 respectively keeping same magnification for all images.

Table 1 – SEM working parameters and their working values defined for the images A to I. SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS

SEM parameters	Aperture 1			Aperture 2			Aperture 3		
	A	B	C	D	E	F	G	H	I
V	HV	HV	HV	HV	HV	HV	HV	HV	HV
AV (kV)	20	20	20	20	20	20	20	20	20
Au_Pd coating	N	N	N	N	N	N	N	N	N
PB	50	50	50	50	50	50	50	50	50
WD (mm)	15	21	26	15	21	26	15	21	26
M (x)	200	200	200	200	200	200	200	200	200
OA (X,Y), stage tilt (T and R) and z (Z) coordinates	X -0.205	X -0.201	X -0.189	X -0.207	X -0.209	X -0.209	X -0.222	X -0.222	X -0.222
	Y 2.264	Y 2.288	Y 2.302	Y 2.261	Y 2.291	Y 2.291	Y 2.264	Y 2.264	Y 2.264
	T 0.00	T 0.00	T 0.00	T 0.00	T 0.00	T 0.00	T 0.00	T 0.00	T 0.00
	Z 13.000	Z 18.000	Z 23.000	Z 13.000	Z 18.000	Z 23.000	Z 13.000	Z 18.000	Z 23.000
	R 0.00	R 0.00	R 0.00	R 0.00	R 0.00	R 0.00	R 0.00	R 0.00	R 0.00

V = Vacuum; HV = High vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation, Z = z, Au_Pd coating = Gold-Palladium coating at sample surface, N = no presence.

2.4 MICROSCOPE MANAGEMENT STEPS

The image acquisition will be conducted with an instructor following the steps bellow:

1. Select a mounted sample from the collection of WD samples developed for this course. You can use materials of your own choosing if they are dry, sputter coated and have been approved by the instructor.
2. Independently secure the sample into the sample holder.
3. Independently insert the sample into the SEM stage.
4. Have Instructor check your work
5. Evacuate the chamber.
6. Identify and state the function of each component and control on the instrument.
7. Adjust the SEM for optimal operation at an accelerating voltage of 10 kV, lens aperture of 1 and WD of 10.
8. Have Instructor check your work
9. Turn off Beam and turn on IR Chamber Camera
10. With help from your instructor to adjust tilt to at least 30º. BE CAREFUL when moving the stage. Watch the sample in the infrared camera scope. DO NOT let the stage our sample touch the column cone or detectors.
11. Turn off IR Chamber Camera and turn on beam.
12. With help from your instructor rotate stage to get desired image.
13. As accomplished in previous labs, perfect the image quality for the WD of 10 and 1200X magnification.
14. Capture images
15. Turn off beam, switch aperture to 3.
16. Turn on beam, and recenter the aperture
17. As accomplished in previous labs, perfect the image quality for the WD of 10 and 1200X magnification.
18. Capture Image
19. Set WD to 20 and Z-focus the sample.
20. As accomplished in previous labs, perfect the image quality for the WD of 20, 1200X, magnification, and same X/Y coordinates as step 17.
21. Turn off beam, switch aperture to 1.

22. Turn on beam, and recenter the aperture
23. As accomplished in previous labs, perfect the image quality for the WD of 20 and 1200X magnification.
24. Capture Image
25. Follow procedures for venting chamber and remove sample.
26. Follow lab procedures for securing SEM and cleanup work area.
27. Have Instructor check your work

3 RESULTS

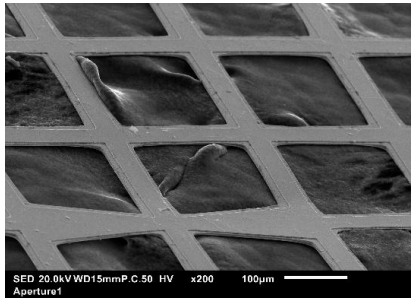
3.1 SEM IMAGES

SEM model JEOL JSM-IT100 InTouchScope™ is capable to achieve max accelerating voltage of 20 kV and magnificalton of 30.000 from the specification. In the following pictures uncoated TEM grids at the stub of SEM was observed instead to apply DOF strategies to keep image quality.

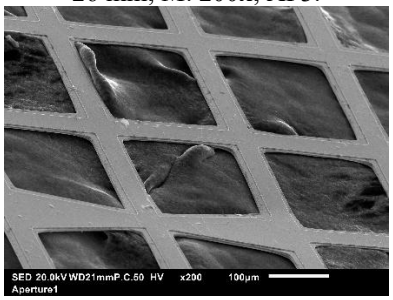
TEM grid is a metal-wire used to observe samples at TEM and to evaluate DOF strategies was adhered to a carbon tape in the top of the stub for SEM. 3 conditions of aperture (AP1; AP2; AP3) was tested to achieve the DOF changing the WD (Work distance) in 3 different conditions as 15, 21 and 26 mm respectively. Each of WD condition was tested in combination with the aperture conditions.

Fig. 2 shows a set of images collected considering the conditions discussed before. 3 conditions of aperture (AP1; AP2; AP3) was tested to achieve the DOF changing the WD (Work distance) in the images A to I. Images and their parameters, AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification are described A to I. A – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP1. B – AV: 20kV; PB: 50; WD: 21mm; M: 200x; AP1. C – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP1. D – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP2. E – AV: 20kV; PB: 50; WD: 21 mm; M: 200x; AP2. F – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP2. G – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP3. H – AV: 20kV; PB: 50; WD: 21 mm; M: 200x; AP3. I – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP3.

Figure 2 – Uncoated TEM grids at the stub of SEM to evaluate DOF. TEM grid is a wire used to observe samples at TEM and to evaluate DOF strategies was adhered to a carbon tape in the top of the stub for SEM imaging using a SEM model JEOL JSM-IT100 InTouchScope™. 3 conditions of aperture (AP1; AP2; AP3) was tested to achieve the DOF changing the WD (Work distance) in the images A to I. Images and their parameters, AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification are described A to I. A – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP1. B – AV: 20kV; PB: 50; WD: 21mm; M: 200x; AP1. C – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP1. D – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP2. E – AV: 20kV; PB: 50; WD: 21 mm; M: 200x; AP2. F – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP2. G – AV: 20kV; PB: 50; WD: 15 mm; M: 200x; AP3. H – AV: 20kV; PB: 50; WD: 21 mm; M: 200x; AP3. I – AV: 20kV; PB: 50; WD: 26 mm; M: 200x; AP3.



A



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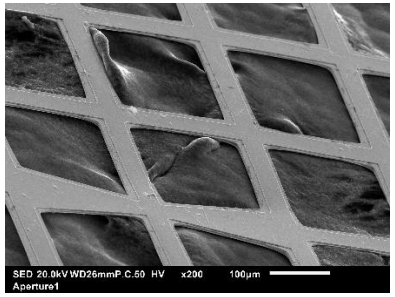

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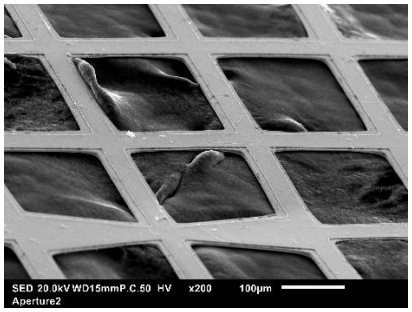

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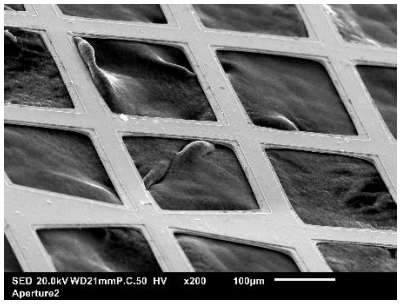

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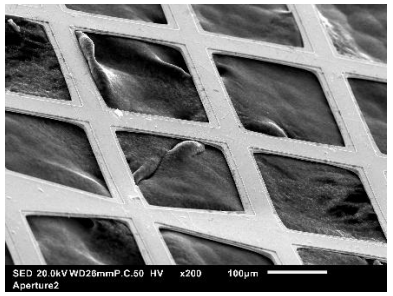

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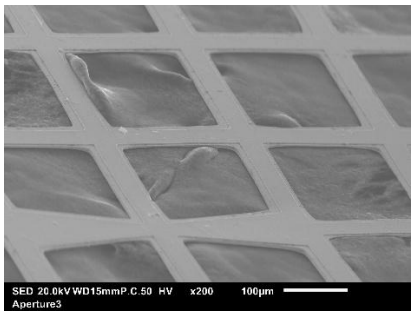

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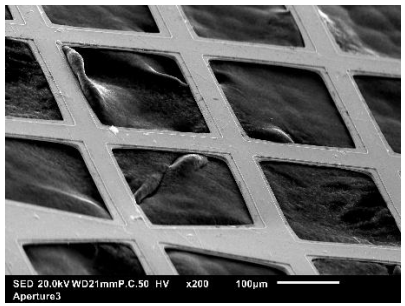

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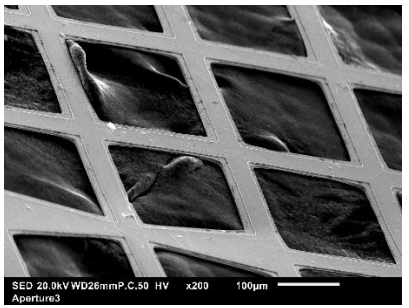

I

4 DISCUSSION

One of the key steps to obtain high quality pictures from SEM sample evaluations is the imaging acquisition step and their management. Many parameters could be changed in this step to achieve better image resolution. Fig. 2 shows a set of images collected according to the concept of DOF. Parameters such as WD, AP, M and PB was changed in each of 9 image conditions.

M at 200x and PB 50 was the 2 main conditions that are kept as constants instead to change during the imaging acquisition. WD was changed in each set of 3 images in the same AP. Images A to C at AP1 was acquired considering WD 15, 21 and 26 respectively. Images D to F at AP2 was acquired considering WD 15, 21 and 26 respectively. And finally, images G to I at AP3 was acquired considering WD 15, 21 and 26 respectively.

DOF increase as we increase the WD and as we are keeping the M constant no decreases in DOF due magnification was observed. Comparing the set of images A, D and G we will observe at same M, PB and WD how the increase in AP (AP1 to AP3) influence in the quality of the imaging. This are the most important change in DOF capture in the set of images from the characterized samples.

5 CONCLUSION

The lack of DOF becomes serious in various applications for image accquisition. DOF increase as we increase the WD and as we are keeping the M constant no decreases in DOF due magnification was observed. Comparing the set of images A, D and G we will observe at same M, PB and WD how the increase in AP (AP1 to AP3) influence in the quality of the imaging. This are the most important change in DOF capture in the set of images from the characterized samples. One of the key conclusions of this practices is that changes in WD versus AP affect DOF differently. AP changes affect more DOF than WD variations.

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7 SELF-ASSESSMENT

7.1 PERSONAL SKILLS

Study DOF at this laboratory practices for me were a significative increase in the understanding of how the change in many variables could really influence in the final quality of the image. DOF becomes for me now a practical concept once the changes in AP and WD visually affect the image and their surface.

7.2 IMAGE QUALITY

Since the beginning of the laboratory practices plan, we are discussing about the image quality and the effects of many parameters in the final imaging. DOF, is one of the technical concepts that due to the combination of setting parameters was possible visualize how important they become. This report is the result of the practical understanding of this important parameter and their significate influence in the imaging acquisition.

BACKSCATTERED ELECTRON IMAGE (BEI)

ABSTRACT

Achieve micro-nano scale from the microscope and their artifacts became the main challenge in the management of equipment operation. Backscattered electrons, which are emitted from the sample after multiple elastic collisions with atoms was the technique used in this report to evaluate the sample of a rock surface. A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM image. 3 images named SED, BEC and BET was acquired applying respectively secondary electrons detector (SED), backscattered electron composition detector (BEC) and backscattered electron topographical detector (BET). Images named a - SED, b - BEC and c - BET. The images and their parameters are described in images “a” to “c”, a – AV: 10kV; PB: 53; WD: 20 mm; M: 60x. b – AV: 10kV; PB: 57; WD: 20 mm; M: 60x. c – AV: 10kV; PB: 57; WD: 20 mm; M: 60x. a-SED show us a rock surface, it was possible observe different textures and regions with peaks, depressions mixed with flat areas in the top and bottom part. BEI strategy allows us to qualitatively characterize regions with different compositions, b-BEC, regions with withe areas and gray transitions to black are defined with different composition of the rest of the rock surface exhibit surface color in black or totally gray. Finally, c-BET, shows the topographical variations in the top of the sample surface. It is relevant comment that a chemical change in the composition was also identified in the peaks of the sample surface evaluated in c-BET. With areas in c-BET are also characterized with different chemical composition. BEI becomes an predictive technique associated with SEM imaging allowing the non-destructive sample evalluation considering many different aspects as surface, structure and chemical composition.

1 INTRODUCTION

Achieve micro-nano scale from the microscope and their artifacts became the main challenge in the management of equipment operation. Exist many techniques that are applied in the operational settings to improve the image quality.

Backscattered electron or interaction (BSE or BEI) imaging based on scanning electron microscopy (SEM) has been widely used in scientific and industrial disciplines[1]. The conservation of energy and charge impose that any system should obey A=1-R-T (where A is the absorbance, R is the reflectance, and T is the transmittance of the system). Thus, when operating a scanning electron microscope (SEM) under T =0 condition, A =1-R will be satisfied; that is, A is complementary to R[1].

Backscattered electrons, which are emitted from the sample after multiple elastic collisions with atoms, are sensitive to the phase composition. Although these electrons travel in a different direction, they possess the similar energy as the incident electrons and exhibit a deeper depth of interaction compared to secondary electrons. In contrast, secondary electrons have much lower energy than incident electrons due to inelastic

scattering between the incident beam and the top electron layers of the atoms in the sample[2].

The electron backscatter diffraction pattern (EBSD), which is revealed by diffracted electrons, provides information about the local orientation of crystalline materials[2], composition and topographical information.

