E-Guide

Sample Preparation

A guide to ideal sample preparation

PHENOMWORLD

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Introduction

Sample preparation is crucial to require good SEM images. Unfortunately, not all samples can be easily imaged, but this guide will help you with tips and tricks to obtain good results from the most common samples.

Feel free to combine different techniques to boost the beneficial effects and do not underestimate your creativity. Scanning electron microscopes are versatile instruments and they can do much more than you would expect.

This sample preparation guide is meant for those who are approaching scanning electron microscopy for the first time, or are relatively new to it. The content is valid for most commonly used types of samples. For more detailed information on specific kinds of samples, please contact your scanning electron microscope manufacturer.

1. Basic sample preparation

Every scanning electron microscope is equipped with a sample holder or a loading chamber where the sample can be inserted. To load a sample in a scanning electron microscope, the use of aluminium stubs is recommended. These come in different sizes and are commercially available.

It is crucial that the sample is firmly mounted to the surface of the stub before placing it in the sample holder or stage.

To place the sample onto the pin stub, you can use:

- doublesided carbon sticker
- conductive paint
- conductive tape
- special clamping
- a combination of the above.

It is also highly recommended that you remove all loose particles from your sample.
To do this, you can:

- Hold the aluminium stub with tweezers, tilt it by 90° and gently tap it on its side.
- Spray dry air on the sample.

Always remember to take the following precautions:

- Be careful while handling your sample to prevent damage.
- Always use tweezers to prevent contamination.
- Make sure that the mounting procedure is reliable so that you do not introduce mechanical vibrations.
- DO NOT spray dry air in the direction of any electronics or scanning electron microscopes, as it might cause fire hazards.
- Make sure there is no condensed liquid in your spray air straw by first spraying away from your sample.

These precautions will dramatically reduce the risk of contamination of your system and sample holder and guarantee better performance over time.

If you deal with any of the samples on the following list, please refer to the dedicated section of this guide for suggestions on how to get the best images.

- Non-conductive samples
- Magnetic samples
- Beam sensitive samples
- Powders and particles
- Samples containing moist or outgassing samples.

2. Non-conductive samples

When a non-conductive material is imaged, the electrons accelerated onto the sample don't have a path to the ground potential and will accumulate on the surface.

This will result in a progressively increasing brightness in the image, until all the details are no longer visible. In worst case, the entire field of view will turn white. Minor movement can also be detected, caused by the interaction between the electrons. This will cause blurriness in the collected image.

Several solutions are widely used:

2.1 Conductive tape or paint

By covering part of the sample with a piece of conductive tape (e.g. copper tape) or some conductive paint, a bridge circuit to the surface of the aluminium stub is created.
This will allow the sample to partially discharge and is enough to image non-conductive samples when imaging areas close to the tape edge.

2.2 Low vacuum

Lowering the vacuum level introduces air molecules into the sample chamber which interact with the electron beam. Positive ions are generated and are being attracted by the sample surface. The interaction between the negatively charged electron and the positively charge ions will lead to the discharging of the sample.

This technique adds some noise to the final image, but allows you to analyse the sample without further processing it, making the analysis faster and inexpensive (no additional instrumentation is needed).

