Standard operating procedure for the operation of SEM-EDX for elemental analysis, with additional applications to biomass

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This document and its content are an aider for our group, and it is not intended to be a replacement for the training, protocols, documents and else provided by the technical team.

Contents

1. Scope

This SOP is for the microscopic and elemental analysis of samples via SEM-EDX. It can be applied to all samples that are adequate for SEM-EDX. Additionally, the scope of this SOP covers the analyses of biomass and/or materials from living systems that are adequate for SEM-EDX.

2. Safety

2.1. PPE:
Ensure upon arrival to the laboratory, that all relevant PPE is worn according to the requirements, such as but not limited to, lab coat, goggles, gloves and other specified equipment specified by the technical team.

2.2. Hazard:
Hazards are present as it's a multi-disciplinary laboratory, therefore, ensure that the risk assessment has been read and signed.

3. Interferences

3.1. Low conductivity material:
Organic materials and other samples normally have low conductivity, and this drawback is overcome by using carbon adhesive discs/tabs with carbon on both sides.

3.2. Moisture:
Samples must be dry as much as possible since the high vacuum inside the equipment will tend to evaporate water, causing damage to the equipment.

3.3. Uneven surface:
Roughness, unevenness and other complex topologies negatively affect the conduction of electrons over the sample's surface. As the sample thickness increases, the penetration of X rays gets more compromised. Therefore, the technique works better in samples that are smooth and thin layered.

3.4. Dust:
The right protocol must be followed with dust samples to avoid damage to the equipment due to flying solids.

4. Materials and equipment

4.1. Scanning Electron Microscope (Hitachi TM4000 Plus)
4.2. Energy dispersive X-ray Spectroscopy (Oxford Instrument Aztec EDS)
4.3. TM4000 software
4.4. AztecOne software
4.5. Carbon conductivity pad
4.6. Metal spatula
4.7. Metal tweezer
4.8. Metal scalpel
4.9. Standard reference
4.10. Pressured air
4.11. USB flash drive
4.12. Height instrument

4.13. Screw
4.14. Stand

5. Method

5.1. Analyse the standard. Always analyse the relevant commercial standard to check that the equipment is working as expected and that the analyses are accurate.
5.2. Turn the SEM-EDX equipment ON (the switch is on the right-hand side when facing the equipment's brand and model)

5.3. Analysis of hexagonal standard:
5.3.1. Use the Allen key provided by the equipment supplier to fit or remove the mounting sample holder, if need be, as depicted by Figure 1.

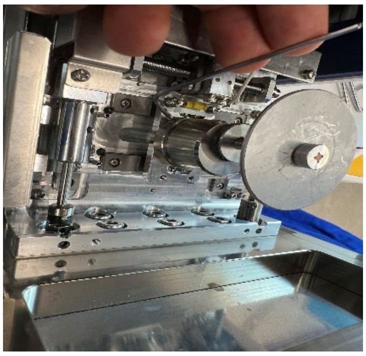

Figure 1 Removal of SEM mount using Allen key.

5.3.2. Standard: use the Cu/Al standard. This cylindrical bolt has high conductivity and thus, can be mounted directly on the mounting sample holder without a sticky carbon pad, as depicted in Figure 2.

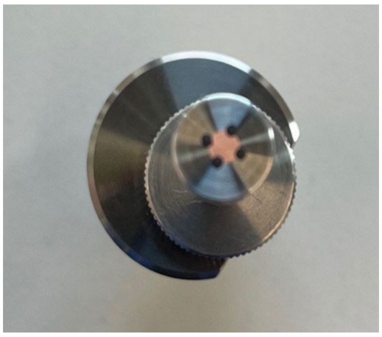

Figure 2 Cu and Al Standard on the mount for SEM-EDX Analysis

5.3.3. Using the measurement instrument, as depicted in Figure 3, measure the correct height of the sample to avoid damage to the equipment. The sample should be to 5 mm below the white plastic cylinder of the measuring device. Change screw size according to height requirements, as depicted in Figure 3, as well as loosen the bolt to the mount holder to change the height.

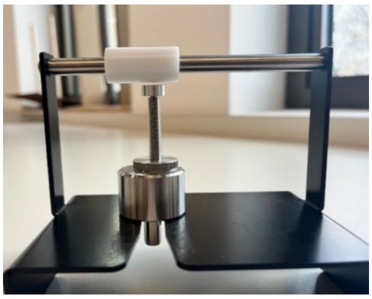

Figure 3 Height measurement instrument for SEM mount.

5.3.4. Remove the standard from the mount, and screw the mount back into the SEM chamber, using the provided Allen key.
5.3.5. Carefully place the standard back into the centre of the mount and close the SEM chamber.