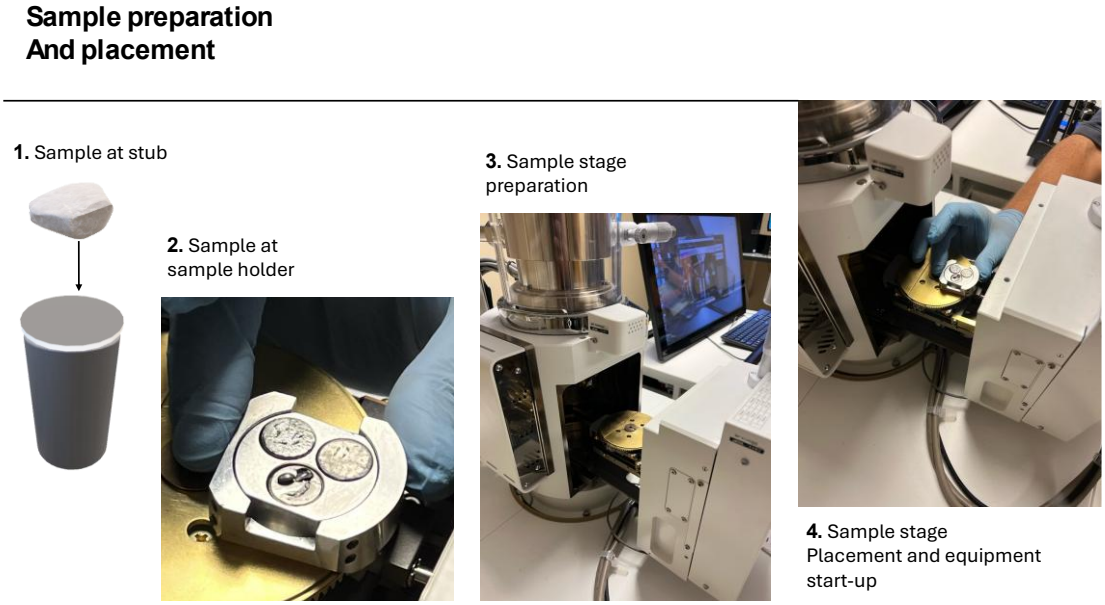
In this report SEM technique will be explored throughout the evaluation of backscattered electron image (BEI) using a piece of rock sample with JEOL JSM-IT100 InTouchScope™ available at Baker laboratory at ESF-SUNY. The lab report will assess the effects of BEI strategy on image quality and demonstrate the instrument's ability to imaging applying different configurations to evaluate the sample, their composition and their topographical structure.

2 METHODS

2.1 SAMPLE PREPARATION

A piece of uncoated rock was placed at the stub for BEI evaluations. Fig. 1 shows the preparation steps and placement at the SEM equipment during this laboratory practices.

Figure 1 – Sample preparation and placement at the sample holder and stage at SEM model JEOL JSM-IT100 InTouchScope™. 4 Steps of placement as following: 1. Sample at stub; 2. Sample at sample holder; 3. Sample stage preparation and 4. Sample stage placement and equipment start-up.



2.2 SEM

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY.

2.3 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to acquire all the images at the same aperture in this lab report using the JEOL JSM-IT100. The electrons detector was changing for each acquired image. 3 images named SED, BEC and BET was acquired applying respectively secondary electrons detector (SED), backscattered electron composition detector (BEC) and backscattered electron topographical detector (BET). The microscope conditions keep the same key parameters as work distance (WD), aperture, accelerating voltage and magnification. Slight variation of probe current could be observed without any significative influence in the image acquisition.

Table 1 – SEM working parameters and their working values defined for the images acquired with secondary electrons detector (SED), backscattered electron composition detector (BEC) and backscattered electron topographical detector (BET). SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS

SEM parameters	**Images**		
	SED	**BEC**	**BET**
V	HV	HV	HV
AV (kV)	10	10	10
Au_Pd coating	N	N	N
PB	53	57	75
WD (mm)	20	20	20
M (x)	60	60	60
Objective aperture (X,Y), stage tilt (T and R) and z (Z) coordinates	X -6.069	X -6.069	X -6.069
	Y -6.903	Y -6.903	Y -6.903
	T 0.00	T 0.00	T 0.00
	Z 27.000	Z 27.000	Z 27.000
	R 0.00	R 0.00	R 0.00

V = Vacuum; HV = High vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation, Z = z, Au_Pd coating = Gold-Palladium coating at sample surface, N = no presence, SED = secondary electrons detector, BEC = backscattered electron composition detector and BET = backscattered electron topographical detector.

2.4 MICROSCOPE MANAGEMENT STEPS

The image acquisition will be conducted with an instructor following the steps bellow:

1. Use the mounted sample of a wood and the sample of different metals prepared for this lab.
2. Secure samples into the sample holder.
3. Insert the sample into the SEM stage.
4. Review the function of each component and its control on the instrument.
5. Adjust magnification to 100, adjust brightness and contrast of main monitor, power up IR Camera and monitor, and follow lab procedures to prepare SEM for operation.
6. Adjust SEM to the following parameters: Accelerating voltage of 20 kV, Working distance 10mm, Aperture 2, Spot size 70.
7. Focus beam on wood sample.
8. Record and report the counts per second (CPS) for each image that is collected.
9. As accomplished in previous labs, perfect the image quality for the parameters above and a minimum of 500X magnification.
10. Switch to BEI-Compo mode.
11. Adjust image for contrast and brightness.
12. Capture Image
13. Switch to BEI-Topo mode.
14. Adjust image for contrast and brightness.

3 RESULTS

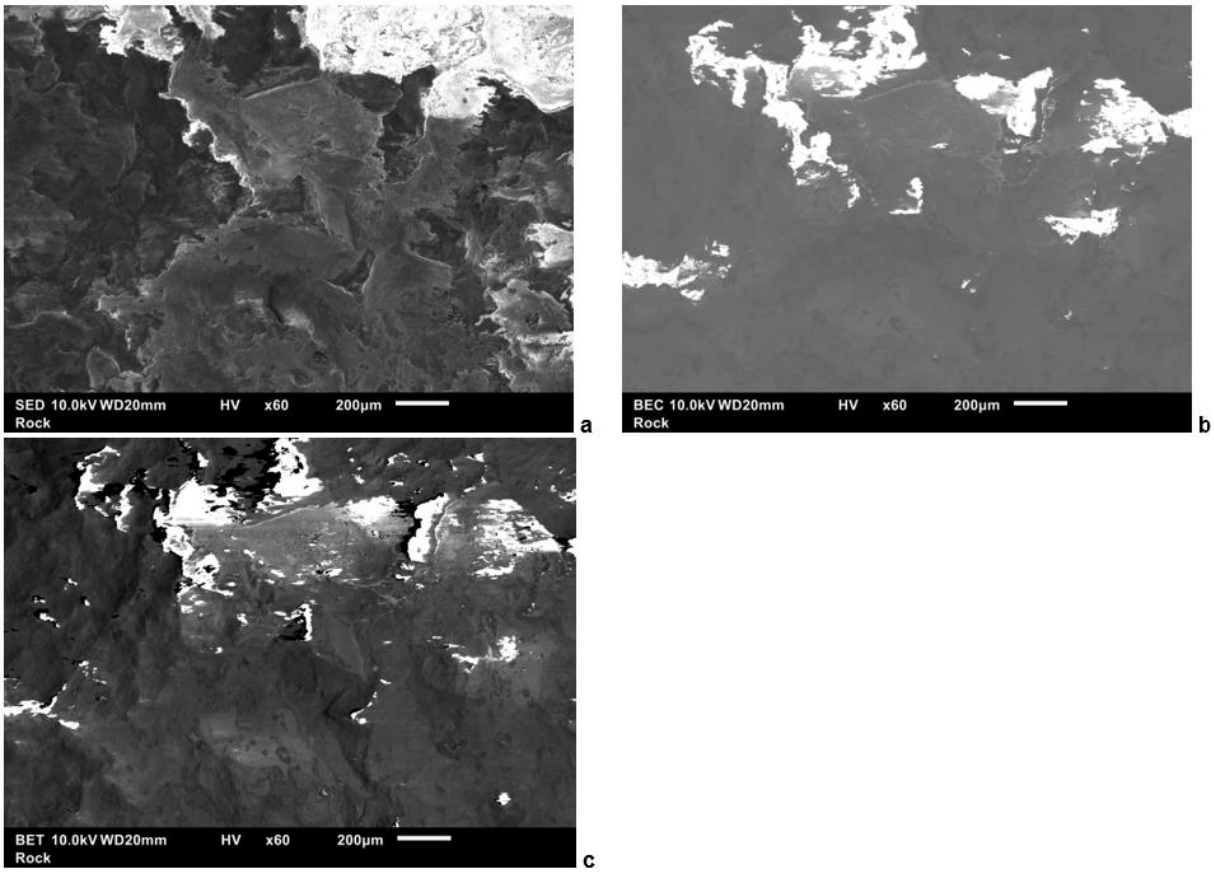
3.1 SEM IMAGES

SEM model JEOL JSM-IT100 InTouchScope™ is capable to achieve max accelerating voltage of 20 kV and magnificalton of 30.000 from the specification. In the following pictures uncoated samples of piece of a rock was placed in a stub and observed applying different backscattered strategies keeping the AV at 10 kV.

The set of images in fig. 2 shows an uncoated rock with differential chemical composition and topography observed with secondary electrons detector (SED), backscattered electron composition detector (BEC) and backscattered electron topographical detector (BET). 3 conditions electron detector was tested to evaluate BEI changing according to the sample structure. Images named a - SED, b - BEC and c - BET.

The images and their parameters are described in images “a” to “c”, a – AV: 10kV; PB: 53; WD: 20 mm; M: 60x. b – AV: 10kV; PB: 57; WD: 20 mm; M: 60x. c – AV: 10kV; PB: 57; WD: 20 mm; M: 60x.

Figure 2 – Uncoated rock with differential chemical composition and topography observed with secondary electrons detector (SED), backscattered electron composition detector (BEC) and backscattered electron topographical detector (BET). SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS. 3 conditions electron detector was tested to evaluate BEI changing according to the sample structure. Images named a - SED, b - BEC and c - BET. Images and their parameters, AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification are described a to c. A – AV: 10kV; PB: 53; WD: 20 mm; M: 60x. b – AV: 10kV; PB: 57; WD: 20 mm; M: 60x. c – AV: 10kV; PB: 57; WD: 20 mm; M: 60x.



4 DISCUSSION

One of the key parameters that influence BEI imaging quality is AV. Increasing the accelerating voltage leads to deeper penetration of backscattered electrons and a larger volume of interaction. Higher accelerating voltages also increase background noise from signals emitted by the inner sample, consequently degrading the spatial resolution[2]. To acquire SED, BET and BEC images in this report 10 kV was applied according to the SEM work parameters. Is recommended to use an accelerating voltage that is 1.5 to 2.0 times the X-ray excitation energy of the heaviest element present in the sample[2].

Fig. 2 a-SED show us a rock surface. It was possible observe different textures and regions with peaks, depressions mixed with flat areas in the top and bottom part. BEI strategy allows us to qualitatively characterize regions with different compositions, b-BEC, regions with withe areas and gray transitions to black are defined with different composition of the rest of the rock surface exhibit surface color in black or totally gray. Finally, c-BET, shows the topographical variations in the top of the sample surface. It is relevant comment that a chemical change in the composition was also identified in the peaks of the sample surface evaluated in c-BET. With areas in c-BET are also characterized with different chemical composition. The BEI strategy adopted for this sample evaluation allow us to identify the chemical composition shift in specific areas with different topographical structure.

5 CONCLUSION

BEI becomes an predictive technique associated with SEM imaging allowing the non-destructive sample evalluation considering many different aspects as surface, structure and chemical composition. Important parameters as AV needs to be considered during the image acquisition in order to improve the outcomes from this technique. The rock sample was characterized with a different topographical structure with specific areas in the top of the peakes and in the transition to the vales with chemical composition shifts.

6 REFERENCE

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Lihui Li, Jian Yang, WangWei Liu, Pengfei Ren, Overview of the application of quantitative backscattered electron (QBSE) image analysis to characterize the cement-based materials, Construction and Building Materials, Volume 406, 2023, 133332, ISSN 0950-0618, https://doi.org/10.1016/j.conbuildmat.2023.133332

7 SELF-ASSESSMENT

7.1 PERSONAL SKILLS

Backscattered electron image (BEI) was an important part of this course since the beginning mainly due to the resilience of this technique for the evaluation sample according

to their chemical composition and topographical structure. This new strategy for sample evaluation becomes an attractive alternative for me as a researcher and PhD student.

7.2 IMAGE QUALITY

Many different strategies for imagining evaluation were discussed in the previous classes. The core of these techniques is linked to the management of the equipment and their electron beam. BEI is one the first artifacts that once applied in the acquired image we can be able to evaluate changes in the chemical composition and external structure of the sample from the interaction of the beam with the sample. Defining composition and structure from the image are an important advancement related to image quality.

ENERGY DISPERSIVE SPECTROMETRY (EDS)

ABSTRACT

Several techniques and artifacts start being applied with microscopy to improve particle studies and characterization. Energy dispersive spectrometer (EDS) accoupled in SEM (1) provides a broad-spectrum characterization of samples that does not require a priori knowledge of the specimen composition. In this report SEM technique will be explored throughout the evaluation of energy dispersive spectrometry (EDS) using parts of 2 watches, W1 and W2. A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images. W1 could be characterized as older than the W2 due to some careful evaluations of the gear's surfaces. W1 shows traces of Ag (Silver), Au (Golden) and Ni (Niquel) in different gears parts. The presence of Au indicates in this case that W1 could be more expensive than W2. W2 presented very interested traces of uniform coating with Mo (Molybdenum) in their watch hand. All images acquired from W2 indicated a lot of C (Carbon) traces in almost all watches parts, indicating possible plastic polymer composition. The gears in the W2 showed a preserved surface indicating that W2 is new compared with W1, and cheaper. EDS evaluations demonstrate enormous potential for chemical mapping and characterization according to the sample structure.

1 INTRODUCTION

New techniques for particle structure and chemical composition studies accoupled to SEM became a great strategy once we didn't have access to equipment's for specific characterizations. Several techniques and artifacts start being applied with microscopy to improve particle studies and characterization.