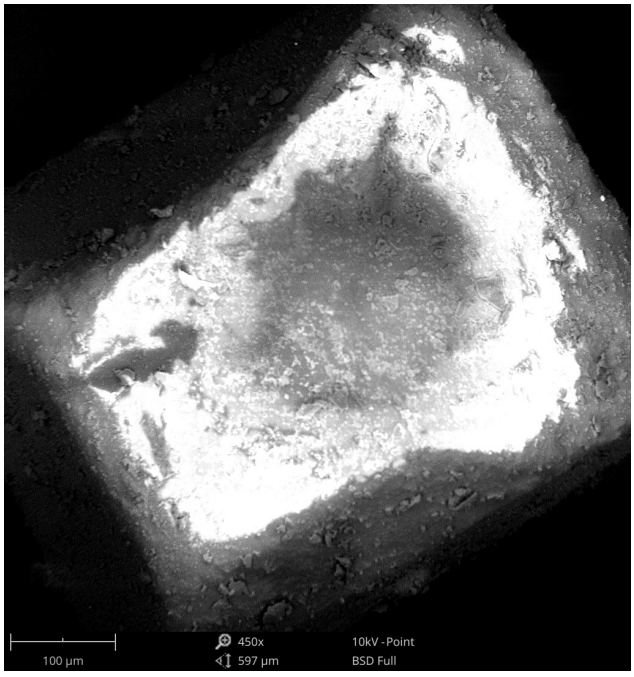

Fig. 1 SEM image of sugar cube charging

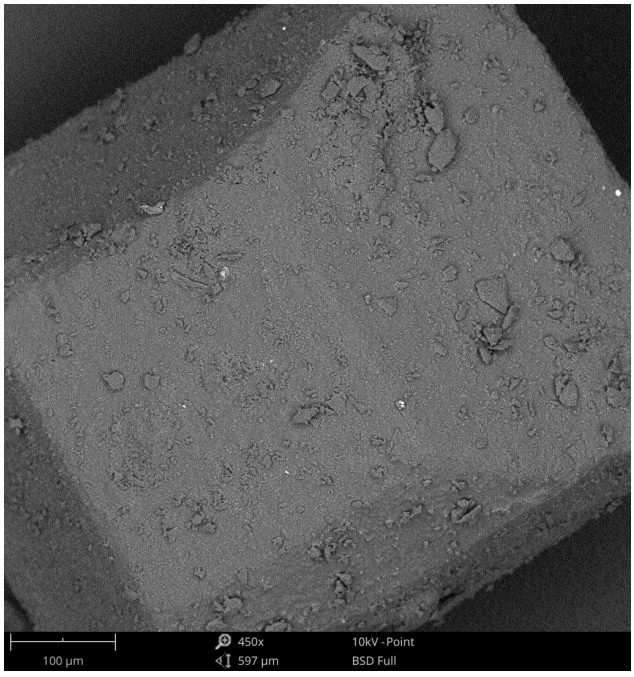

Fig. 2 SEM image of sugar cane in low vacuum

2.3 Sputter coating

By sputter coating, it is possible to create a thin layer of conductive material on the sample's surface. This creates a connection between the sample and the surface of the aluminium pin and therefore a bridge circuit to the ground potential.

The choice of the coating material is strongly dependent on what kind of analysis needs to be performed on the sample. Gold or Platinum, due to their extremely high conductivity, are the ideal elements for high resolution images. Lighter elements, such as Carbon, can be used when EDS analysis on non-organic samples is required. ITO (indium – doped tin oxide) can create transparent, conductive layers, and can be used on glasses used often in other microscopic methods to make them suitable for SEM.

The disadvantage of using a sputter coater is that additional instrumentation is required, the analysis becomes more time consuming and the samples undergo more pumping cycles. In addition, any advantage of using a BSD detector to image the sample is lost, as the contrast becomes very homogeneous and there is no difference in grey intensity for different elements.

Fig. 3 SEM image of paper with no precaution (charging)

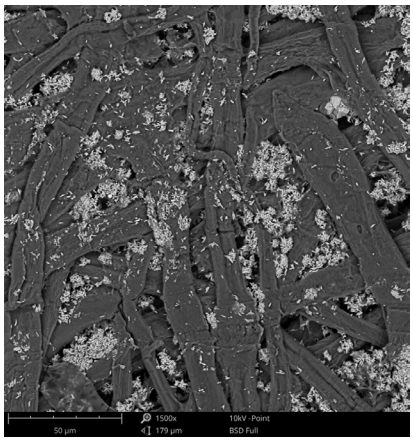

Fig. 4 SEM image of paper in low vacuum mode

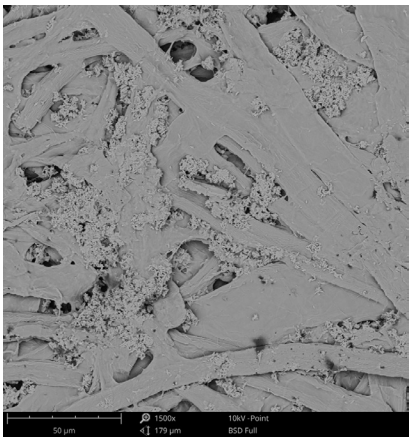

Fig. 5 SEM image of paper with gold coating

3. Magnetic samples

Samples that generate a magnetic field can interfere with the electron beam, reshaping it and consequently producing deformed images, usually elongated along one axis and blurry.
This problem is known as astigmation and consists of an increase in the eccentricity of the beam cross section.

