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SEM Technology: A Comprehensive Guide

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An introduction to electron microscopy

Luigi Raspolini, Application and Product Engineer

Electron Microscopy is a technique that makes use of the interactions between a focused electron beam and the atoms composing the analyzed sample to generate an ultra-high magnification image. This technique, when compared to normal light microscopy, has the advantage of breaking the limit of resolution that comes with light microscopy and allows for resolution that can reach the atomic level.

Light microscopy is, in fact, limited by the wavelength of light, which is physically set in a defined range. When going below the lower limit of this range, the image becomes blurry and it is no longer possible to distinguish details.

Ernst Abbe, a German scientist largely operating in the world of optical microscopes, was the first one to formalize this limit and put it into a mathematical equation. As always happens with every discovery, the paternity of the idea has been promptly doubted and the limit broken with advances in modern technology, although it remains true for traditional optical microscopes.

Electron microscopy bypasses this limitation completely, using the dimension of the electron beam as the limiting factor for the best resolution value. Since the date of its invention, in 1931, the electron microscope has been continuously improved, and the first commercial models became available thanks to the developments that happened in the Philips research laboratories in the Netherlands.

How is it possible that a company that shined for the production of light bulbs started developing such a complex piece of technology? Simple: The first models of electron microscope required a tungsten filament to emit electrons and a high vacuum so that the electron could reach the surface of the sample undisturbed. Notice any similarities with light bulbs?

Over time, different kinds of electron microscopes were developed

- Transmission electron microscopes (TEM): These use high acceleration voltages (typically >30kV) to generate an electron beam that can penetrate the sample. Samples are very thin, which allows the electrons to be transmitted across the sample and collected from a detector at the bottom of the tool. TEMs provide the best (lowest) resolution value but have very tight requirements in terms of sample preparation and analysis conditions.
- Scanning electron microscopes (SEM): These use lower acceleration voltages. The electron from the beam can get reflected (backscattered) or new electrons can be generated (secondary electrons) and collected by the detector to generate an image. The resolution value is not as good as a TEM, but the technique is more versatile.

The following chapters will describe the working principle of a ccanning electron microscope, focusing on the single components of the device and how the tool can be used to analyze different kind of samples.

Chapter 1: Guidelines for selecting the best microscope for your research

In recent years, electron microscopy has found more and more applications. Each sample has a combination of best settings that need to be used to optimize the results of the analysis. This section will tackle one by one, all the main aspects that you need to take into account when imaging samples and will explain a bit of the physics and mathematics behind them.

1.1 Magnification

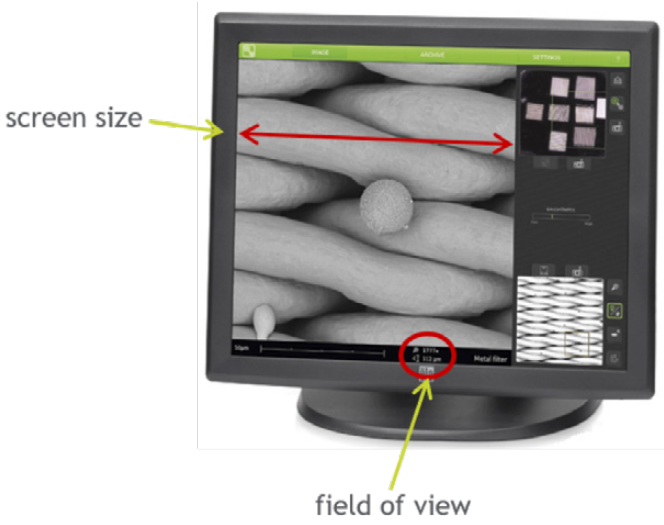
The first magnifying glasses date back to the Greeks, with Aristophanes describing the first attempt to look at small details as a leisure activity for kids. This was when the word magnification entered human language for the very first time.

Time has passed, and the interest of science for the micro and nano world has exponentially increased, creating the need for a quantification of magnification.

The modern definition of magnification is the ratio between two measurements, which implies that two objects are needed for a correct evaluation of the value.