There have been a series of advancements in electron beam instruments and x-ray detectors which may make it possible to improve significantly the quality of results from the quantitative electron-probe analysis of individual particles. These advances include: (1) field- emission gun electron beam instruments such as scanning electron microscopes (FEG-SEMs) that have high brightness electron guns with excellent performance at low beam energies, $E0$ 10 keV and (2) high-resolution energy-dispersive x-ray spectrometers[2].

Energy dispersive spectrometer (EDS) accoupled in SEM (1) provides a broad-spectrum characterization of samples that does not require a priori knowledge of the specimen composition[1].

In this report SEM technique will be explored throughout the evaluation of energy dispersive spectrometry (EDS) using parts of 2 watches, W1 and W2, with JEOL JSM-IT100 InTouchScope™ available at Baker laboratory at ESF-SUNY. The lab report will assess the effects of energy-dispersive x-ray system to qualitatively analyze the elemental

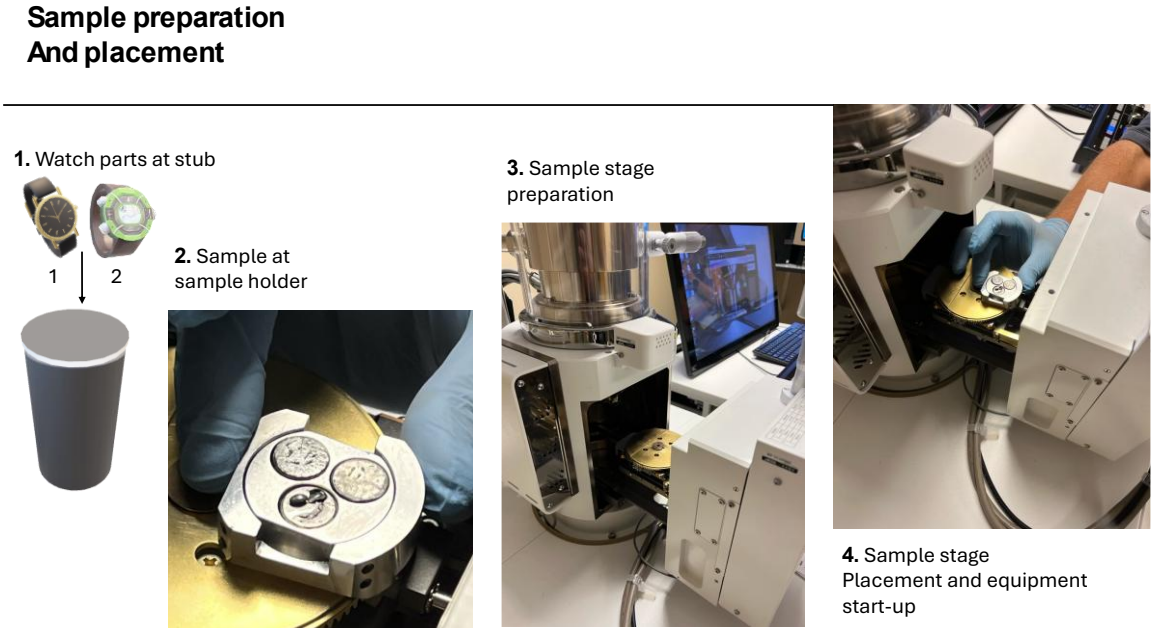
composition of materials and demonstrate the instrument's ability to imaging applying different configurations to evaluate the sample.

2 METHODS

2.1 SAMPLE PREPARATION

Fig. 1 shows the preparation steps and sample placement at the SEM equipment during this laboratory practices. Two watches parts was observed at the stub. In this laboratory practices the watches will be named as 1 and 2. At the end of this report the watches 1 and 2 will be identified according to their age and composition material.

Figure 1 – Sample preparation and placement at the sample holder and stage at SEM model JEOL JSM-IT100 InTouchScope™. 4 Steps of placement as following: 1. Sample at stub; 2. Sample at sample holder; 3. Sample stage preparation and 4. Sample stage placement and equipment start-up.



2.2 SEM

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY.

2.3 ENERGY DISPERSIVE SPECTROMETRY (EDS)

JEOL Energy Dispersive Microanalysis System with peltier cooled silicon. Drift Detector (SDD) and EDS software. This system includes all the hardware and software

components for multi-point analysis, image capture, hyper spectral mapping, line scan, drift correction, elemental quantification and reporting.

2.4 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to acquire all the images at the same aperture in this lab report using the JEOL JSM-IT100 and EDS.

Table 1 – SEM working parameters and their working values defined for the watch images. Watch 1(W1.1 to W1.4) and Watch 2 (W2.5 to W2.7). Images acquired with secondary electrons detector (SED) and energy dispersive spectrometry (EDS). SEM JEOL JSM-IT100 InTouchScope™ equipped with EDS

SEM parameters	**SED and EDS Images**						
	W1.1	**W1.2**	**W1.3**	**W1.4**	**W2.5**	**W2.6**	**W2.7**
V	HV	HV	HV	HV	HV	HV	HV
AV (kV)	20	20	20	20	20	20	20
Au_Pd coating	N	N	N	Y	N	N	N
PB	60	60	60	58	60	60	60
WD (mm)	10	9	10	10	13	13	13
M (x)	370	160	95	180	110	100	170
Objective aperture (X,Y), stage tilt (T and R) and z (Z) coordinates	X -3.225	X -2.205	X -1.003	NR	X 8.844	X 9.034	X 6.440
	Y -2.957	Y -7.515	Y -2.761	NR	Y -4.202	Y -7.095	Y -3.922
	T 0.000	T 0.000	T 0.000	T 0.000	T 0.000	T 0.000	T 0.000
	R 0.000	R 0.000	R 0.000	R 0.000	R 0.000	R 0.000	R 0.000
	Z 14.000	Z 14.000	Z 14.000	Z 14.000	Z 14.000	Z 14.000	Z 14.000

V = Vacuum; HV = High vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation, Z = z, Au_Pd coating = Gold-Palladium coating at sample surface, N = no presence, Y = presence, NR = non-registered.

2.5 MICROSCOPE MANAGEMENT STEPS

The image acquisition will be conducted with an instructor following the general steps bellow and their selected mode (Spectrum, line and mapping):

1. Obtain the samples of watch parts mounted on stubs.
2. Follow the instructions as in Lab 1 for inserting the specimen and setting the observation conditions (select EDS).
3. Observe the sample in Backscattered mode, focus and find the field of view. Use 15 to
4. 20 kV, objective aperture 2 or 3, WD 10, and select a larger probe current (60). 5. Adjust focus, astigmatism, align the objective aperture, and adjust contrast and brightness.
5. Select the EDS icon (Fig. 1) to go to the EDS page.

A. Spectrum

a) Select SPECTRUM and press START.
b) Note the counts per second (cps) and dead time (DT) to include in your report.
c) Collect spectrum; with table of elements, turn elements on/off as needed. Double
d) click on the elements desired, Ag, Au, Cu, Ni, Zn, and C.
e) Collect Spectrum and save report to your folder as a Word file. (Select PRINT).

B. Line

a) Use point tool to select multiple points on the sample.
b) Collect point analysis spectra.
c) A spectrum will be collected for each point Select PRINT to save the report as a Word file.

C. Mapping

a) Use the Map tool to collect x-ray maps for specific elements, Ag, Au, Cu, Ni, Zn, and C.
b) Select PRINT to save the report as a Word file
c) At any time, to EXIT EDS, click on OBS icon (same as EDS icon)

3 RESULTS

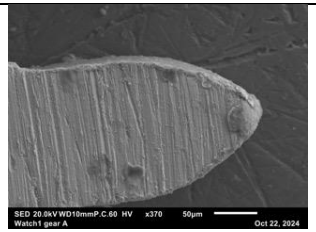
3.1 SEM IMAGES

SEM model JEOL JSM-IT100 InTouchScope™ is capable to achieve max accelerating voltage of 20 kV and magnificalton of 30.000 from the specification. In the following pictures random parts of a two watches, W1 and W2, was placed in a stub and observed applying different EDS strategies keeping the AV at 20 kV.

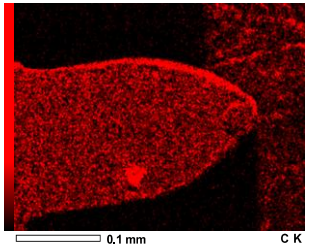
The set of images in fig. 2 shows uncoated and coated watches parts, W1 and W2. Watch 1(W1.1 to W1.4) and Watch 2 (W2.5 to W2.7). Images acquired with secondary electrons detector (SED) and energy dispersive spectrometry (EDS). W1.1 – image set 1 of uncoated gear tooth from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cu (Copper), Zn (Zinc), Ag (Silver), Au (Golden). Images accquired with AV: 20 kV, WD: 10 mm, PB: 60, M: 370x. W1.2 – image set 2 of uncoated gear base and tooth from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), Fe (Iron) and Ni (Niquel). Images accquired with AV: 20 kV,

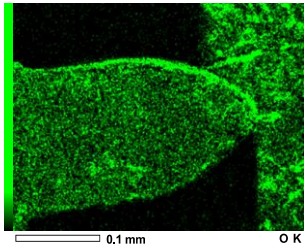
WD: 9 mm, PB: 60, M: 160x. W1.3 – image set 3 of uncoated gear center hole from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cl (Chlorine), Cu (Copper), Zn (Zinc). Images accquired with AV: 20 kV, WD: 10 mm, PB: 60, M: 95x. W1.4 – image set 4 of coated unknow component from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cu (Copper), Ag (Silver). Images accquired with AV: 20 kV, WD: 10 mm, PB: 58, M: 180x. W2.5 – image set 5 of uncoated internal gears from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Fe (Iron), Ni (Niquel). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 110x. W2.6 – image set 6 of uncoated watch hand from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), S (Sulfur), Ni (Niquel), Zi (Zinc), Mo (Molybdenum). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 100x. W2.7 - image set 7of uncoated internal gear from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Alluminium), Si (Silica) and Cl (Chlorine). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 170x.

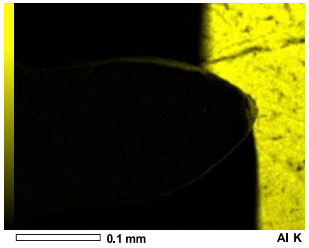
Figure 2 – Uncoated and coated watches parts, W1 and W2. Watch 1(W1.1 to W1.4) and Watch 2 (W2.5 to W2.7). Images acquired with secondary electrons detector (SED) and energy dispersive spectrometry (EDS). SEM JEOL JSM-IT100 InTouchScope™ equipped with EDS. W1.1 – image set 1 of uncoated gear tooth from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cu (Copper), Zn (Zinc), Ag (Silver), Au (Golden). Images accquired with AV: 20 kV, WD: 10 mm, PB: 60, M: 370x. W1.2 – image set 2 of uncoated gear base and tooth from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), Fe (Iron) and Ni (Niquel). Images accquired with AV: 20 kV, WD: 9 mm, PB: 60, M: 160x. W1.3 – image set 3 of uncoated gear center hole from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cl (Chlorine), Cu (Copper), Zn (Zinc). Images accquired with AV: 20 kV, WD: 10 mm, PB: 60, M: 95x. W1.4 – image set 4 of coated unknow component from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cu (Copper), Ag (Silver). Images accquired with AV: 20 kV, WD: 10 mm, PB: 58, M: 180x. W2.5 – image set 5 of uncoated internal gears from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Fe (Iron), Ni (Niquel). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 110x. W2.6 – image set 6 of uncoated watch hand from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), S (Sulfur), Ni (Niquel), Zi (Zinc), Mo (Molybdenum). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 100x. W2.7 - image set 7of uncoated internal gear from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Alluminium), Si (Silica) and Cl (Chlorine). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 170x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification.

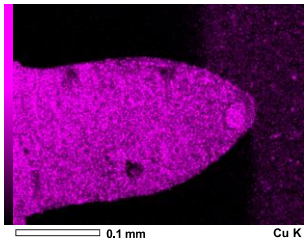


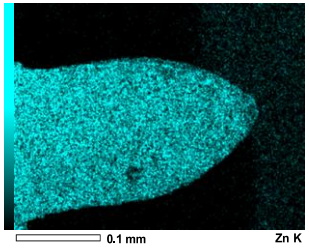
W1– parts composition map (Gear tooth)

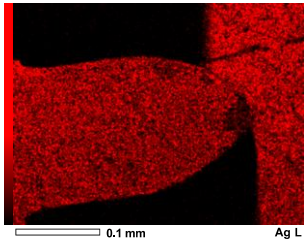


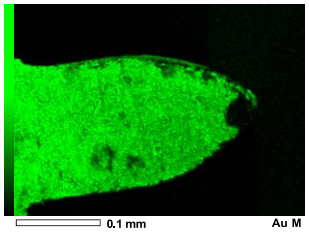


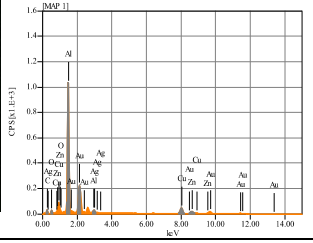




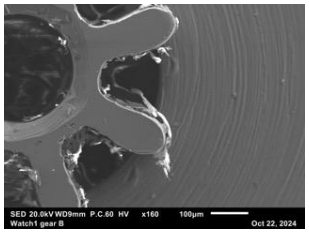




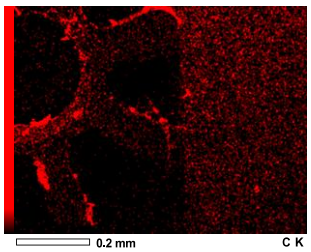


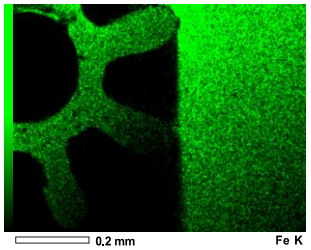


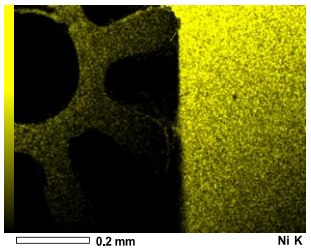
W1.1 – image set 1 of uncoated gear tooth from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cu (Copper), Zn (Zinc), Ag (Silver), Au (Golden). Images accquired with AV: 20 kV, WD: 10 mm, PB: 60, M: 370x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification.