3.1 Stigmation correction

All scanning electron microscopes offer the possibility to tune for astigmation. Certain instruments require the user to fix stigmation values every time, others can store standard values that are valid for most samples.

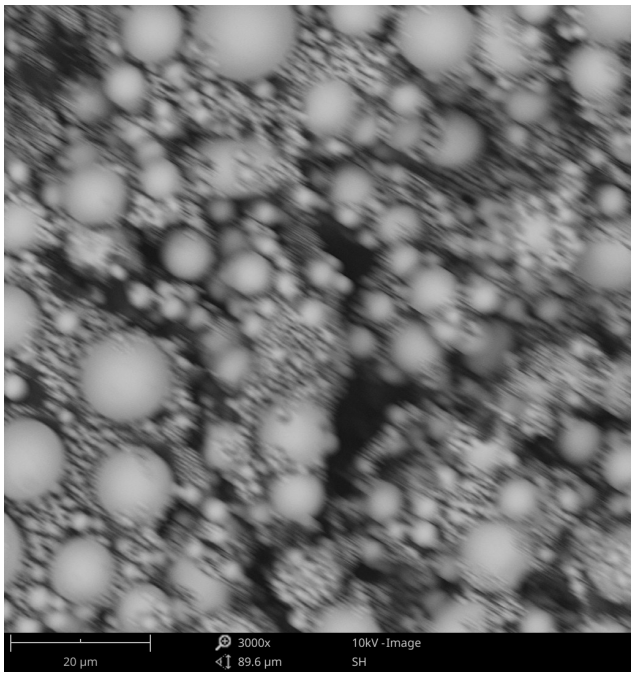

Fig. 6 Astigmatic image of tin balls

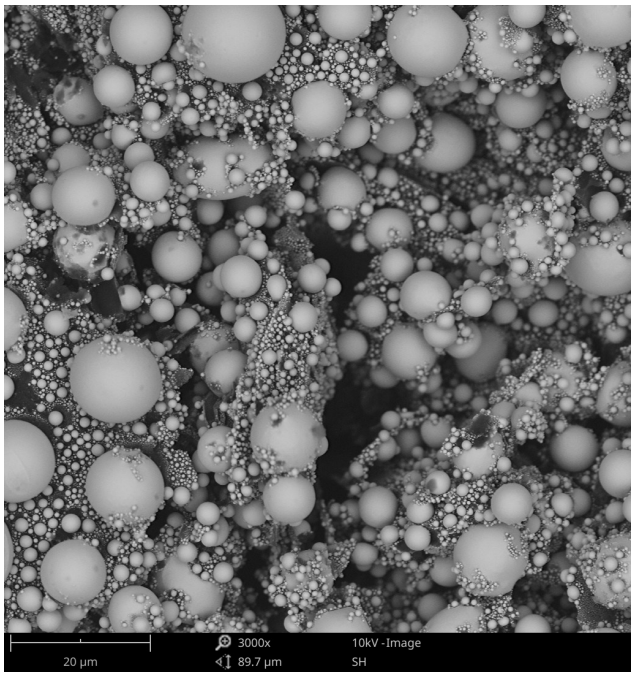

Fig. 7 SEM image of tin balls in focus

Astigmatic image of tin balls. The procedure alters the lenses' magnetic field which are responsible for beam reshaping. Only in the case of a circular beam shape, the best image can be produced.

When changing the stigmation, it will be necessary to fine-tune the focus again.

3.2 Demagnetization

Sometimes the magnetic field is just too extreme and stigmation correction will not inhance the outcome. In these cases, a demagnetizer can be used. This device can reduce the magnetic field of the sample to a level where the SEM can be used for imaging without further changes.