The first object is obviously the sample. The second is a picture of it. Although the sample will not change its size, the picture can be printed in an infinite number of different sizes.

$$\text{Magnification} = \frac{\text{size of the sample on the picture}}{\text{real size of the sample}}$$



This means that printing a picture of an apple that fits on a standard printer sheet and printing it again to fit on a poster that will be used to cover a building, will change the magnification value dramatically (much larger in the second case).

A more scientific example can be applied to microscopy: when storing a digital image of the sample, resizing the image causes the magnification number to become ostensibly wrong. Magnification is thus a relative number and it is of no practical use in the scientific field.

What scientists use is a couple of parameters that describe the actual imaged area (field of view — the area that the microscope points at) and how sharp this image is (resolution). The formula of magnification also changes accordingly:

$$\text{Magnification} = \frac{\text{size of the image viewed on screen}}{\text{field of view}}$$

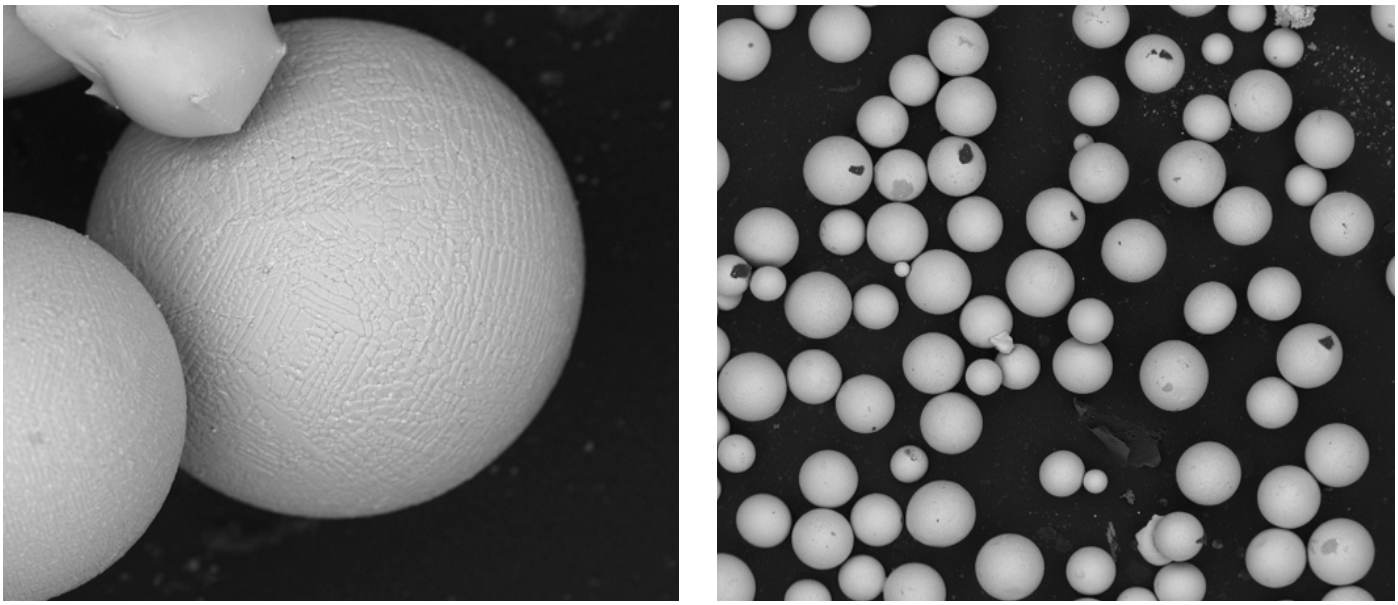
As you can see, the formula still offers a vague description and does not consider the resolution. This means that scaling the same image to a bigger screen will cause the magnification number to change.

The field of view defines the size of the feature to be imaged. This value typically ranges between some millimeters (a bug) to few microns (the hair of a bug) and a couple of nanometers (the molecular macrostructure of the exoskeleton). With modern instruments, objects in the range of few hundred picometers can be imaged and that is the average size of an atom.

But how do you define what is the required field of view to image my samples?