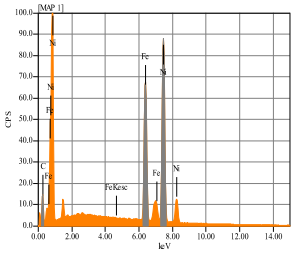


W1– parts composition map (Gear base and tooth)

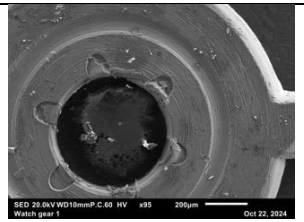




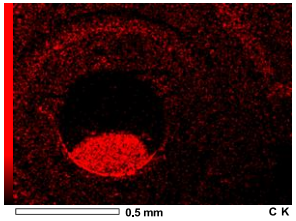


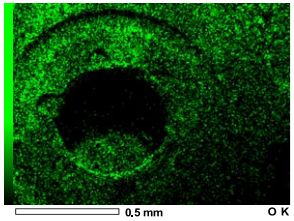


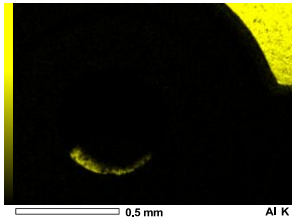
W1.2 – image set 2 of uncoated gear base and tooth from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), Fe (Iron) and Ni (Niquel). Images accquired with AV: 20 kV, WD: 9 mm, PB: 60, M: 160x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification

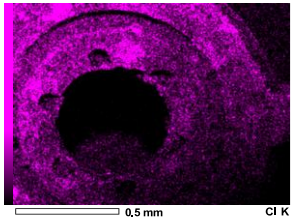


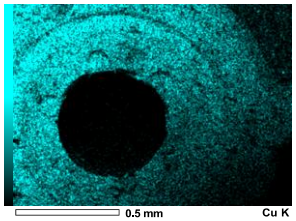
W1 – parts composition map (Gear center hole)

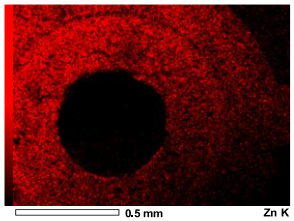


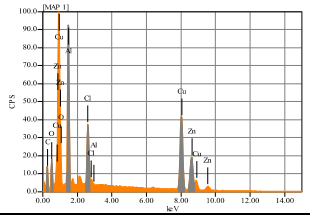




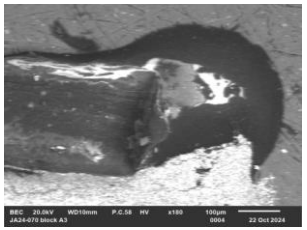




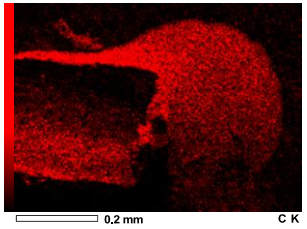


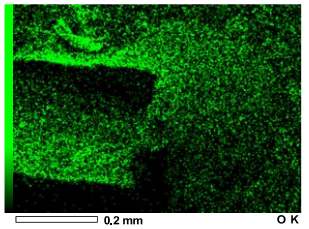


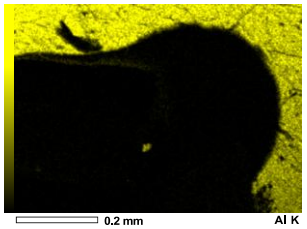
W1.3 – image set 3 of uncoated gear center hole from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cl (Chlorine), Cu (Copper), Zn (Zinc). Images accquired with AV: 20 kV, WD: 10 mm, PB: 60, M: 95x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification.

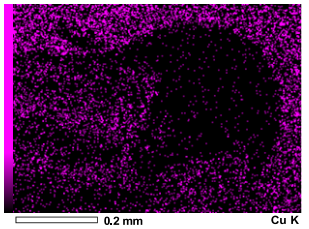


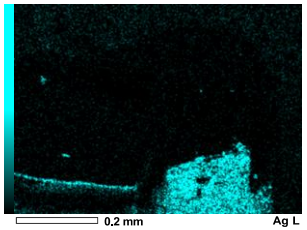
W1 – parts composition map (Unknow component)

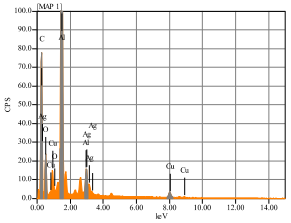




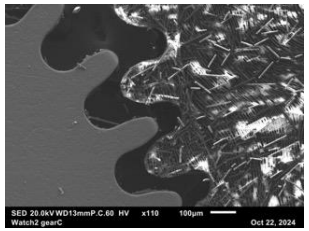




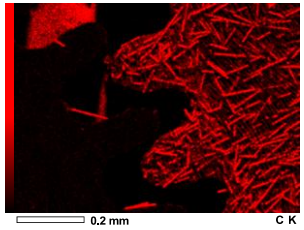


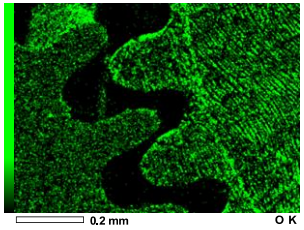


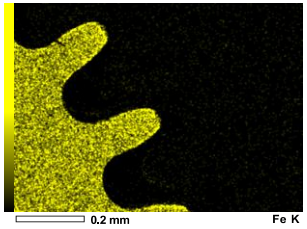
W1.4 – image set 4 of coated unknow component from W1. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Aluminium), Cu (Copper), Ag (Silver). Images accquired with AV: 20 kV, WD: 10 mm, PB: 58, M: 180x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification.

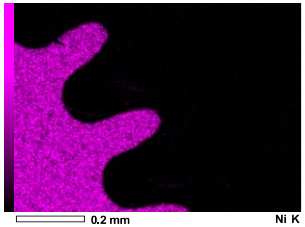


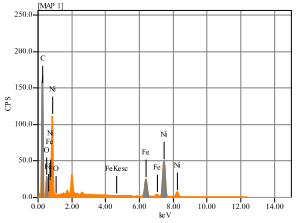
W2 – parts composition map (Internal gears)



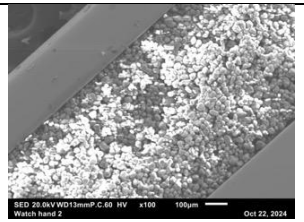




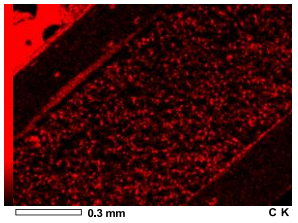


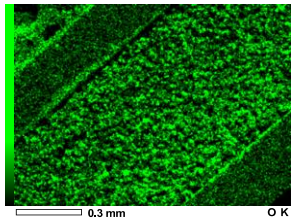


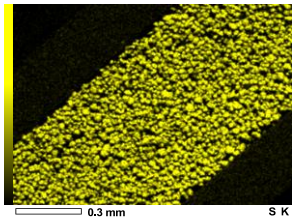
W2.5 – image set 5 of uncoated internal gears from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Fe (Iron), Ni (Niquel). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 110x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification.

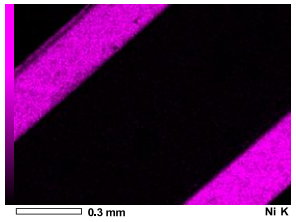


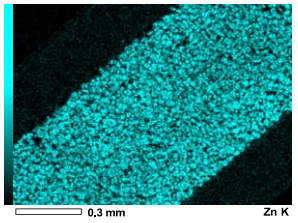
W2 – parts composition map (Watch hand)

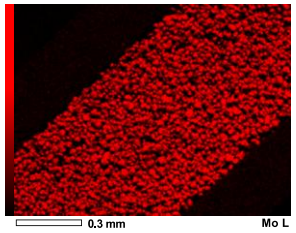


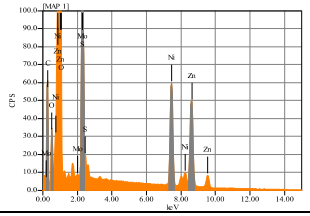




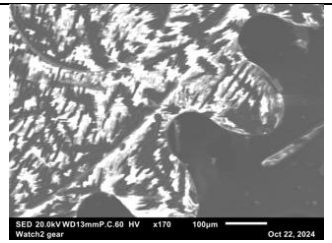




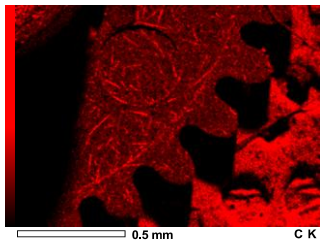


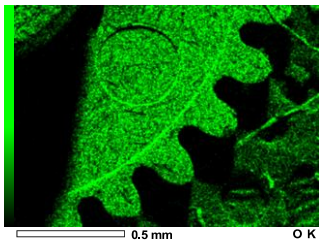


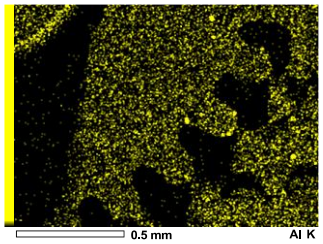
W2.6 – image set 6 of uncoated watch hand from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), S (Sulfur), Ni (Niquel), Zi (Zinc), Mo (Molybdenum). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 100x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification.

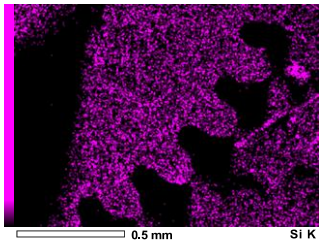


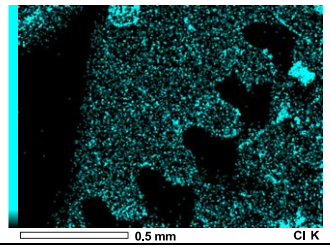
W2 – parts composition map (Internal gear)

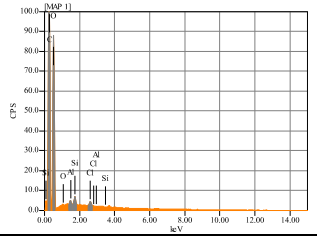












W2.7 - image set 7of uncoated internal gear from W2. Top to bottom, left to rigth: SED (Secondary electron transmission); EDS (Energy dispersive spectrometry) showing chemical composition of C (carbon), O (Oxygen), Al (Alluminium), Si (Silica) and Cl (Chlorine). Images accquired with AV: 20 kV, WD: 13 mm, PB: 60, M: 170x. AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification.

4 DISCUSSION

EDS allow us during this laboratory practices discover the different components and structures of each of this watches W1 and W2. This technique proof being efficient to determine in a short period of analysis the chemical composition and their traces of the W1 and W2.

W1 could be characterized as older than the W2 due to some careful evaluations of the gear's surfaces. W1 shows traces of Ag (Silver), Au (Golden) and Ni (Niquel) in different gears parts. The presence of Au indicates in this case that W1 could be more expensive than W2.

W2 presented very interested traces of uniform coating with Mo (Molybdenum) in their watch hand. All images acquired from W2 indicated a lot of C (Carbon) traces in almost all watches parts, indicating possible plastic polymer composition. The gears in the W2 showed a preserved surface indicating that W2 is new compared with W1, and cheaper.

5 CONCLUSION

This practices use of the materials from 2 types of watches in different stages of life cycle and made from different materials and probably different costs, as samples to evaluate the application of EDS technique accoupled to SEM. EDS evaluations demonstrate enormous potential for chemical mapping and characterization according to the sample structure.

6 REFERENCES

(1) Brintlinger, Todd & Buckhout-White, Susan & Bassim, Nabil & Mathur, Divita & Samanta, Anirban & Robinson, Jeremy & Idrobo, Juan-Carlos & Stroud, Rhonda & Goldman, Ellen & Ancona, Mario. (2020). Chemical Mapping of Unstained DNA Origami Using STEM/EDS and Graphene Supports. ACS Applied Nano Materials. 3. 10.1021/acsanm.9b02574

(2) Small, J. (2002). The Analysis of Particles at Low Accelerating Voltages (10 kV) With Energy Dispersive X-Ray Spectroscopy (EDS). Journal of Research of the National Institute of Standards and Technology. 107. 10.6028/jres.107.047.

7 SELF-ASSESSMENT

7.1 PERSONAL SKILLS

Energy dispersive spectroscopy (EDS) was one of the most important advanced tools accoupled to the SEM equipment we evaluate since the begging of our semester. The

opportunity to have different techniques to complete sample study of structure and chemical composition in only one equipment is “priceless”. The learning from this technique was a significate improvement for my technical development as an nanotechnologist.

7.2 IMAGE QUALITY

Resolution is one the first sentences that come to my mind when we discuss image quality. In this case, applying EDS won’t result in better image qualities, however, show significative information’s linked to the sample structure and composition. This characteristics already discussed in these report becomes as unique strategy accoupled to the SEM equipment for samples evaluations.

STEM

INSTRUMENT BASICS: SECONDARY ELECTRON IMAGING QUALITY IMAGE FOR PUBLICATION

ABSTRACT

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory practices. The outcome for this report is a high quality image of an ladybug mandible for publication with descriptive figure legend. The image acquired in the secondary electron detector mode (SE) shown a detailed of the mandible area and their whiskers applying AV: 10 KV, WD:13 mm, Magnification: 1000x and Scale: 10µm. The image acquisition represents a key step to obtain pictures that real represent the structure that we are evaluating in the equipment. This image is an example of application of secondary electron detector in SEM to acquire pictures using different microscopy settings and image quality treating for publication.

1 INTRODUCTION

The purpose of this laboratory is to learn the basic operation of the microscope and how to acquire a secondary electron image. You will learn how to manipulate the electron beam to produce high quality images. A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory practices, SEM is located at Backer Laboratory at ESF – SUNY, the outcome for this report is a high quality image for publication with descriptive figure legend.