4. Beam sensitive samples

Delicate samples, as for instance thin polymeric foils or biological samples, can be damaged by the electron beam due to either heat generated in the interaction area or the rupture of chemical bonds. This will result in either a hole in the observed structure or a progressive deformation of the scanned area.

4.1 Beam settings

The easiest way to reduce this effect is to use lower values for acceleration voltage and current. In these cases, the smallest possible values are recommended.

4.2 Sputter coating

In the worst cases, a thin coating layer can be applied to the sample in order to shield the sensitive surface. Increased conductivity will also improve image resolution.

4.3 Cooling

Thermal effects can be reduced by using a temperature-controlled device. Removing the heat generated by the beam will protect the sample from thermal-induced surface modifications.

4.4 Time

Spending a long time on a specific position might cause damage to the sample, over time. Being agile during the analysis will prevent excessive alterations, but might not produce the best results in terms of image quality.

4.5 Magnification

Zooming-in implies having the same number of electrons shot on a smaller surface area. The thermal drift therefore my be increased and the deformation effects will become more evident. Whenever possible, low magnification is recommended.

5. Powders and particles

When imaging particles, information as for instance particle size or shape are crucial for the design of the process flow.

The easiest way to prepare a powder or particles sample is to collect a small amount of sample with a spoon and let it fall on a carbon double-sided sticker, removing the excess particles later using spray air.

Unfortunately, this method will cause many particles to overlap, hiding important features, or to be blown off, introducing errors in particle counting routines.

5.1 Particles disperser

The best way to prepare a powder sample is by using a particle disperser unit. This will allow an homogenous distribution of the sample on the sticker, reducing the chances of having of overlapping particles and generating a pattern that can be used for granulometry.

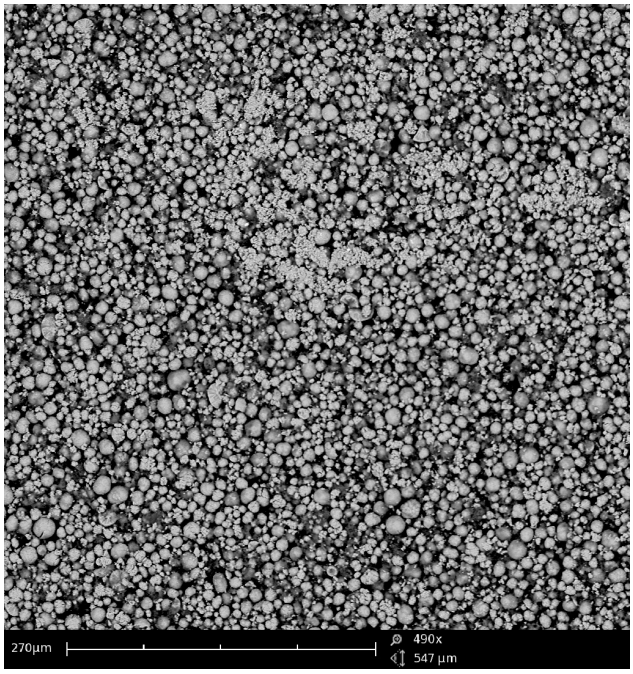

Fig. 8 SEM image of powder. SEM images of powder samples using a simple method.

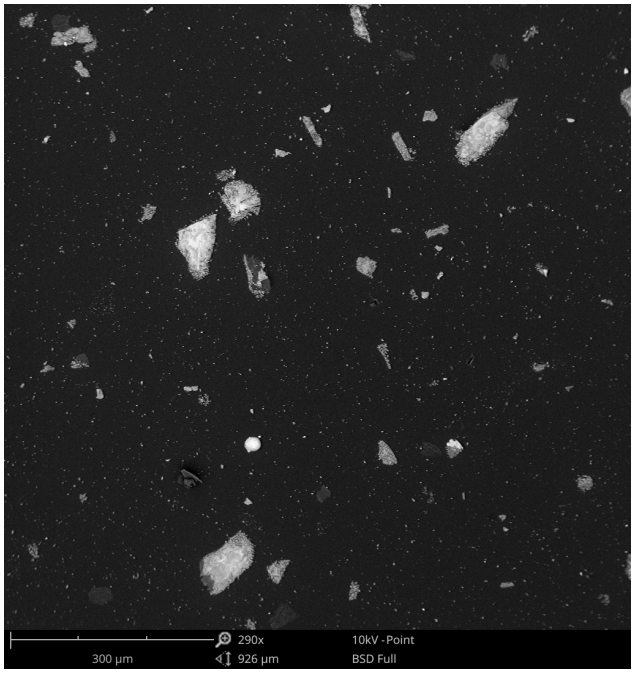

Fig. 9 SEM image of powder sample using a disperser.