5.4. Analysis of dust:
5.4.1. Using the spatula, scoop the dust sample onto the carbon pad.
5.4.2. Further spread and level out the dust sample using the spatula, ensuring that the carbon pad is completely covered.
5.4.3. Tip over the excess dust sample into a disposal bin, and using an air cannister; remove any residual dust debris.
5.4.4. Repeat step 4.3.3. to ensure that height of the mount is appropriate for SEM-EDX analysis.
5.4.5. Repeat step 4.3.5.

5.5. SEM Analysis:
5.5.1. Press “Evac/Air” button.
5.5.2. Once the TM4000 changes from white solid light to blue solid light, it indicates that the equipment is ready to analyse.
5.5.3. Launch the TM4000 software.
5.5.4. Once the software opens up, it will request for the selection of a specimen holder type, select accordingly, as depicted by Figure 4.

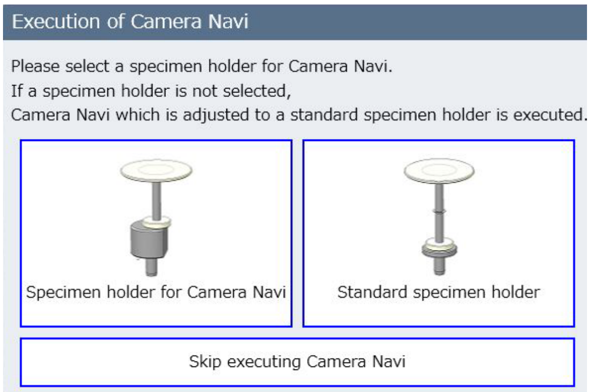

Figure 4 Execution of camera navi for selection of specimen holder.

5.5.5. Allow time for the software to automatically detect the standard, then confirm the image capture using the “confirm” button of the popup, as depicted below in Figure 5.

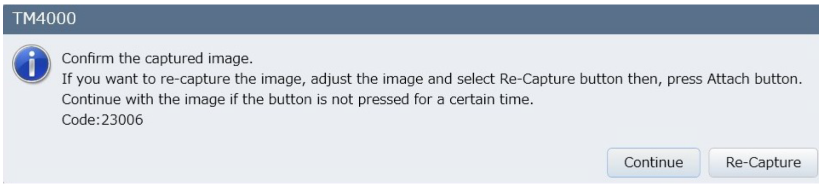

Figure 5 Capturing image of SEM sample.

5.5.6. Click “Start”, located on the top left corner of the software, ensuring that the Voltage is 15 KW mode 3, Conductor (H) vacuum and BSE detector for conductive material, as depicted by Figure 6. Whereas vacuum is charged red L for non-conductive material.

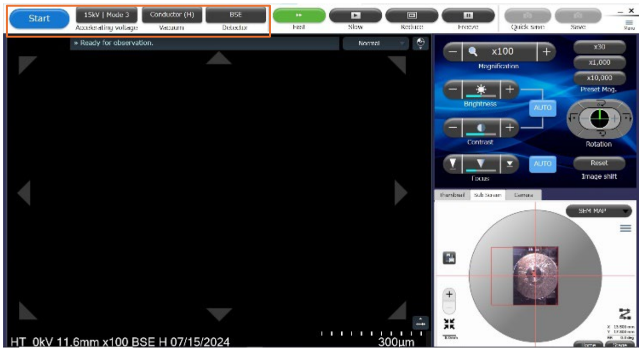

Figure 6 Start of TM4000 software for scanning of sample.

5.5.7. Using the arrows on the screen, move the camera. Once a desired scan is rendered, and adjusted using focus, magnification and brightness; save the scan to an appropriate location, as depicted by Figure 7.

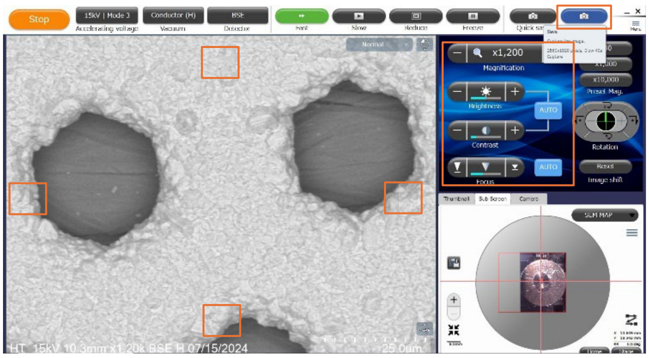

Figure 7 Capturing a high resolution of the rendered scan.

5.5.8. Then, click on “slow” which is located at the top of the screen, as saving results in the image to be “frozen”, as depicted by Figure 8.