2 METHODS

2.1 SAMPLE

The purpose of this laboratory is to learn the basic operation of the microscope and how to acquire a secondary electron image. You will learn how to manipulate the electron beam to produce high quality images. A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory practices. An image in the mandible area was acquired for treatment and figure legend description.

2.2 SEM

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY.

2.3 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to capture the selected image in this lab report using the JEOL JSM-IT100.

Table 1 – SEM working parameters and their working values

SEM* parameters	Figure 1
Vacuum (V) (Pa)	HV
Accelerating Voltage (AV) (kV)	10
Probe Current (PB)	50
Working Distance (WD) (mm)	13
Magnification (M) (x)	1000
Objective aperture (OA) (X,Y), stage tilt (T), rotation (R) and z (Z).	X -3.313
	Y 1.682
	T 0.000
	Z 15.000
	R 0.000

*SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS. V = Vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation and Z =z.

2.4 MICROSCOPE MANAGEMENT STEPS

With assistance from the instructor follow the steps described below:

1. Select a sample from the prepared samples developed for this course.
2. Secure the sample into the sample holder.
3. Look at the stage Z control (working distance or height) on the front of the chamber, and
4. make sure it is at 20 mm. This is to ensure that the stage does not hit and damage the
5. backscatter detector. Z should never be less than 10 mm.
6. Open the InTouch software. Select CHANGE icon to open the sample chamber and insert the sample.
7. Follow the software instructions to insert the sample. When prompted by the software, measure sample height, adjust stage height (Z), and take a picture of the stage.
8. The software will then ask you to select the appropriate Observation Conditions for your sample. With instructor assistance, select the conditions.

9. When prompted, close the chamber door, and evacuate the chamber.
10. The software will automatically provide the Observation Conditions for the sample type that you selected: accelerating voltage, and Probe Current and detector.
11. With assistance adjust kV to 10, magnification to 500, focus and auto-adjust the brightness and contrast (ABC)
12. Use the stage navigation system (SNS button), move the stage to place your sample under the electron beam.
13. Focus image and adjust ABC as needed.
14. Select objective aperture 2 (on column)
15. Center the objective aperture:

a) Press “wobbler” icon.
b) Adjust knobs on aperture holder (on column) until movement is minimized in both the X and Y directions.

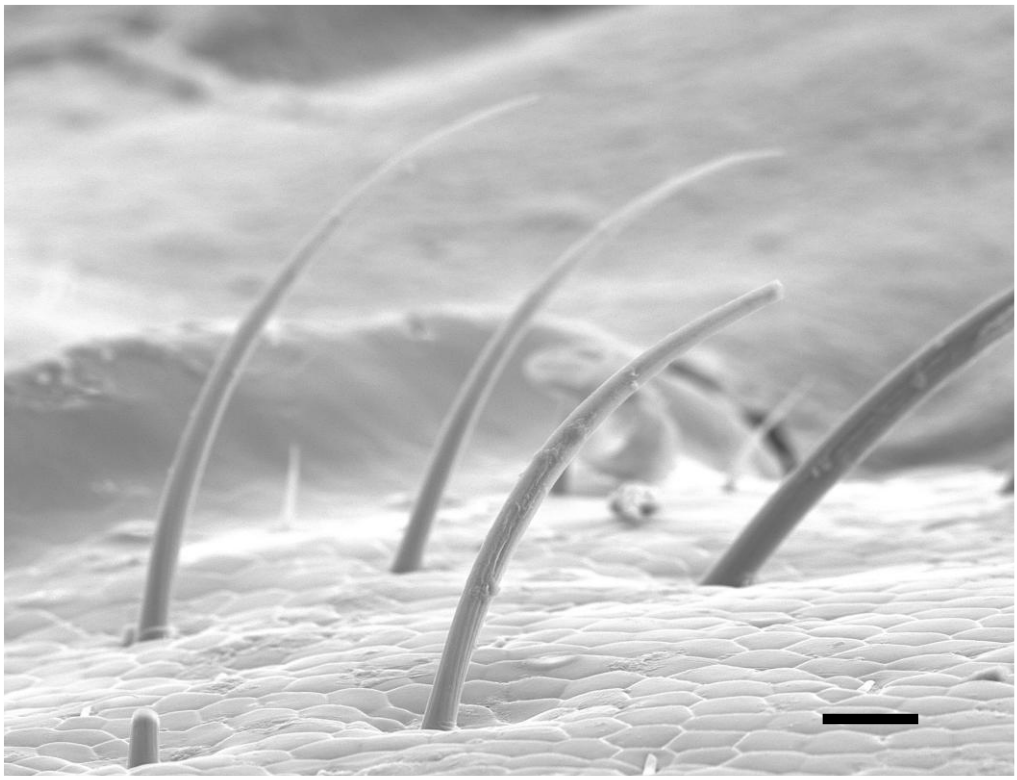
16. For the first image, set the probe current (P.C) to 30.
17. To focus, increase magnification to higher than desired, and focus.
18. Optimize focus, brightness, and contrast.
19. Adjust astigmatism.
20. Return to desired magnification.
21. To save images to your folder, and label your image, go to the Conditions button; instructor will assist.
22. Collect the image (Photo scan) and save to your folder.
23. With assistance, turn off the beam (OBSERVE off), and vent the chamber, or use the CHANGE button to vent the chamber.
24. Before opening the chamber, return Z to 20 mm. 26. Remove sample and evacuate the chamber.

3 RESULTS

3.1 IMAGE FOR PUBLICATION

Figure 1 shows a Ladybug mandible whiskers. This image acquired in the secondary electron detector mode (SE) shown a detailed of the mandible area and their whiskers applying AV: 10 KV, WD:13 mm, Magnification: 1000x and Scale: $10\mu m$.

Figure 1 – Ladybug mandible whiskers. Image acquired in the secondary electron detector mode (SE). Detailed of the mandible area and their whiskers are shown. AV: 10 KV. WD:13 mm. Magnification: 1000x and Scale bar: $10\mu m$.



4 CONCLUSION

The image acquisition represents a key step to obtain pictures that real represent the structure that we are evaluating in the equipment. This image is an example of application of secondary electron detector in SEM to acquire pictures using different microscopy settings and image quality treating for publication.

5 SELF-ASSESSMENT

5.1 PERSONAL SKILLS

This laboratory practice reinforces my ability to evaluate micro and nanoscale materials form biological and non-biological sources. In this case my experience from the last semester was applied into this process to acquire the image in this class.

5.2 IMAGE QUALITY

The first step after download and receive the digital picture acquired from the SEM was start treating the image and add a properly figure legend. Significative parameters as brightness were changed and finally the most representative area cropped to illustrate the focused aimed area.

POLYMER NEGATIVE STAINING USING STEM MODE

ABSTRACT

Why stain cellulosic material in the laboratory? Numerous reasons have emerged over the course of time, and some of the more important are listed as identification of fiber type, chemical components; detection of microstructure, fibrils, mycelia, biofilms; and qauntification and estimation of degree of fibrilation, changes due process, surface area, pores and size. A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the STEM images. Uranyl acetate (UO2(CH3COO)2'2H2O) and lead citrate (Pb3(C6H5O7)2) will be used for NCC coating and further observation in electron microscopy, thus the purpose of this laboratory is to analyze the effect of negative stains on the qualities of a scanning transmission electron microscope (STEM) image. The staining was divided in 4 major steps: (1) NCC dispersion in water and drop formation in the surface of an glass slide; (2) Sample placement at SEM cupper grid; (3) Grid+sample coating in uranyl acetate immersion and (4) Last step of Grig+sample coating in lead citrate immersion. After these treatments, the grid was left to dry and storage to further observation. Image acquired in the secondary electron detector mode (SE) for STEM and NCC in the grid surface was observed at AV: 20 KV; WD:12 mm magnification: 60000x and scale bar: 0.2 μm.

1 INTRODUCTION

Analytical staining methods can be regarded as relatively simple, rapid, low-cost, and widely practiced for identification of fibers. Why stain cellulosic material in the laboratory? Numerous reasons have emerged over the course of time, and some of the more important are listed as identification of fiber type, chemical components; detection of microstructure, fibrils, mycelia, biofilms; and qauntification and estimation of degree of fibrilation, changes due process, surface area, pores and size [5].

Staining is one of the more traditional methods to identify a component or polymer in a sample. Since 1940`s various techniques have been adapted from protein chemistry to the cytological demonstration of proteins *(e.g.,* ninhydrin reaction [1]; Millon reaction [2] and Sakaguchi reaction [3,4]. Exist a number of techniques for protein separation and identification and each of then present their protocol.

The process of staining a material inherently involves several different aspects.

Some of these involve the colorant, including why certain compounds absorb light of different wavelengths. One also needs to be concerned with how and to what extent the colorant is retained on the target material. Presumably the amount of uptake will be closely related to the depth of coloration. Uptake of dye clearly depends on both chemical and physical aspects, and such issues will be considered [5].

Uranyl acetate (UO2(CH3COO)2'2H2O) and lead citrate (Pb3(C6H5O7)2) will be used for NCC coating and further observation in electron microscopy, thus the purpose of this laboratory is to analyze the effect of negative stains on the qualities of a scanning transmission electron microscope (STEM) image.

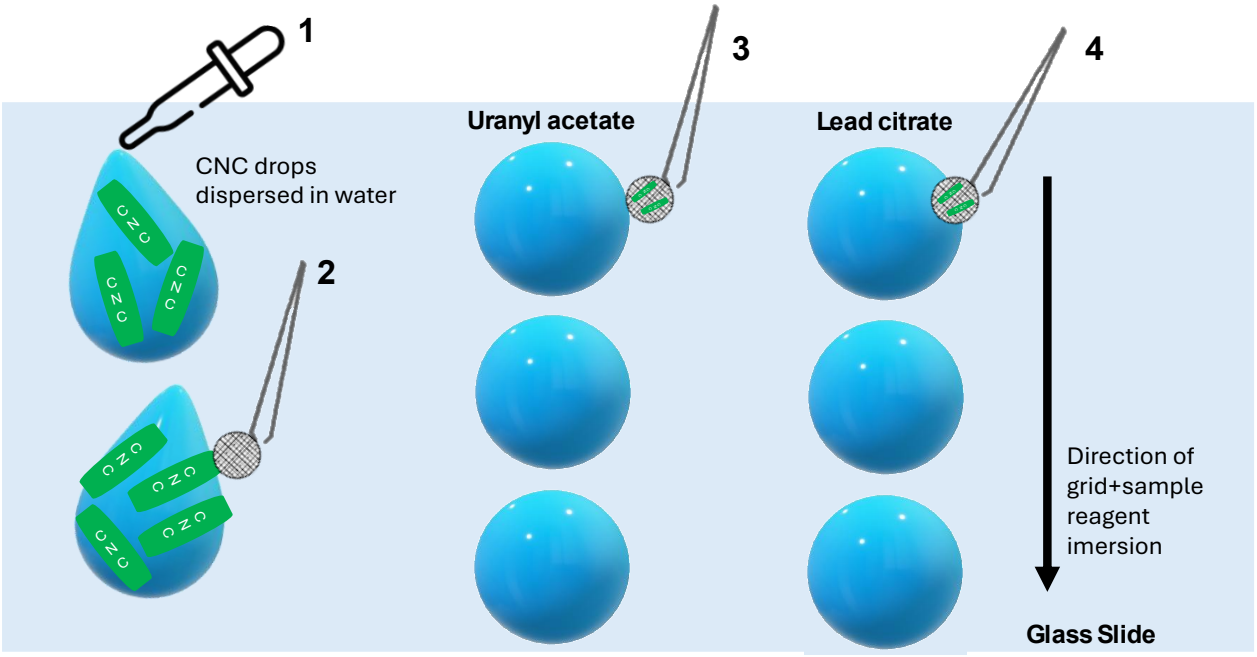
2 METHODS

2.1 SAMPLE PREPARATION

Nanocrystalline cellulose (NCC) with and without negative staining. This will be accomplished by producing micrographs of drop-casted NCC in the transmission mode using the highest magnification possible.

Figure 1 shows how the NCC dispersed in water was treated with the negative stains of uranyl acetate and lead citrate in 4 steps.

Figure 1 - Nanocrystalline cellulose (NCC) negative staining. The staining was divided in 4 major steps: (1) NCC dispersion in water and drop formation in the surface of an glass slide; (2) Sample placement at SEM cupper grid; (3) Grid+sample coating in uranyl acetate immersion and (4) Last step of Grig+sample coating in lead citrate immersion. After these treatments, the grid was left to dry and storage to further observation.



2.2 STEM MODE

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer

Laboratory at ESF – SUNY. An STEM adaptor was used to place the grid for image acquisition and observation.

2.3 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to capture the selected image in this lab report using the JEOL JSM-IT100.

Table 1 – STEM working parameters and their working values

STEM* parameters	Parameters
Vacuum (V) (Pa)	High vacuum
Accelerating Voltage (AV) (kV)	20
Probe Current (PB)	35
Working Distance (WD) (mm)	12
Magnification (M) (x)	60000
Objective aperture (OA) (X,Y), stage tilt (T), rotation (R) and z (Z).	2.704
	-0.315
	0.000
	0.000
	23.000

*STEM mode using SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS. V = Vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation and Z =z.

2.4 MICROSCOPE MANAGEMENT STEPS

With assistance from the instructor follow the steps described below:

1. The nanocrystalline cellulose (NCC) was dried overnight at 105 ^{0}C and stored in a dissector.
2. A small quantity of NCC (< 1 mg) was suspended in a drop of DI water.
3. A formvar treated TEM grid was dipped into the droplet and then allowed to air dry.
4. With assistance, following published instructions, load the TEM grid into the STEM holder.
5. Load the STEM adapter safely into the SEM.
6. Evacuate the chamber.
7. Identify and state the function of each component and control on the instrument.
8. The STEM adapter is 11mm, tall above the stage.
9. With assistance set the working distance to 21 mm and Z-focus the instrument

10. Manipulate the stage to place your sample under the electron beam at a working distance of 10.
11. Set accelerating voltage to 20 kEV
12. With assistance center aperture, optimize filament saturation, and gun alignment
13. Focus image at low magnification.
14. Zoom/Focus the image centering on particles stuck on the formvar.
15. Continue to zoom in until the copper grid in outside the field of view.
16. Adjust Brightness and Contrast
17. Adjust probe current
18. Optimize stigma, focus, brightness, and contrast
19. Capture images of sample.
20. Vent Chamber
21. Remove sample
22. Treat the NCC by dipping the same grid, as in previous steps, 3 times in 1% Urinal Acetate and 3 times in 1% lead citrate.
23. Independently secure the stained grid onto the STEM holder.
24. Independently insert the holder into the SEM stage.
25. Have Instructor check your work
26. Evacuate the chamber.
27. Identify and state the function of each component and control on the instrument.
28. Independently optimize instrument.
29. Adjust Brightness and Contrast
30. Set the probe current to optimize image
31. Focus image at low magnification.
32. Zoom/Focus the image centering on particles stuck on the formvar.
33. Continue to zoom in until the copper grid in outside the field of view.
34. Perfect image quality
35. Capture image
36. Remove sample from Stage and evacuate the chamber
37. Follow lab procedures for securing SEM and cleanup work area.
38. Have Instructor check your work.

