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Sample preparation

The extended guide to sample preparation to obtain high-quality analysis outcomes

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Sample preparation

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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 never underestimate your creativity. Scanning electron microscopes (SEMs) 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 SEM manufacturer.

Basic sample preparation

Every SEM is equipped with a sample holder or a loading chamber where the sample can be inserted.

To load a sample in a SEM, 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:

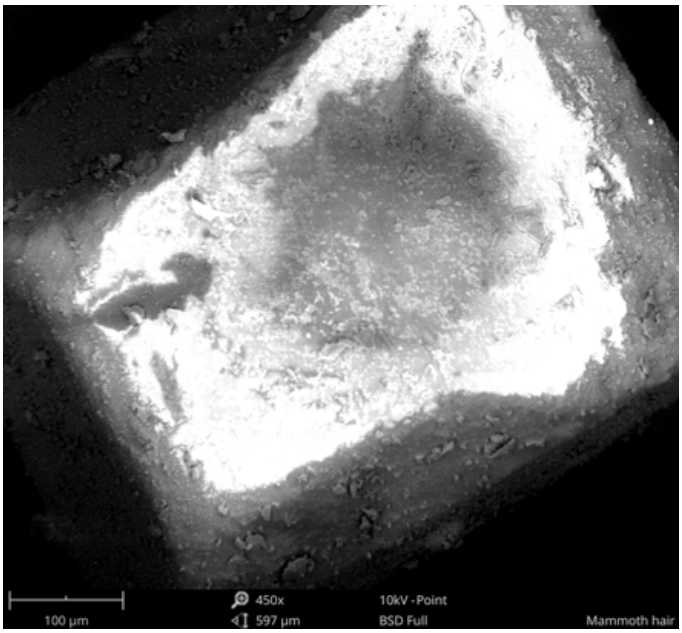
- Be careful while handling your sample to prevent damage.
- Always use tweezers to prevent contamination.
- Make sure that the mounting procedure is solid, so that you do not introduce mechanical vibrations due to incorrect mounting.
- **DO NOT** spray dry air in the direction of any electronics or scanning electron microscope, because 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 will guarantee better performance over time.

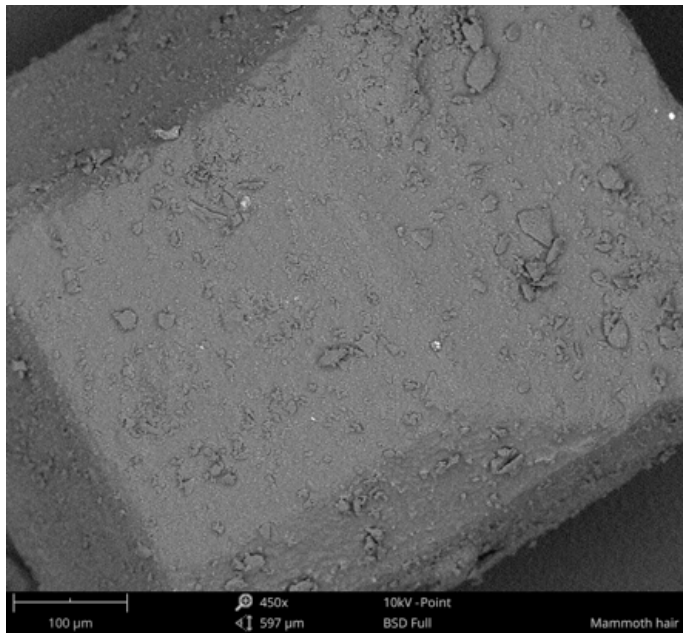
For any of the samples on the following list, please refer to the dedicated section of the guide for suggestions on how to get the best images.

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

Non-conductive samples



SEM image of sugar cube charging.



SEM image of sugar cane in low vacuum.

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

The image will become increasingly bright or entirely white until details are no longer visible. 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 aluminum stub is created.