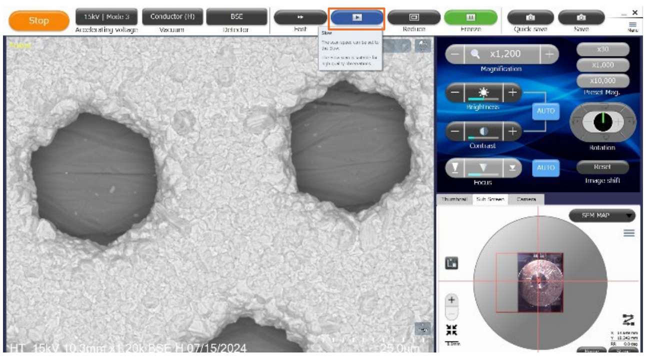

Figure 8 Unfreeze for electron image scanning.

5.6. EDX Analysis:

5.6.1. Open AztecOne software and create a new project, as depicted by Figure 9.

Figure 9 Creation of a new project for elemental analysis.

5.6.2. Click on pre-defined elements and select to include major elements that are expected for the sample, as depicted by Figure 10.

Figure 10 Including and excluding Elements for optimising EDX.

5.6.3. On site 1, click on “scan image”; which will take around 2 minutes to complete, as depicted by Figure 11.

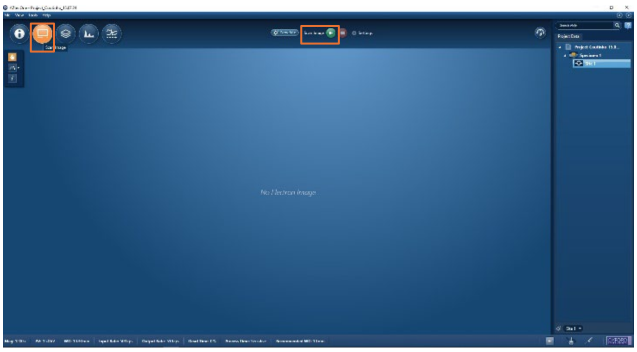

Figure 11 Acquiring an electron scan image of the SEM render.

5.6.4. Then move to the next section, and click on “acquire map data”, which will complete after 10 scans of the surface, as depicted by Figure 12.

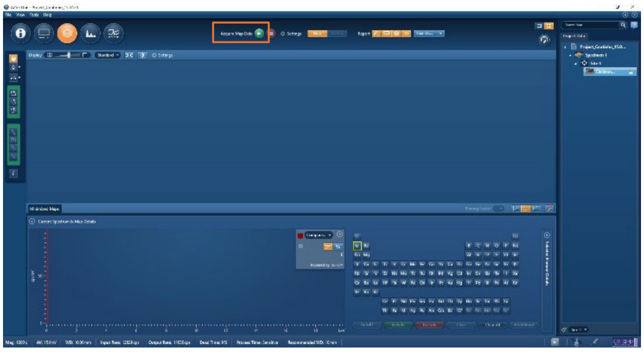
Figure 12 Acquiring map data of the electron scan image.

5.6.5. Then move to the next section, and using the rectangular region tool, select the desired area for spectrum creation to analyse the chemical composition at the elemental level, as depicted by Figure 13, below. Then click "acquire spectrum" for a global EDS layered image.

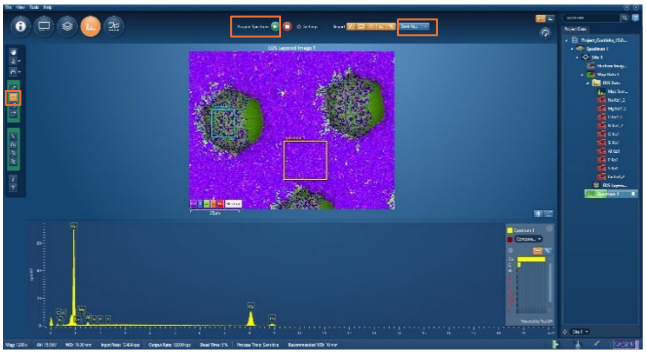
Figure 13 Acquiring global EDS layer spectrum and region spectrum.

5.6.6. Using the "save as" button located on the top right hand corner of the screen, save the spectrum acquired, which will create a document consisting of the map sum spectrum, and the weight % of the identified elements, as demonstrated in Figure 14, above.

5.7. End of Analysis:
- 5.7.1. Save the project and close AztecOne, then click "stop" on the TM4000 software.
- 5.7.2. Once the TM4000 stops fully, press "Evac" on the SEM and allow for the blue light to become a solid white light.
- 5.7.3. Open the chamber and place the standard back into its container.
- 5.7.4. Close the chamber, press on "Evac" again and allow the chamber to become vacuum, then switch off SEM, with the button located at the left side of the machine.

6. References

1. Hitachi High-Technologies Corporation (2002) "Instruction Manual for Model S-4800 Field Emission Scanning Electron Microscope"
2. Hernandez, E., Kheirandish, K. (2023), "Protocol for Biomass Preparation for SEM-EDX". CCU.