It depends. For example, if you have particles with an average size of 1 micron and you want to count them, it is ok to have 20 particles per image, rather than wasting time by imaging one particle at a time. Even taking into account empty space between particles, a field of view of 25-30 microns is enough for such a sample.

On the other hand, if your interest lies in the structure of a particle, a close up is needed and the observed area must be closer to 2-3 microns, if not smaller.



Images of particles. A close-up of a particle (left) shows the surface topography (FOV=92.7µm). A larger field of view (right) enables more particles to be imaged (FOV= µm).

1.2 Resolution

In microscopy, resolution is defined as the minimum distance between two objects that still allows the observer to distinguish them as separate entities.

And this is where microscopes come in. Microscopes allow us to reach outstanding resolutions, in some cases enabling the user to distinguish even atoms.

Thanks to their incredible resolution, desktop SEMs - particularly when compared to standard optical microscopes - are extremely powerful tools to analyze small features. With an average resolution easily lower than 10nm, and a price range similar to that of a high-end optical device, desktop SEMs are slowly revolutionizing the industry, realigning production standards to a new level of miniaturization.

It is important to remember that the resolution of a microscope is not the size of the smallest feature that can be imaged. This means that using a device with a resolution of 10nm to image and measure samples with an average size of 50-100 nm provides good results. Smaller features will look blurry and would require a far more sophisticated device to be imaged.

In other words, the resolution of the device should be 5 to 10 times lower than the size of the feature to be imaged.

When spots are far enough apart to be distinguished, they are resolved. If they are too close, the edges will seem to overlap, and the objects will be unresolved.

Scaning electron microscope images are stored in an image file (*e.g.* JPEG, TIFF) with a user-defined number of pixels the resolution. A SEM will scan small areas with an electron beam, which means the portions of the surface will become a pixel of the final image. More pixels result in a longer processing time, and samples can be affected by such a long analysis process.

1.3 Electron source

The electron source — or cathode, filament or electron gun — is one of the most important modules of a desktop SEM. Its purpose is to provide a stable beam of electrons. There are two groups of electron sources used in SEM, varying in the amount of current they produce into a small beam size (spot), the stability of the beam, and the lifetime of the source.

This section will focus on a type of electron source that is being used in desktop SEM: the thermionic electron source. More specifically, we focus on (the differences of) two types thermionic electron sources: Tungsten and Cerium Hexaboride (CeB_6).

What is a thermionic electron source?

When any solid material is heated, electrons will be emitted by thermionic emission. The emission becomes significant when the thermal energy of the electrons is sufficient to exceed the work function of the material. The cathode is made from a high melting point material with a relatively low work function in order to emit many electrons.

The electron beam that is projected onto your sample is created by the emitted electrons being accelerated from the high negative potential of the source to ground potential at the anode inside the electron column. This process can, of course, only happen inside the vacuum of an electron column and by using lenses to control the beam.

Before analyzing and comparing the Tungsten and CeB_6 source, it is useful to know which properties of an electron source are key when determining its performance. We will focus on the most important properties:

1. **Brightness of the electron source**
   Brightness is defined as the beam current per unit area per solid angle. The more current/electrons you have available in a small spot size, the better you can achieve high resolution (quality) images at high magnification.

   The brightness increases linearly with the acceleration voltage. For example, every electron source is ten times as bright at 10kV as it is at 1kV.

   The spot size of the electron beam can be made smaller to improve the resolution, but at some point, the limitation is the signal-to-noise-ratio necessary to get a good quality image.

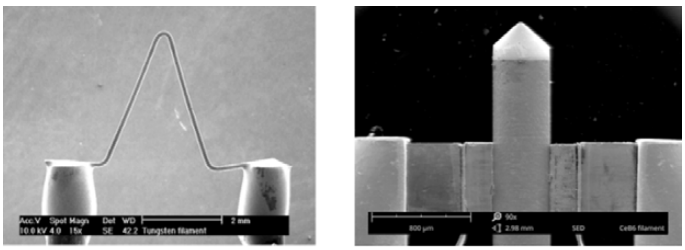
2. **Source size**
   As mentioned before, a small spot size contributes to good image resolution and therefore high- quality images. The lenses (mainly facilitated by the condenser lens) inside the electron column are responsible for demagnifying the beam diameter (or spot size). You can imagine that a smaller physical size of your source leads to less (complex) demagnification.