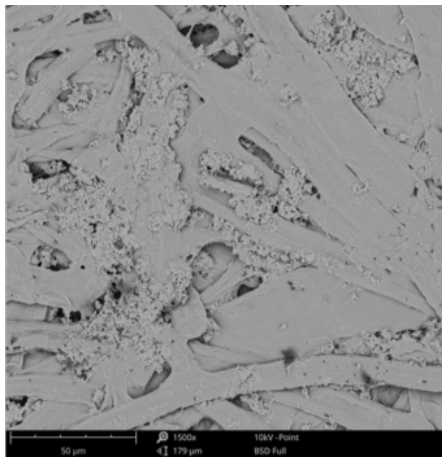
This will allow the sample to partially discharge and is enough to image mildly non-conductive samples when imaging areas close to the tape edge.

Low vacuum

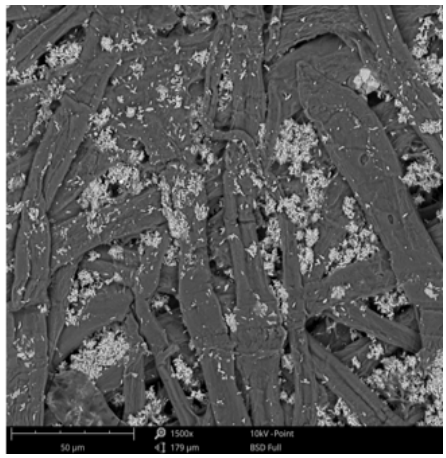
Introducing an atmosphere in the sample chamber allows beam interaction with air molecules. Positive ions are generated and attracted by the large number of electrons on the sample surface. The ions will further interact with the electrons, discharging the sample.

While this technique adds some noise to the final image, you can analyze the sample faster and inexepensively without further processing.

SEM image of paper imaged with no precaution (charging.)



SEM image of paper imaged with gold coating.



SEM image of paper in low-vacuum model.

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 between the surface of the aluminum pin and the ground potential.

The choice of coating material is strongly dependent on the kind of analysis to be performed on the sample. Gold and platinum are ideal materials for high-resolution images because both have extremely high conductivity. Lighter elements, like carbon, can be used when Energy Dispersive Spectroscopy (EDS) analysis on non-organic samples is required. An alloy of indium oxide and titanium oxide (ITO) can create transparent, conductive layers, to be used on optical glasses to make them suitable for SEM.

However, there are disadvantages to using a sputter coater: Additional instrumentation is required, the analysis becomes more time consuming, and the samples undergo more pumping cycles. Also, any advantage of using a backscatter electron detector (BSD) to image the sample is lost, as the contrast becomes very homogeneous and there is no difference in gray intensity for different elements.

Magnetic samples

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

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

Stigmation correction

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

The procedure alters the magnetic field of the lenses, which reshapes the beam. 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.