When using a disperser the particles are easy to detect and evenly spread.

Operational parameters, such as the vacuum level and the amount of sample needed, depend largely on the nature of the powders. Some general guidelines:

- Delicate samples might break due to strong pressure outburst.
- Hydrophilic samples might need a higher vacuum burst to be separated.

6. Samples containing moist or outgassing samples

As electron microscopes operate in high vacuum levels, every wet sample which is loaded in the imaging chamber will outgas. Certain samples have microstructures that will resist the phase change, providing excellent results without major concerns. A typical example is a fresh leaf. If the sample does not have a rigid structure, it can be imaged, in case that one of the following techniques is used to prepare it.

6.1 Force drying

To verify whether the sample will resist the vacuum, the use of another instrument, such as a desiccator or a sputter coater, is recommended. Eventual changes in the sample should be immediately noticeable.

6.2 Critical point drying

Critical point drying (also known as supercritical point drying) describes a process in which liquid is transformed into gas to dry up samples. This either can be done with low temperatures and low pressures or high temperatures and high pressures. The last option tries to circumvent a phase change while passing a supercritical state.

6.3 Cooling

Cooling is an alternative to drying techniques to preserve the structure of the sample completely intact by freezing the sample.

If the phase change is quick enough, the liquids in the sample will not build crystals and the structure will be perfectly preserved.

It is important to consider that the phase change is not permanent and a prolonged exposure to a high vacuum will increase the evaporation rate.

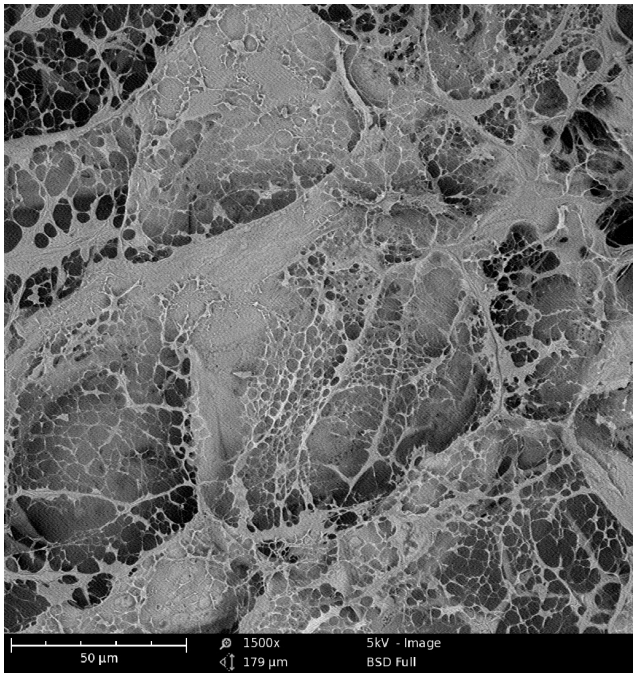

Fig. 10 SEM image of a cucumber's intact structure.