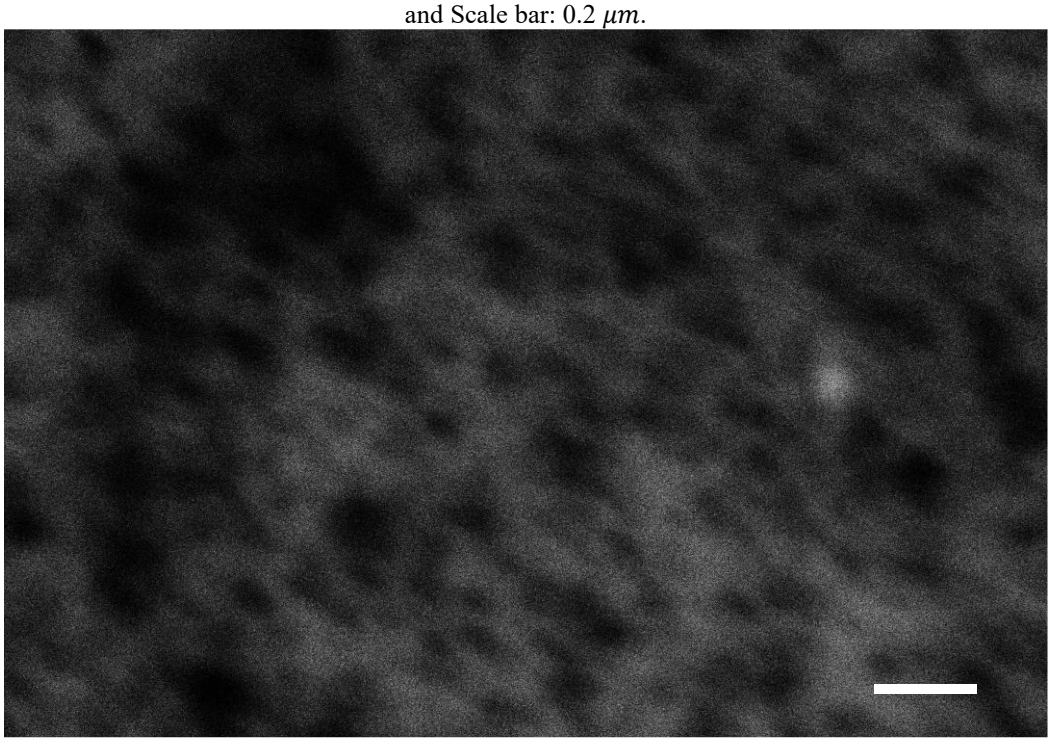
3 RESULTS

3.1 STEM IMAGES OF NCC NEGATIVE STAINING

There are many references indicating a preferential adsorption of direct dyes onto cellulose, either in the pure form of cellulose or when it is believed to be exposed at surfaces accessible to the aqueous media. NCC is one of this cases, once this nanomaterial is even more accesible thus the fiber surface.

Figure 2 shows Nanocrystalline cellulose (NCC) negative staining. Image acquired in the secondary electron detector mode (SE) for STEM. Detailed of NCC in the grid surface. AV: 20 KV. WD:12 mm. Magnification: 60000x and Scale bar: 0.2 μm.

Figure 2 – Nanocrystalline cellulose (NCC) negative staining. Image acquired in the secondary electron detector mode (SE) for STEM. Detailed of NCC in the grid surface. AV: 20 KV. WD:12 mm. Magnification: 60000x and Scale bar: 0.2 μm.



4 CONCLUSION

NCC dispersed in water was coated using uranyl acetate (UO2(CH3COO)2'2H2O) and lead citrate (Pb3(C6H5O7)2) and the image acquired presented a poor resolution due to the high magnification.

5 SELF-ASSESSMENT

5.1 PERSONAL SKILLS

This laboratory practice reinforces my ability to understanding the chemical interactions with different tissues and materials. Many types of staining`s strategies exist accordingly the interaction the functional groups of the molecules and the dyes or specific chromophores agents choose for this evaluation.

5.2 IMAGE QUALITY

For staining evaluation the image was an good exercise, however the quality was poor due to the high magnification assumed to acquire from the sample.

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WOOD SECTIONS IMBEDDED IN RESIN

ABSTRACT

Biological samples could be classified as challenge materials due to their specificity and 3D structure. Thus, find the right techniques to prepare and make the correct structures self-available for further observation is a key to obtain great microscope pictures. A wood sample from sugar maple (Acer saccharum) and their preparation, sectioning, their tool manipulation and image acquisition in the STEM mode using an SEM is the objective of this laboratory report. 3 major steps was followed: 1 – Sample and tools (Sugar Maple (Acer saccharum) wood samples has been treated with hot water (80 0C for 3 hrs.), dried (105 0C for 24 hrs.), and embedded into a resin block of Arldite 502 stained in Osmium. The knife tools: a bolt with the cutting knife prepared to be used in the ultramicrotomy and an apparatus with a wooden toothpick prepared to individualize the thin wood sections in the water reservoir in the bolt). 2 – Ultramicrotomy sectioning and sample transfer (Embedded wood was placed at the support in the ultramicrotomy following the knife orientation and the thin sections was prepared. The thin wooden section was transferred for an cupper grid for further evaluation). 3 – Grid storage (With the grid and the wooden sample in the top, the excess of water was removed and the conjunct storage in the grid box). Detailed transversal wood sections in the grid surface was obtained using AV: 20 KV, WD: 20 mm, magnification: 150x and scale bar: 100 μm. and AV: 20 KV, WD: 21 mm, magnification: 8500x and scale bar: 2 μm. Sample preparation, sectioning and placement in the grid for STEM observation play a role to guarantee enough resolution in the acquired pictures using electron microscopy. The sectioning step produced a nice thin slices of wood samples, however their observation using STEM mode result in pictures with lower resolution instead to observe the wood structure in SEM mode.

1 INTRODUCTION

Many sectioning, staining and chemical techniques are available for biological sample preparation and evaluation using electron (SEM, STEM, TEM), light (LM), fluorescence (FM) and confocal microscopy (CM). Biological samples could be classified as challenge materials due to their specificity and 3D structure. Thus, find the right techniques to prepare and make the correct structures self-available for further observation is a key to obtain great microscope pictures.

To sectioning in ultrathin films the ultramicrotomy permits the preparation of thin sections to observe the actual structure in a bulk material [1]. For sectioning, an ultra-microtome, equipped with a diamond knife or a glass knife, is normally preferred [1].

In adition to the sectioning step for sample prepare, is the process of staining a material inherently involves several different aspects. Some of these involve the colorant, including why certain compounds absorb light of different wavelengths. One also needs to be concerned with how and to what extent the colorant is retained on the target material [2].

Light microscopes are the stand by instruments of wood anatomists even in these days of electron microscopy and ultraviolet microspectroscopy. However, the great advantage of the SEM over the LM and TEM is that, having a vastly greater depth of field, the subject is viewed in its three-dimensional structure and more detailed observations are possible due to the higher resolution [3].

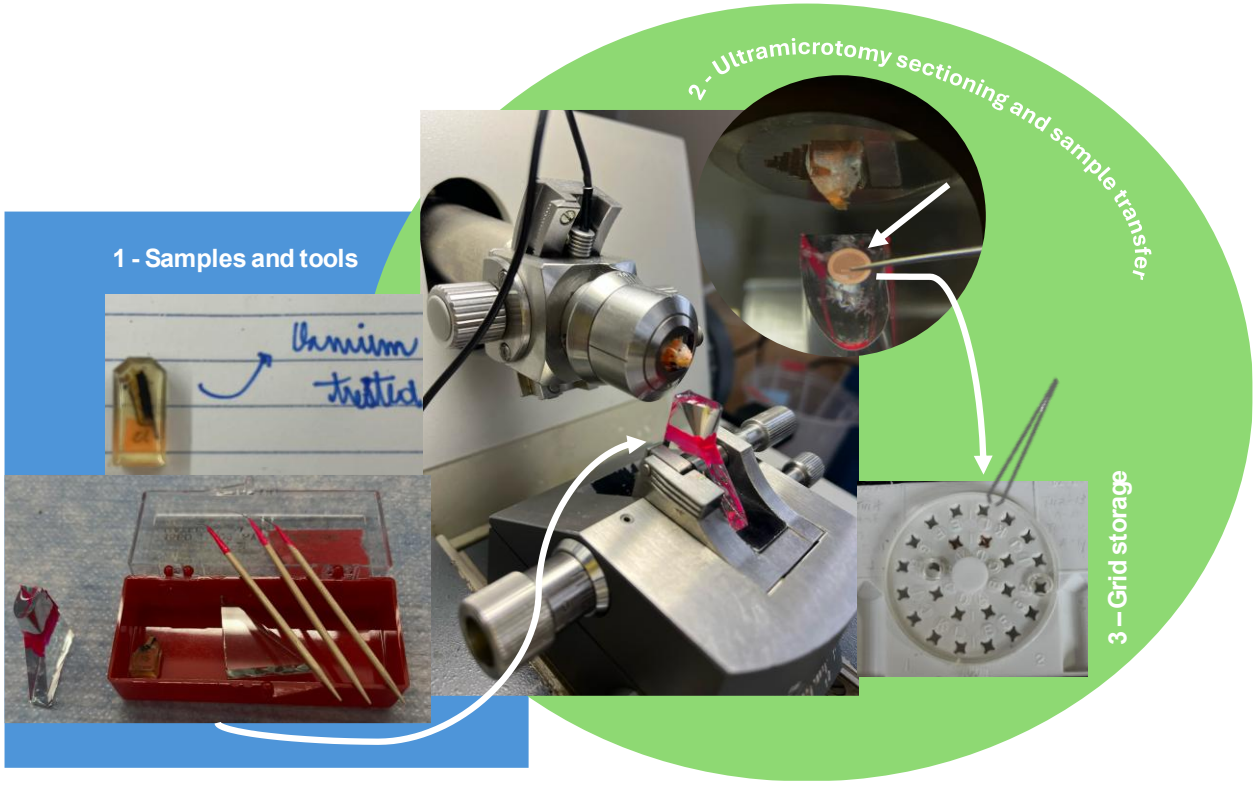
A wood sample from sugar maple *(Acer saccharum)* and their preparation, sectioning, their tool manipulation and image acquisition in the STEM mode using an SEM is the objective of this laboratory report.

2 METHODS

2.1 SAMPLE PREPARATION

Figure 1 shows the 3 major steps to prepare thin sections of the embedded wood sample to prepare and STEM grid for electron microscopic evaluation: A – Sample and tools (Sugar Maple *(Acer saccharum)* wood samples has been treated with hot water (80 ^{0}C for 3 hrs.), dried (105 ^{0}C for 24 hrs.), and embedded into a resin block of Arldite 502 stained in Osmium. The knife tools: a bolt with the cutting knife prepared to be used in the ultramicrotomy and an apparatus with a wooden toothpick prepared to individualize the thin wood sections in the water reservoir in the bolt). B – Ultramicrotomy sectioning and sample transfer (Embedded wood was placed at the support in the ultramicrotomy following the knife orientation and the thin sections was prepared. The thin wooden section was transferred for an cupper grid for further evaluation). C – Grid storage (With the grid and the wooden sample in the top, the excess of water was removed and the conjunct storage in the grid box).

Figure 1 – Sample preparation for STEM evaluation. 3 major steps was followed: 1 – Sample and tools (Sugar Maple *(Acer saccharum)* wood samples has been treated with hot water (80 ^{0}C for 3 hrs.), dried (105 ^{0}C for 24 hrs.), and embedded into a resin block of Arldite 502 stained in Osmium. The knife tools: a bolt with the cutting knife prepared to be used in the ultramicrotomy and an apparatus with a wooden toothpick prepared to individualize the thin wood sections in the water reservoir in the bolt). 2 – Ultramicrotomy sectioning and sample transfer (Embedded wood was placed at the support in the ultramicrotomy following the knife orientation and the thin sections was prepared. The thin wooden section was transferred for an cupper grid for further evaluation). 3 – Grid storage (With the grid and the wooden sample in the top, the excess of water was removed and the conjunct storage in the grid box).



2.2 STEM MODE

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY. A STEM adaptor was used to place the grid for image acquisition and observation.

2.3 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to capture the selected image in this lab report using the JEOL JSM-IT100.

Table 1 – STEM working parameters and their working values

STEM* parameters	Parameters for figure 1 and 2
Vacuum (V) (Pa)	High vacuum
Accelerating Voltage (AV) (kV)	20 and 20
Probe Current (PB)	35 and 56
Working Distance (WD) (mm)	20 and 21
Magnification (M) (x)	150 and 8500
Objective aperture (OA) (X,Y), stage tilt (T), rotation (R) and z (Z).	2.573 and 3.035
	-1.180 and -0.142
	0.000 and 0.000
	0.000 and 0.000
	30.000 and 30.000

*STEM mode using SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS. V = Vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation and Z =z.