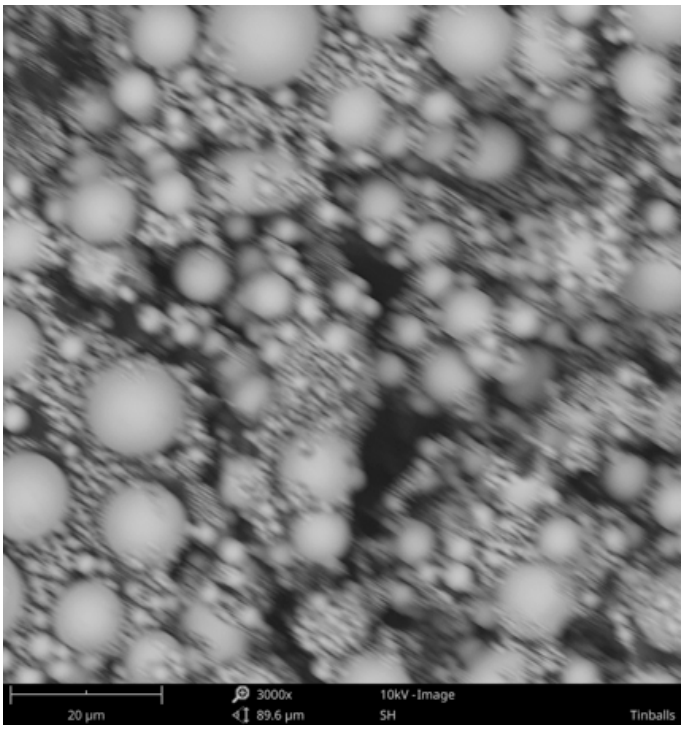
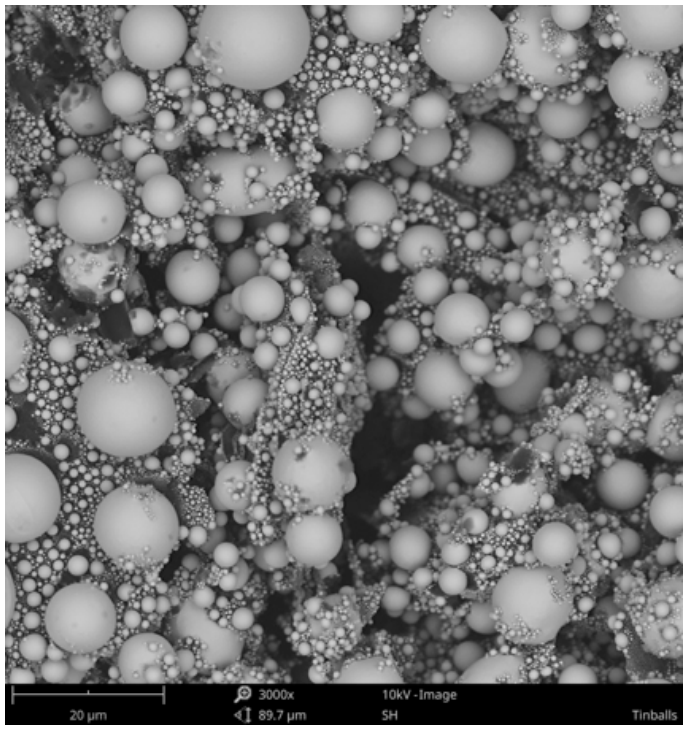


Image acquired with wrong stigmation settings. The image appears blurry and the particles (supposedly spheres) slightly deformed.



After correcting the stigmation, the image looks sharp and rich in details.

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.

