Sample preparation

The extended guide to sample preparation to obtain high-quality analysis outcomes
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I joined Phenom-World as an Application Engineer chasing my passion for science and photography.

Electron microscopy gave me the chance to merge my interests and investigate all kind of materials from a very different (and closer) point of view. My job consists of investigating new uses of a scanning electron microscopes to provide completely new sets of information to the academic and industrial communities and new tools to discover the world and improve our technologies.

I want to thank my colleagues Karl Kersten, Jasmin Zahn, Antonis Nanakoudis, Marijke Scotuzzi, who inspire me and push me daily to discover more. A big thank you also to Lorelei de Boer, coordinator of the project, who continuously pushes us to share our knowledge.

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Sample preparation

Sample preparation is crucial if you require a good SEM image. 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 don 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 macro categories of samples. For more detailed information on specific kinds of samples, please contact your scanning electron microscope manufacturer.
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, standard sizes and are readily available on a commercial basis.

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

To stick the sample to the pin stub, you can use:
- Double-sided carbon sticker
- Conductive paint
- Conductive tape
- Special clamps
- A combination of the above.

It is also 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.

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 solid so that you do not introduce mechanical vibrations due to incorrect mounting.
- DO NOT spray dry air in the direction of any electronics or scanning electron microscope, as it might be flammable.
- 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 the 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
Non-conductive samples

The first magnifying glasses date back to the Greeks, with Arist.

When a non-conductive material is imaged, the electrons shot on the sample surface 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 the worst case, the entire field of view will turn white. Mild movement can also be detected, caused by the mutual interaction of the electrons. This will cause blurriness in the collected image.

Several solutions are widely used:

**Conductive tapes or paints**
By covering part of the sample with a piece of conductive tape (e.g. copper tape) or some conductive paint, a bridge to the surface of the aluminium stub is created.

This will allow the sample to partially discharge and is enough to image mildly non-conductive samples when imaging areas close to the tape edge.

**Low vacuum**
Introducing an atmosphere in the sample chamber allows beam interaction with air molecules. Positive ions are generated and attracted by the large number of electrons on the sample surface. The ions will further interact with the electrons, discharging 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 instrument is needed).
Sputter coating

By using a sputter coater, it is possible to create a thin layer of a conductive material on the sample surface. This creates a connection with the surface of the aluminium pin and therefore to the ground potential.

The choice for 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 material for high resolution images. Lighter elements, like Carbon, can be used when EDS analysis on non-organic samples is required. ITO (an alloy of indium oxide and titanium oxide) can create transparent, conductive layers, and can be used on optical glasses to make them suitable for SEM.

The disadvantage of using a sputter coater is that additional instrumentation is required, the analysis become 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.
Magnetic samples

Samples that generate a magnetic field can interfere with the accuracy of the electron beam, reshaping it and consequently producing deformed images, usually elongated along one axis and blurry.

This problem is known as stigmation alteration and consists of an increase in the eccentricity of the beam cross section.

**Stigmation correction**

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

The procedure alters the lenses magnetic field, which are responsible for beam reshaping. When the shape is circular again, the best image can be produced.

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

**Demagnetization**

Sometimes the magnetic field is just too intense and stigmation will not cope with it. 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 image it.
Beam sensitive samples

Delicate samples, like thin polymeric foils or biological samples, can be damaged by the electron beam due to the heat generated in the interaction area or the rupture of chemical bonds.

This will result in either a hole in the surface or a progressive deformation of the scanned area.

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

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

**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.

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

**Magnification**
Zooming-in implies having the same number of electrons shot on a smaller area. The thermal drift is increased and the deformation effects will become more evident. When possible, low magnification is recommended.
When imaging particles, information like particle size or shape are crucial in 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, inducing errors in particle counting routines.

**Particles disperser**

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

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

- Fine powders require a smaller amount of sample.
- Delicate samples might break due to strong pressure outburst.
- Hydrophilic samples might need a higher vacuum burst to be separated.
As electron microscopes operate in high vacuum levels, every wet sample that is loaded in the imaging chamber will immediately start to 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 provided that one of the following techniques is used to prepare it.

**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.

**Critical point drying**
Also known as supercritical drying, this technique forces the liquids in the sample to evaporate, maintaining a low temperature. The evaporation is driven by the pressure level, which is brought below the vapour tension of the liquid in the sample.

During this process, the liquids will create fracture in the sample, causing modifications in the structure.
Cooling
This is an alternative to drying techniques that will 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 form 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.

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 be enough to collect images. The overall image quality will be lower, but the sample can be imaged in its original state.

Small amount of sample
Using a small quantity of sample is sometimes enough to contain the effects of vacuum and evaporation. The sample can be collected with a toothpick and a veil of it can be deposited on the stub.

This technique is particularly effective with gels and emulsions.
Sample preparation is just the starting point for faster & better analysis

Learn how to improve your process even more by speaking with an SEM expert.

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