2.4 MICROSCOPE MANAGEMENT STEPS

With assistance from the instructor follow the steps described below:

1. Use a new safety edge razor blade to trim their sample in the resin block into a trapezoid needed for effective sectioning.
2. Wearing goggles follow the procedures to cut class bars into sharp glass knives.
3. Using metallic tape attach a boat to your sharpest knives.
4. With assistance, following published instructions to load the trimmed resin block into the ultramicrotome and use a facing knife to prepare the surface of the trapezoid.
5. With assistance, following published instructions to use a cutting knife with boat to produce sections and collect them on a mesh TEM Grid.
6. Stain the grid with 1% urinal acetate and 5% lead citrate.
7. Load a grid into the STEM adapter
8. Load the STEM adapter safely into the SEM.
9. Evacuate the chamber.
10. Identify and state the function of each component and control on the instrument.
11. The STEM adapter is 11mm, tall above the stage.
12. With assistance set the working distance to 21 mm and Z-focus the instrument
13. Manipulate the stage to place your sample under the electron beam at a working distance of 10.

14. Set accelerating voltage to 20 kEV
15. With assistance center aperture, optimize filament saturation, and gun alignment
16. Focus image at low magnification.
17. Zoom/Focus the image centering on particles stuck on the formvar.
18. Continue to zoom in until the copper grid in outside the field of view.
19. Adjust Brightness and Contrast
20. Adjust probe current
21. Optimize stigma, focus, brightness, and contrast
22. Capture images of sample.
23. Vent Chamber
24. Remove sample
25. Repeat steps 1 through 24 until you have obtained a quality STEM image of the wood sample.
26. Follow lab procedures for securing SEM and cleanup work area.

3 RESULTS

3.1 STEM IMAGES OF *ACER SACCHARUM* WOOD SAMPLES

Figures 2 and 3 bellow shows sugar maple *(Acer saccharum)* wood sample. Transversal sectioning prepared from wood embedded into a resin block of Araldite 502 stained in Osmium. Image acquired in the secondary electron detector mode (SE) for STEM. For figure 2 detailed of transversal section in the grid surface followed: AV: 20 KV, WD: 20 mm, magnification: 150x and scale bar: 100 μm. For figure 3 detailed of transversal section in the grid surface followed: AV: 20 KV, WD: 21 mm, magnification: 8500x and scale bar: 2 μm.

Figure 2 – Sugar Maple (Acer saccharum) wood sample. Transversal sectioning prepared from wood embedded into a resin block of Araldite 502 stained in Osmium. Image acquired in the secondary electron detector mode (SE) for STEM. Detailed of transversal section in the grid surface. AV: 20 KV. WD: 20 mm. Magnification: 150x and Scale bar: 100 μm.

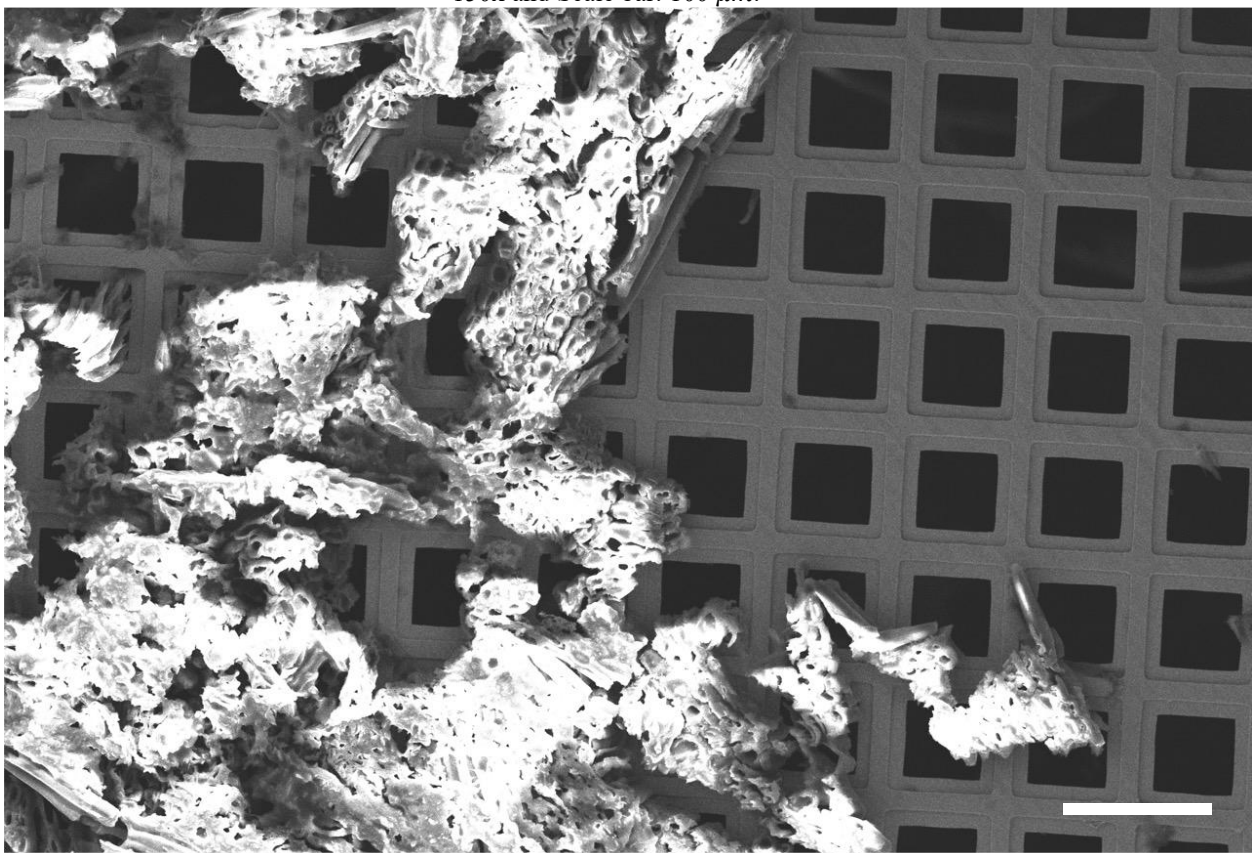
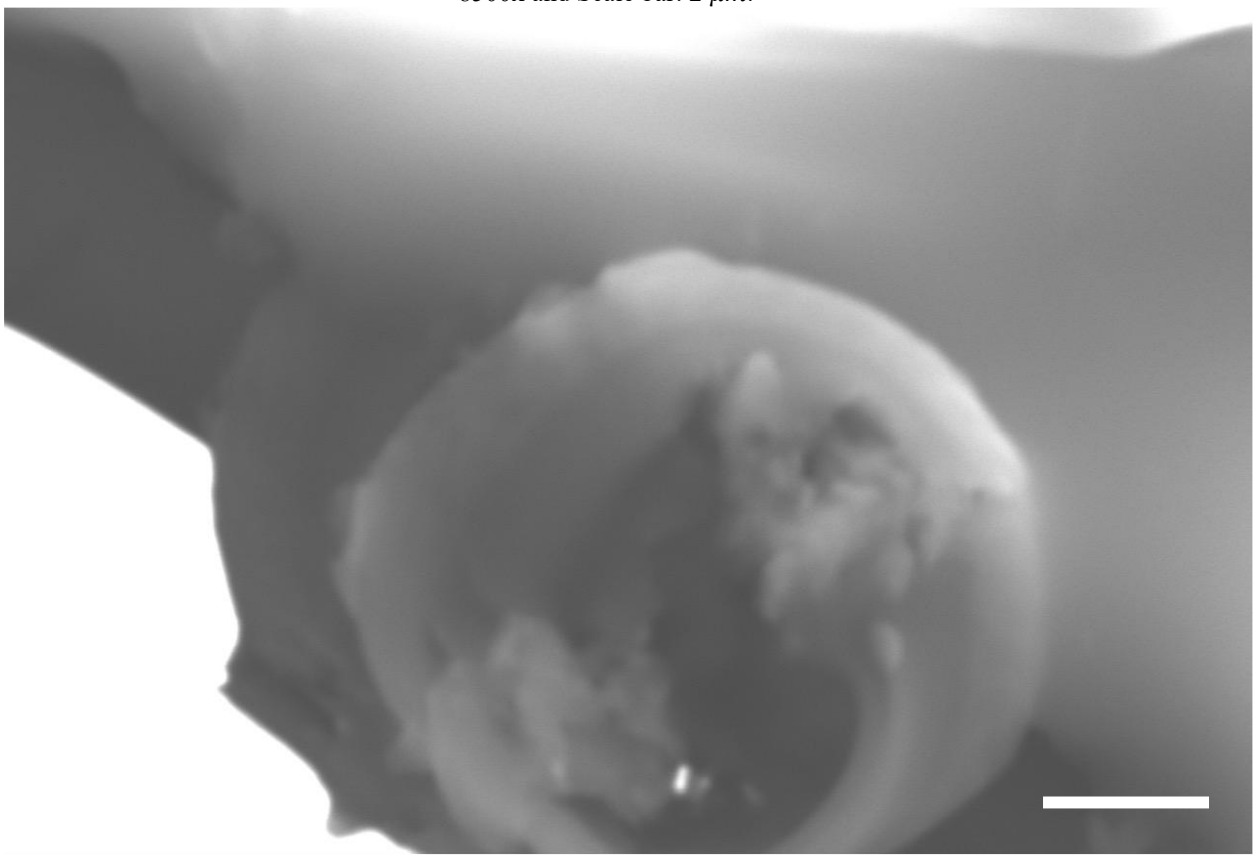


Fig. 2 was acquired in the are out of the beam in STEM mode, reflecting SEM quality and resolution to evaluate this type of sample. It is evident and clearly the transversal section of wood exemplified by the fiber agglomerates and their lumens in the picture. Unfortunately, due the lack of time during the class this sample was not explored once the STEM mode was the objective as showed in fig. 3 bellow.

Fig. 3 shows and magnified region in the wooden sample without enough resolution to explain their structure in this laboratory practices.

Figure 3 – Sugar Maple (Acer saccharum) wood sample. Transversal sectioning prepared from wood embedded into a resin block of Araldite 502 stained in Osmium. Image acquired in the secondary electron detector mode (SE) for STEM. Detailed of transversal section in the grid surface. AV: 20 KV. WD: 21 mm. Magnification: 8500x and Scale bar: 2 μm.



4 CONCLUSION

Sample preparation, sectioning and placement in the grid for STEM observation play a role to guarantee enough resolution in the acquired pictures using electron microscopy. The sectioning step produced a nice thin slices of wood samples, however their observation using STEM mode result in pictures with lower resolution instead to observe the wood structure in SEM mode. High magnification could be one of the factors that have contribute for the poor resolution in the STEM mode.

5 SELF-ASSESSMENT

5.1 PERSONAL SKILLS

Sample preparation step and their tools become a key step for this image production. The management of this ultrathin and sectioning tools improve our hand skills to understanding the correct manipulation for this samples production.

5.2 IMAGE QUALITY

The sectioning step produced a nice thin slices of wood samples, however their observation using STEM mode result in pictures with lower resolution instead to observe the wood structure in SEM mode. High magnification could be one of the factors that have contribute for the poor resolution in the STEM mode

6 REFERENCES

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TEM SAMPLE PREPARATION AND PROCESSES

ABSTRACT

The purpose of this laboratory is to produce STEM micrographs of flower components. Flower components will be harvested from fresh flowers, fixed, dehydrated and embedded in Araldite 502 resin. A pollen holder was collected from the flower sample and prepared accordingly for further STEM image acquisition. Sample preparation in this laboratory practices was divided in 12 sub steps as: Dissection (1.Flower samples, 2.Polen holder dissection); Staining (3.Safranine staining, 4.KMnO4, 5. Isotonic Phosphate buffer storage); Dehydration (6.Ethanol series, 7.Ethanol resin substitution); Embedding (8.Embedding+24h 60□, 9.Resin block) and Sectioning (10.Sectioning in ultramicrotomy, 11.Sample transfer to grid + post staining and 12.Grid storage). After this using a STEM convertor for SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS the set of images was acquired. Images 2.1 to 2.8 of pollen holder sectioning was acquired in the secondary electron detector mode (SE) with STEM converter Figure 2 shows as: 2.1: AV: 20 KV. WD:11 mm. Magnification: 220x and Scale bar: 100 µm. 2.2: AV: 20 KV. WD:11 mm. Magnification: 4500x and Scale bar: 5 µm. 2.3: AV: 20 KV. WD:11 mm. Magnification: 4500x and Scale bar: 5 µm. 2.4: AV: 20 KV. WD:11 mm. Magnification: 750x and Scale bar: 20 µm. 2.5: AV: 20 KV. WD:11 mm. Magnification: 190x and Scale bar: 100 µm. 2.6: AV: 20 KV. WD:11 mm. Magnification: 800x and Scale bar: 20 µm. 2.7: AV: 20 KV. WD:11 mm. Magnification: 500x and Scale bar: 50 µm. 2.8: AV: 20 KV. WD:11 mm. Magnification: 1000x and Scale bar: 10 µm. The critical steps during all the practices were sectioning and embedding. Sectioning due to the size and material orientation in the Araldite resin block and finally embedding due to the stiffness of some resins formulations that result in a rigid block of resin to follow sectioning steps. Nevertheless, all this preparation was essential to carry out the images in this laboratory report.

1 INTRODUCTION

Sample preparation is one of the most critical and important steps for TEM/STEM image acquisition. Many sectioning, staining and chemical techniques are available for biological sample preparation and evaluation using electron (SEM, STEM, TEM), light (LM), fluorescence (FM) and confocal microscopy (CM). Biological samples could be classified as challenge materials due to their specificity and 3D structure. Thus, find the right techniques to prepare and make the correct structures self-available for further observation is a key to obtain great microscope pictures.