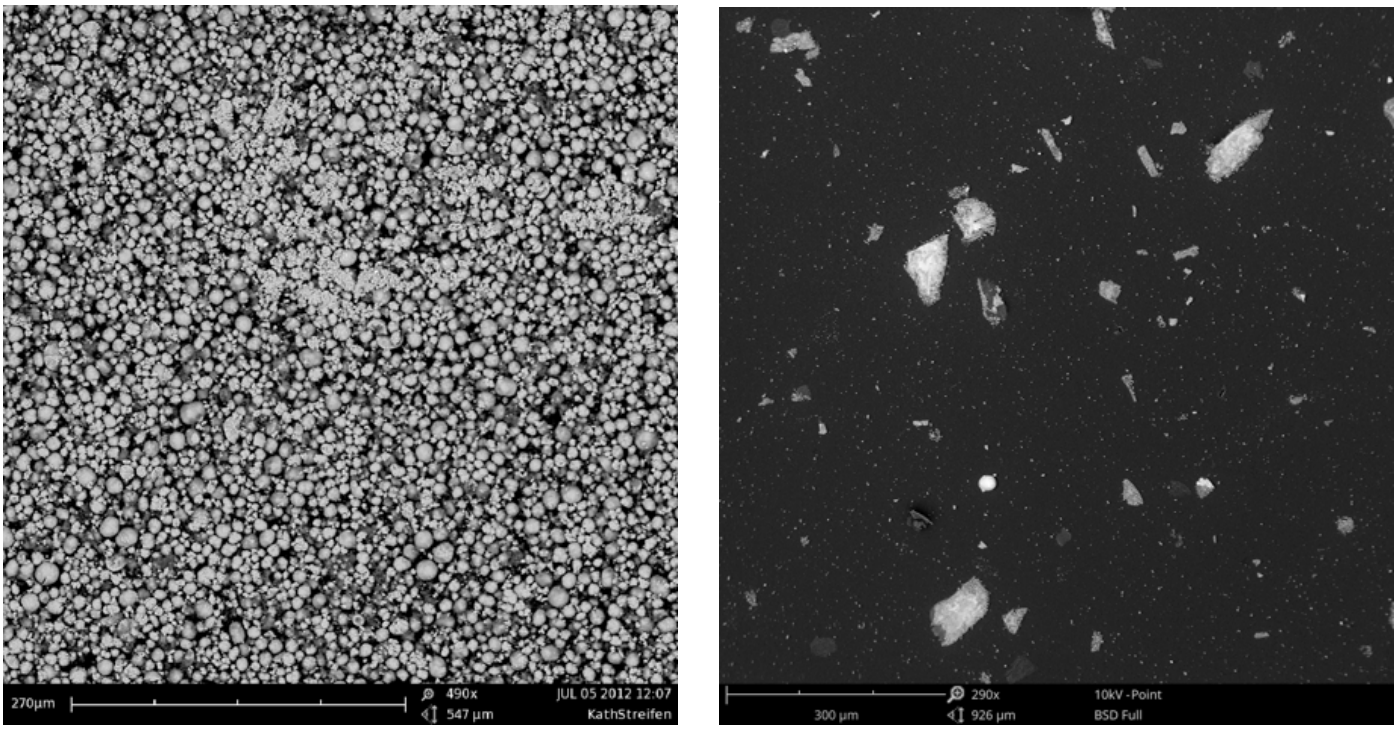
Time

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.

Powders and particles



SEM image of powder samples using a spoon and SEM image of powder sample using a disperser. When using a disperser, the particles are clearly and evenly spread, and a software can be used to count them.

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, then using spray air to remove the excess particles.

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 us the vacuum level and the amount of sample needed, depend largely on the nature of the powder. Factors to consider:

- 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.

Samples containing moist or outgassing elements

When 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. A sample without a rigid structure can be imaged if force drying or critical point drying is used to prepare it.

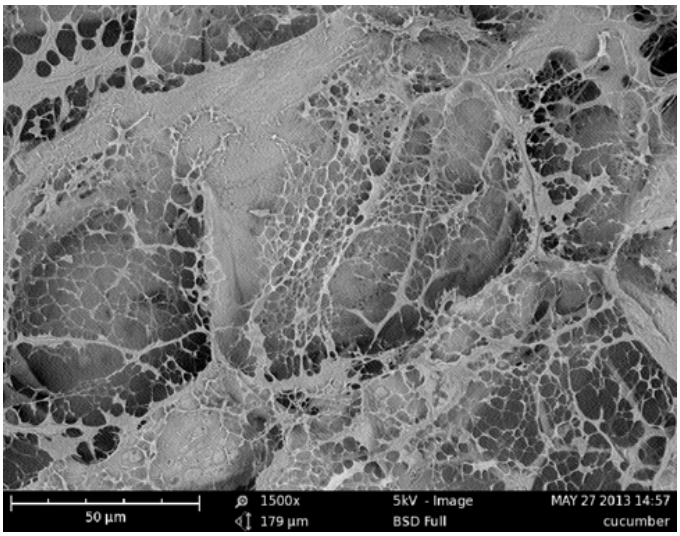
Force drying

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.