3. **Source temperature**
   The source temperature is the operational temperature which overcomes the work function in order to emit electrons. The operational temperature for thermionic sources lies between 1800 and 2800 Kelvin.

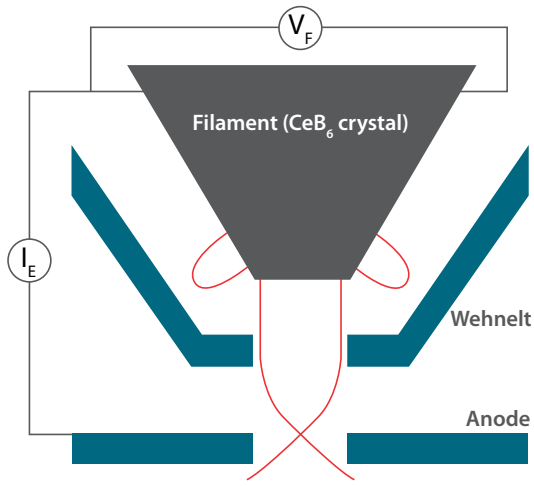
   3a. Electron beam energy spread
   The electron beam energy spread is the spread in electron energies leaving the source. Chromatic aberration becomes the dominant aberration at low acceleration voltage when the energy spread of the source is large. Chromatic aberration is an effect that causes a less focused beam due to electrons with slightly different energy leaving the source.

   3b. Lifetime
   Lifetime represents the lifespan of an electron source before it breaks or needs to be replaced. Preferably, you want a source that is durable and for which you can accurately predict the moment of replacement.

We can now start our tungsten and CeB_6 comparison based on the most important properties of an electron source.



Tungsten versus CeB_6 filament



Cross-section view of an electron column with a schematic view of the source assembly.

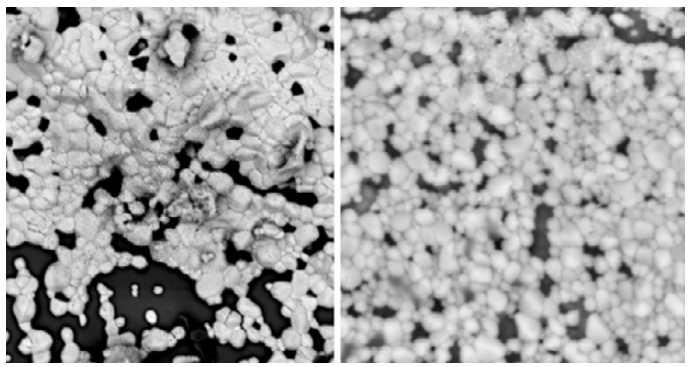
Tungsten filaments are widely used in scanning electron microscopy. Of all metals in pure form, Tungsten has the highest melting point, the lowest vapor pressure, the lowest thermal expansion and a very high tensile strength, which are ideal properties for making an electron source.

But as you will notice in the comparison, tungsten has some fundamental disadvantages compared to a Cerium Hexaboride (CeB_6) electron source:

1. **Brightness of the electron source**
   When we look at brightness, the tungsten source provides 106 A/cm^2 sr. The lower work function of a CeB_6 filament results in higher beam currents at lower cathode temperatures than Tungsten, which means greater brightness at all acceleration voltages. To concretize this: a CeB_6 cathode provides ten times the brightness compared to tungsten: 107 A/cm^2 sr. This gives the CeB_6 source two advantages over a tungsten source:

   More current available in the same focused spot, which means a better signal-to-noise ratio at the same spot size.

   At the same signal-to-noise ratio the CeB_6 spot can be made smaller, which means that a better resolution can be achieved.



Left image: TiO2 powder made with CeB6 system.Right image: TiO2 powder made with the tungsten system.

2. **Source size**
   AThe source size is of tungsten is elliptically shaped with a dimension ranging from 50µm to 100µm, depending on the source configurations and operating conditions. Compared to a CeB_6 source, which has a dimension of <25µm, it means that considerable electron optic demagnification is required for a tungsten source to achieve a small electron probe needed for good resolution in SEM.