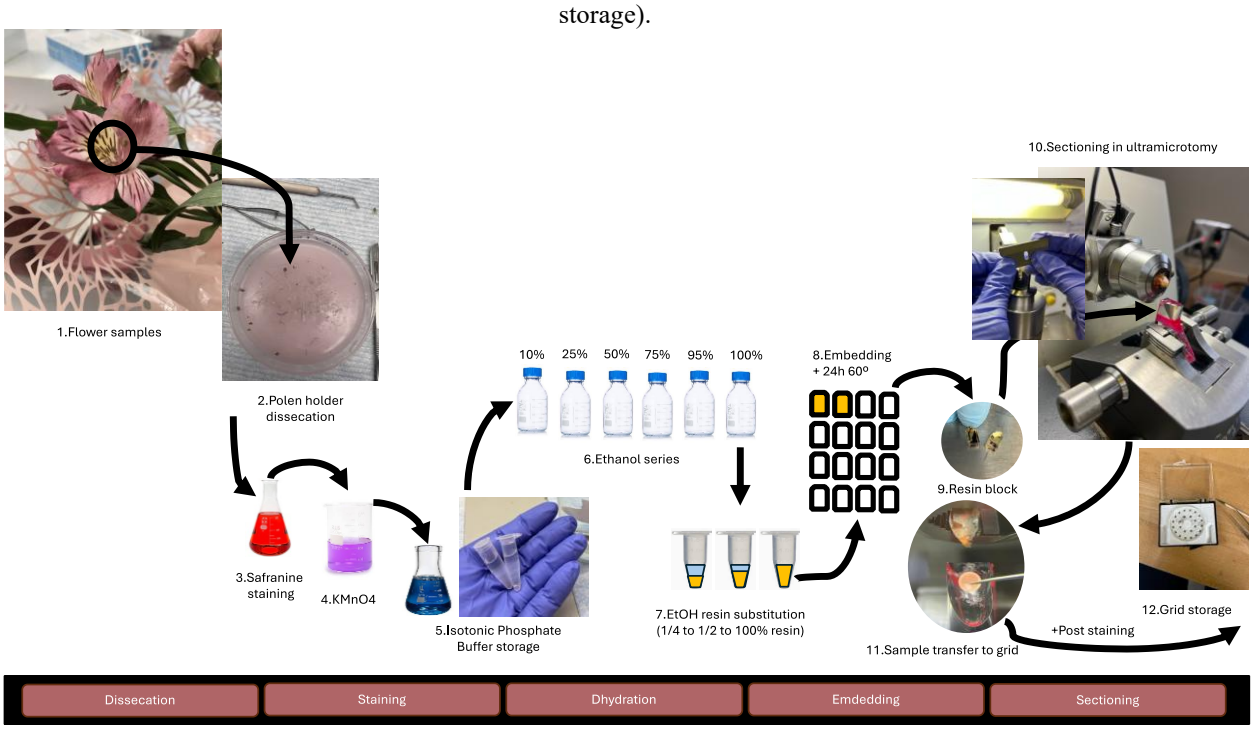
The purpose of this laboratory is to produce STEM micrographs of flower components. Flower components will be harvested from fresh flowers, fixed, dehydrated and embedded in Araldite 502 resin. An pollen holder was collected from the flower sample and prepared accordingly for further STEM image acquisition.

2 METHODS

2.1 SAMPLE PREPARATION

Fig. 1 shows the 5 major steps for flower sample preparation in this laboratory practices divided in 12 substeps. Dissection (1.Flower samples, 2.Polen holder dissection); Staining (3.Safranine staining, 4.KMnO4, 5. Isotonic Phosphate buffer storage); Dehydration (6.Ethanol series, 7.Ethanol resin substitution); Embedding (8.Embedding+24h 60º, 9.Resin block) and Sectioning (10.Sectioning in ultramicrotomy, 11.Sample transfer to grid + post staining and 12.Grid storage).

Figure 1 – Polen holder from flower dissecation sample preparation in 5 major steps: Disecation (1.Flower samples, 2.Polen holder dissecation); Staining (3.Safranine staining, 4.KMnO4, 5. Isotonic Phosphate buffer storage); Dhydration (6.Ethanol series, 7.Ethanol resin substitution); Embedding (8.Embedding+24h 60o, 9.Resin block) and Sectioning (10.Sectioning in ultramicrotomy, 11.Sample transfer to grid and 12.Grid storage).



2.2 STEM MODE

A SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS was used to acquire the SEM images in this laboratory report. The SEM is located at Backer Laboratory at ESF – SUNY. An STEM adaptor was used to place the grid for image acquisition and observation.

2.3 MICROSCOPE WORKING PARAMETERS

Table 1 shows the working parameters applied to capture the selected image in this lab report using the JEOL JSM-IT100.

Table 1 – STEM working parameters and their working values for the images collected from this laboratory practices

SEM parameters	Images							
	1	2	3	4	5	6	7	8
Vacuum	HV	HV	HV	HV	HV	HV	HV	HV
Accelerating Voltage (kV)	20	20	20	20	20	20	20	20
Au_Pd coating	NA	NA	NA	NA	NA	NA	NA	NA
Probe Current	25	42	42	55	60	60	56	53
Working Distance (mm)	11	11	11	11	11	11	11	11
Magnification (x)	220	4500	4500	750	190	800	500	1000
Objective aperture (X,Y), stage tilt (T and R) and z (Z) coordinates	X 2.764	X 2.520	X 2.532	X 2.587	X 2.761	X 2.574	X 2.937	X 2.936
	Y 0.063	Y 0.021	Y 0.011	Y 0.786	Y -0.010	Y -0.008	Y 0.011	Y -0.045
	T 0.000	T 0.000	T 0.000	T 0.000	T 0.000	T 0.000	T 0.000	T 0.000
	R 0.000	R 0.000	R 0.000	R 0.000	R 0.000	R 0.000	R 0.000	R 0.000
	Z 22.000	Z 22.000	Z 22.000	Z 22.000	Z 22.000	Z 22.000	Z 22.000	Z 22.000

*STEM mode using SEM JEOL JSM-IT100 InTouchScope™ equipped with a JEOL-made EDS. NA = non applicable; V = Vacuum; AV = Accelerating voltage; PB = Probe current; WD = Work distance; M = Magnification; OA = Objective aperture; T = Stage tilt; R = Rotation and Z =z.

2.4 MICROSCOPE MANAGEMENT STEPS

With assistance from the instructor follow the steps described below:

1. Load a grid into the STEM adapter
2. Load the STEM adapter safely into the SEM.
3. Evacuate the chamber.
4. Identify and state the function of each component and control on the instrument.
5. The STEM adapter is 11mm, tall above the stage.
6. With assistance set the working distance to 21 mm and Z-focus the instrument
7. Manipulate the stage to place your sample under the electron beam at a working distance of 10.
8. Set accelerating voltage to 20 kEV
9. With assistance center aperture, optimize filament saturation, and gun alignment
10. Focus image at low magnification.
11. Zoom/Focus the image centering on particles stuck on the formvar.
12. Continue to zoom in until the copper grid in outside the field of view.
13. Adjust Brightness and Contrast
14. Adjust probe current
15. Optimize stigma, focus, brightness, and contrast
16. Capture images of sample.
17. Vent Chamber
18. Remove sample
19. Repeat steps 1 through 24 until yo have obtained a quality STEM image of the wood sample
20. Follow lab procedures for securing SEM and cleanup work area.
21. Have Instructor check your work

3 RESULTS

3.1 STEM

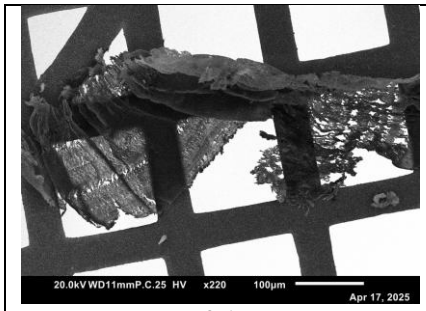
Figure 2 shows the set of images 2.1 to 2.8 of pollen holder sectioning. It is important emphasizing that this sectioning's was carefully divided in 12 substeps. Dissection (1.Flower samples, 2.Polen holder dissection); Staining (3.Safranine staining, 4.KMnO4, 5. Isotonic

Phosphate buffer storage); Dehydration (6.Ethanol series, 7.Ethanol resin substitution); Embedding (8.Embedding+24h 60^{o}, 9.Resin block) and Sectioning (10.Sectioning in ultramicrotomy, 11.Sample transfer to grid + post staining and 12.Grid storage).

The set of images presented was obtained after many sample manipulations due to the needs to obtain good sections from a biological material. It is relevant to mention the step 8, 9 and 10 was repeated more than 3 times to deliver a quality section for STEM image acquisition.

Images 2.1 to 2.8 of pollen holder sectioning was acquired in the secondary electron detector mode (SE) with STEM converter Figure 2 shows as: 2.1: AV: 20 KV. WD:11 mm. Magnification: 220x and Scale bar: 100 μm. 2.2: AV: 20 KV. WD:11 mm. Magnification: 4500x and Scale bar: 5 μm. 2.3: AV: 20 KV. WD:11 mm. Magnification: 4500x and Scale bar: 5 μm. 2.4: AV: 20 KV. WD:11 mm. Magnification: 750x and Scale bar: 20 μm. 2.5: AV: 20 KV. WD:11 mm. Magnification: 190x and Scale bar: 100 μm. 2.6: AV: 20 KV. WD:11 mm. Magnification: 800x and Scale bar: 20 μm. 2.7: AV: 20 KV. WD:11 mm. Magnification: 500x and Scale bar: 50 μm. 2.8: AV: 20 KV. WD:11 mm. Magnification: 1000x and Scale bar: 10 μm.

Figure 2 – Set of images 2.1 to 2.8 of pollen holder sectioning. Image acquired in the secondary electron detector mode (SE) with STEM converter. Detailed of sectioning of pollen holder imbedded in Araldite resin in the grid surface. 2.1: AV: 20 KV. WD:11 mm. Magnification: 220x and Scale bar: 100 μm. 2.2: AV: 20 KV. WD:11 mm. Magnification: 4500x and Scale bar: 5 μm. 2.3: AV: 20 KV. WD:11 mm. Magnification: 4500x and Scale bar: 5 μm. 2.4: AV: 20 KV. WD:11 mm. Magnification: 750x and Scale bar: 20 μm. 2.5: AV: 20 KV. WD:11 mm. Magnification: 190x and Scale bar: 100 μm. 2.6: AV: 20 KV. WD:11 mm. Magnification: 800x and Scale bar: 20 μm. 2.7: AV: 20 KV. WD:11 mm. Magnification: 500x and Scale bar: 50 μm. 2.8: AV: 20 KV. WD:11 mm. Magnification: 1000x and Scale bar: 10 μm.



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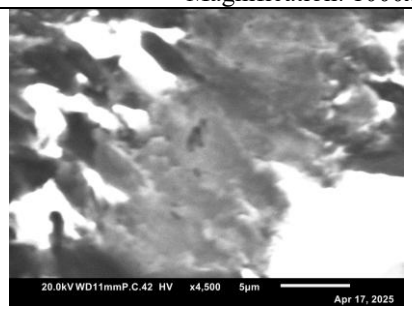

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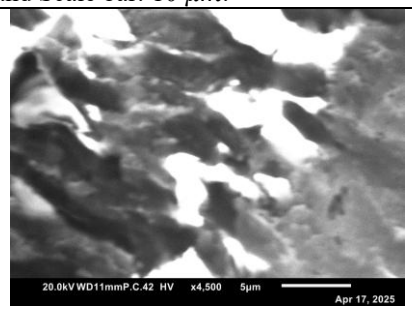

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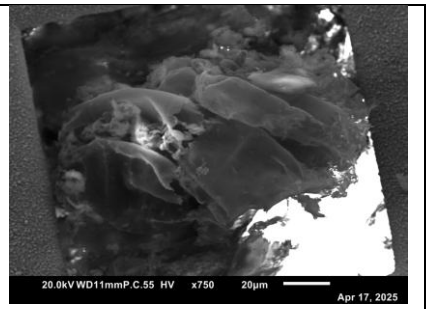
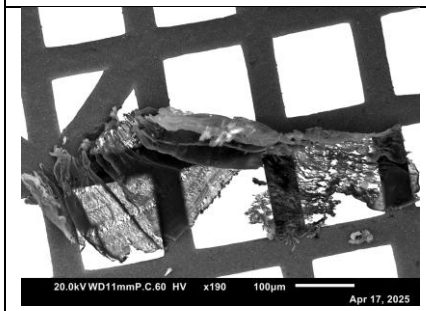


2.4



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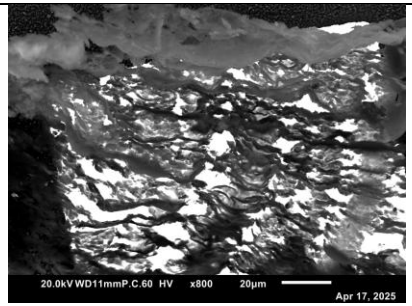

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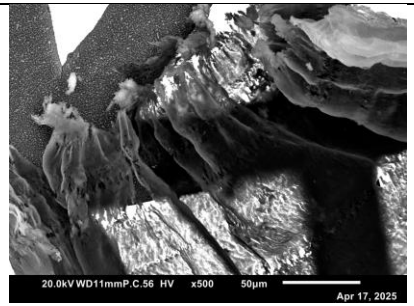

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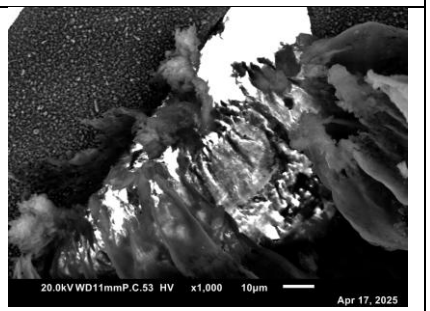

2.8

4 CONCLUSION

Sectioning is the crucial step for an great image outcome for TEM. This report illustrate how important is this essential step during the process of imagining for TEM even using a STEM convertor. The critical steps during all the practices were sectioning and embedding. Sectioning due to the size and material orientation in the Araldite resin block and finally embedding due to the stiffness of some resins formulations that result in a rigid block of resin to follow sectioning steps. Nevertheless, all this preparation was essential to carry out the images in this laboratory report. The biological sample observed wasn't very clear due to the lack of resolution in the microscopy and the thickness of the sections.

5 SELF-ASSESSMENT

5.1 PERSONAL SKILLS

This laboratory practice reinforced my ability to prepare biological samples preserving their structure and keeping sectioning's after the material embedding. As personal experience, I have repeated the sectioning and embedding step more than 3 times and my sectioning still need improvement. This laboratory practices illustrate how important is the real practices for the student formation instead only demonstrate the procedure. This entire semester was a great practical experience.

5.2 IMAGE QUALITY

The acquired images presented a good quality at lower magnification. To consider a very high magnification in TEM, still exist a lack of quality for this equipment. However, this is an great start.

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