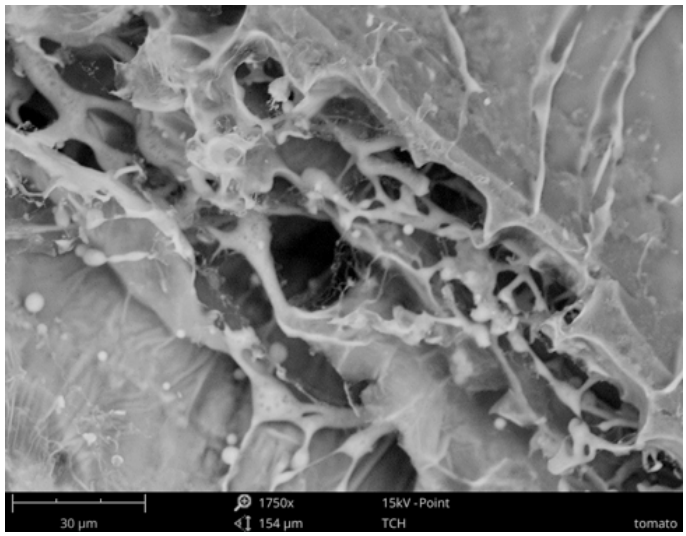
Critical point drying

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 vapor tension of the liquid in the sample.

During this process, the liquids will create fractures in the sample, causing modifications in the structure.



SEM images of a cucumber's intact structure.



SEM image of a tomato's peel and interior 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.

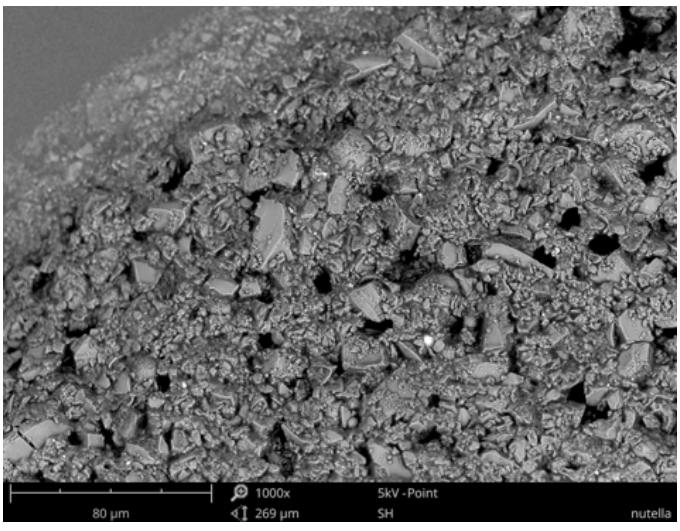
Low vacuum

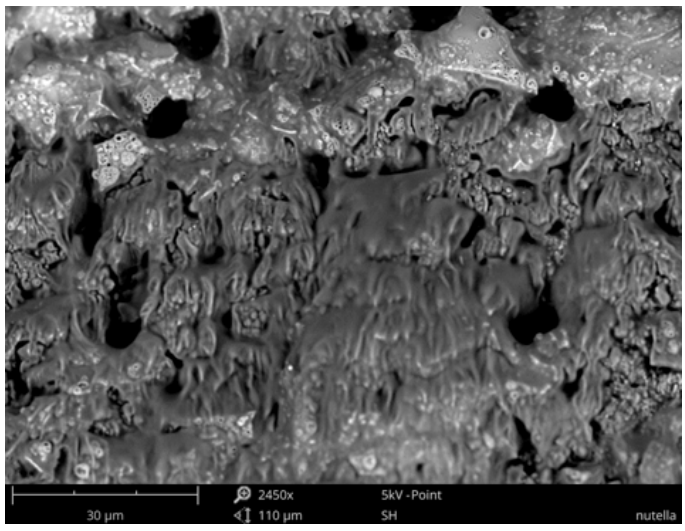
If the sample does not have a particularly high moisture content, using a small amount of sample at a reduced vacuum level can be enough to collect images. The overall image quality will be lower, but the sample can be imaged in its original state.