3. **Electron source temperature**
   The operational temperature of the tungsten filament lies around 2800 Kelvin, where the CeB6 source has an operational temperature of 1800 Kelvin. The difference in temperature has a direct effect on the source.

   3a. Electron beam energy spread
   The higher temperature setting of the tungsten source causes a larger energy spread than a CeB_6 source. Typically, the energy spread of a tungsten source is about 2.5eV, whereas the CeB_6 is about 1eV, resulting in better image quality especially at lower acceleration voltages.

   3b. Electron source lifetime
   A tungsten filament operates at white-hot temperatures, which means it gradually evaporates with time. Eventually, the tungsten wire becomes thin and breaks which always happens during imaging. The breaking of the tungsten wire can possibly contaminate the upper part of the electron column. This is why, when replacing the tungsten filament, it is advised to replace or clean other source-related parts inside the column as well.

The advantage of a CeB_6 source: you can predict its lifetime ending as it slowly degrades in time. You will know when it is time to replace your CeB_6 filament and can do so between operating sessions. You will not end in up in a situation where you have to terminate your analysis because of a broken filament and, more importantly, you do not have to worry about contamination of the column due to debris. Using a CeB_6 source also minimizes the need to replace other source-related parts along with your source.

The lifetime comparison for tungsten and CeB_6: the average lifetime of a tungsten source is about 100 hours, depending on the vacuum. A CeB_6 source typically provides more than fifteen times the service life, at 1500+ hours.

1.4 Voltage

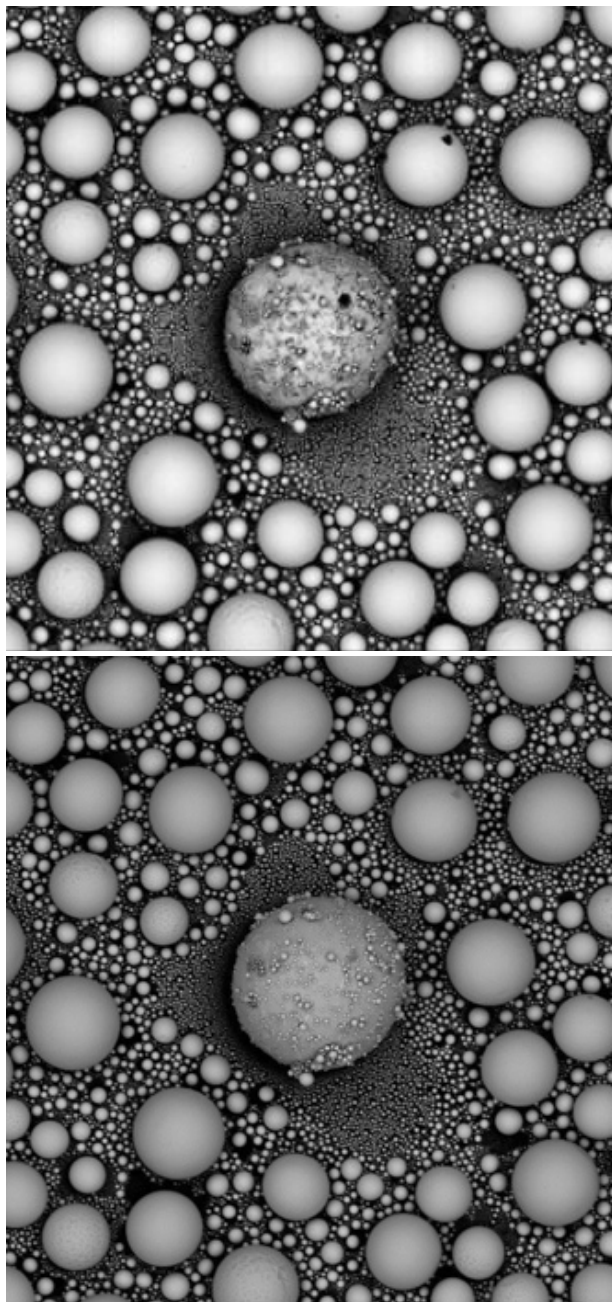
The voltage is an indication of the electrons' energy content: this will therefore determine what kind of interaction the beam will have with the sample. As a general guideline, a high voltage corresponds with a higher penetration beneath the surface of the sample — also known as bigger interaction volume.