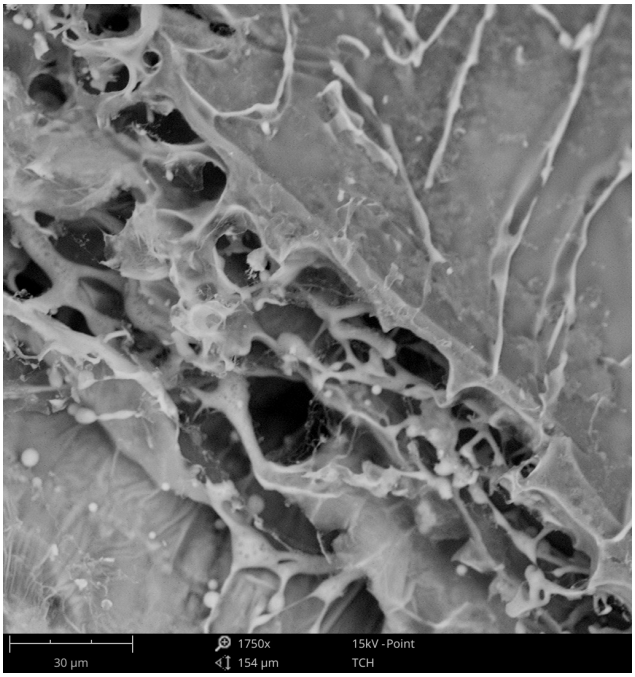

Fig. 11 SEM image of a tomato's peel and inside structure.

6.4 Low vacuum

If the sample does not have a particularly high moisture content, using a small amount of sample at a reduced vacuum level can turn out to be enough to obtain images. The overall image quality will be lower, but the sample can be imaged in its original state.

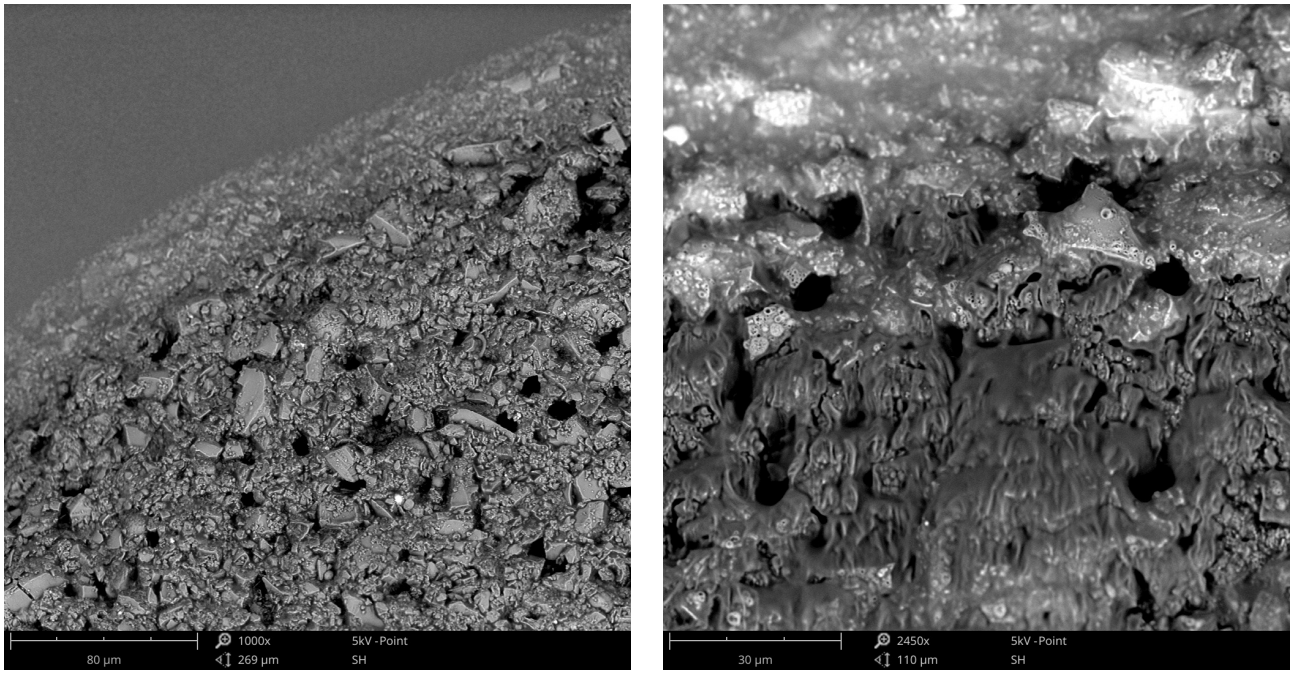

Fig. 12 & 13 SEM images of Nutella

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