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.





SEM images of (left) spreadable hazelnuts and (right) cacao cream.

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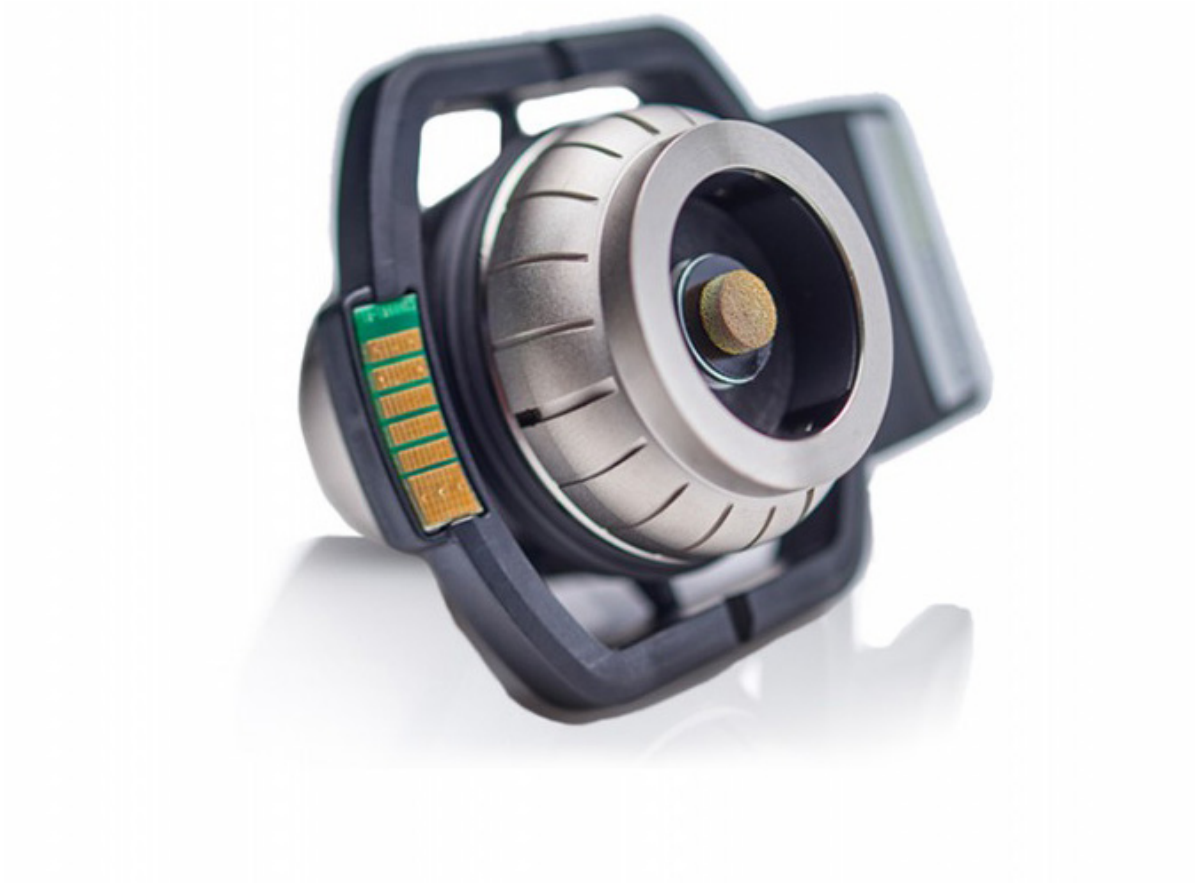

Sample preparation is just the starting point for faster and better analysis

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

Get free SEM advice

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