This means that the electrons will have a larger and deeper propagation within the sample and generate signals in different parts of the affected volume. The chemical composition of the sample also has an impact on the size of the interaction volume: light elements have fewer shells, and the electrons' energy content is lower. This limits the interactions with the electrons from the electron beam, which can therefore penetrate deeper into the sample, compared to a heavier element.

When analyzing the outcoming signals, different results can be obtained. In desktop instruments, three kinds of signals are normally detected: backscattered electrons (BSE), secondary electrons (SE), and X-rays.

The effect of voltage in SEM imaging

The effect of voltage within the BSE and SE imaging is comparable: low voltages enable the surface of the sample to be imaged; high voltages provide more information on the layer beneath the surface. This can be visualized in the images below, where low voltages make surface sample contamination clearly distinguishable, while higher tensions reveal the structure of the surface underneath the contamination layer.



BSE images of tin balls at 5kV (top) and at 15kV (bottom). With the lower voltage, the carbon contamination on top of the sample becomes visible. When the voltage is increased, the deeper penetration enables the imaging of the tin ball surface underneath the carbon spots.

The nature of the sample is also hugely important in the choice of the appropriate voltage. Biological samples, several polymers, and many other (mostly organic) samples are extremely sensitive to the high energy content of the electrons. Such sensitivity is further enhanced by the fact that the SEM operates in vacuum. This is the leading reason why the focus of SEM developers is moving towards increasing the resolution value at lower voltages, providing important results even with the most delicate samples.

The main difficulty that is encountered in this process is the physics principle behind the imaging technique: in a similar way to photography, there are in fact several kinds of distortion and aberration that can affect the quality of the final output. With higher voltages, the chromatic aberrations become less relevant, which is the main reason why the previous trend with SEM was to turn towards the highest possible voltage to improve imaging resolution.

The generation of X-rays

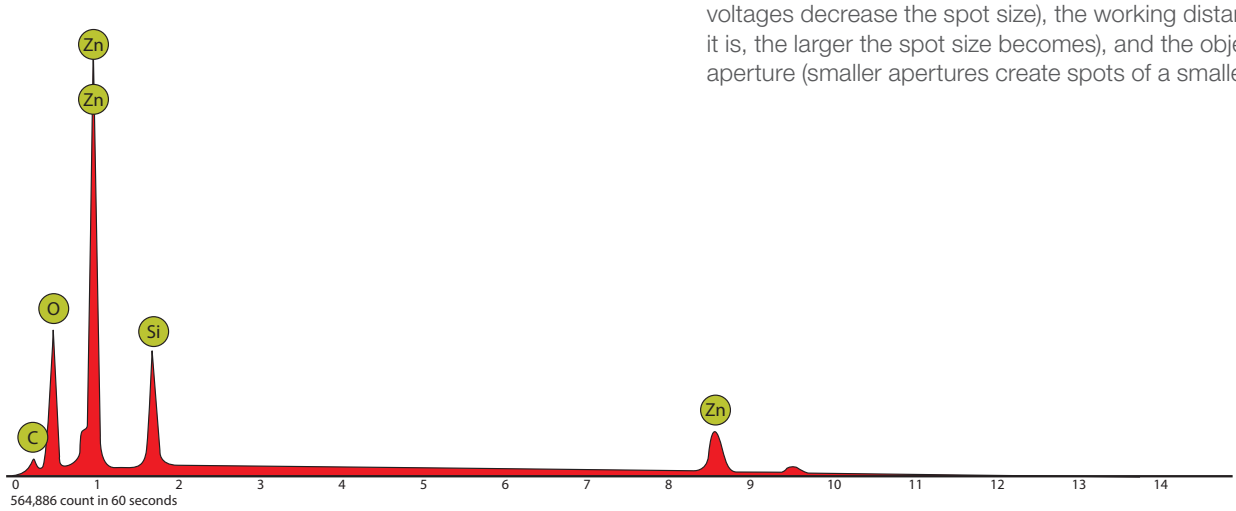
When it comes to X-ray generation, the story is totally different: a higher voltage is responsible for a higher production of X-rays. The X-rays can be captured and processed by an EDS (energy dispersive spectroscopy) detector to perform compositional analysis on the sample.

The technique consists of forcing the ejection of an electron in the target sample by means of the interaction with the electrons from the electron beam (primary electrons).

A charge vacancy (hole) can be generated in the inner shells of an atom, and it is filled by an electron with a higher energy content from an outer shell in the same atom. This process requires the electron to release part of its energy in the form of an X-ray. The energy of the X-ray can finally be correlated to the atomic weight of the atom through the Moseley's law, returning the composition of the sample.

Key factors in X-ray production

- **Overvoltage:** The ratio between the energy of the incoming beam and the energy necessary to ionize the targeted atom.
- **Interaction volume:** Defines the spatial resolution of the analysis.



This example shows an EDS spectrum collected at 15kV. The peaks highlight the presence of an element, and a complex algorithm is applied to convert the signal coming from the detector into chemical composition.

The ideal analysis requires a minimum overvoltage value of 1.5, which means that by increasing the voltage, the maximum number of detectable elements increases. On the other hand, a high voltage corresponds with higher chances of sample damage and, even more importantly, a larger interaction volume.

This not only means that the sample reliability could be compromised, but also that the generation of X-rays interests a much larger volume. In the case of multilayers, particles, and generally non-isotropic materials, a larger interaction volume will generate signals coming from portions of the sample with a different composition, compromising the quality of the results.

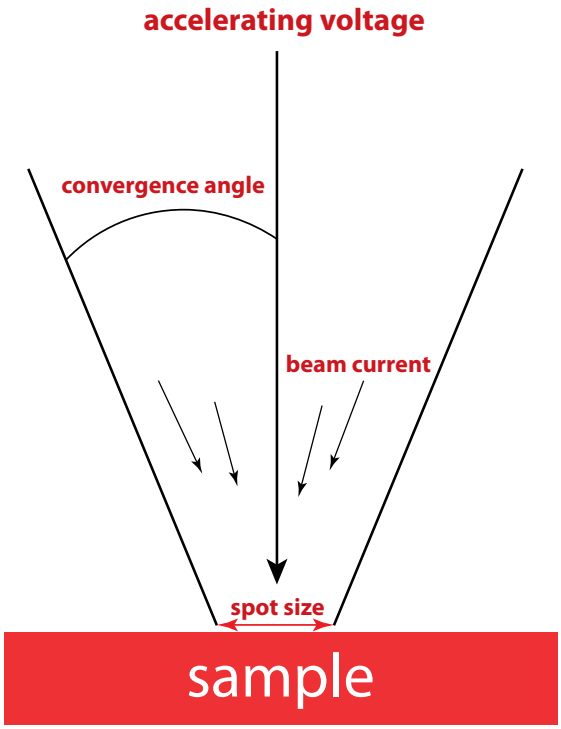
Typical recommended tension values for the analysis range between 10 and 20kV, to balance the two effects. Choosing the ideal value depends on an additional aspect of EDS analysis that is known as 'peak overlap'. X-rays generated by electrons moving from different shells of different elements can have comparable energy contents.

This requires more advanced integration processes to deconvolute the peaks and normalize the results, or use the higher energy content lines (coming from one of the two elements with overlapping peaks). While the former is already implemented in most EDS software, the latter is not always possible, considering that the higher energy level line for a very common element such as lead would require a voltage higher than 100kV.

1.5 Current intensity

In any modern scanning electron microscope, the user has the ability to control the size of the electron probe. This is mainly achieved by adjusting the condenser and the objective lenses of the system and by selecting different apertures.

Electrons are flowing through electromagnetic lenses (which simply consist of coils of wires inside metal pole pieces) and the user is able to control the electron's path by tuning the current that is applied to the lenses. Moreover, the spot size is dependent on the acceleration voltage (high accelerating voltages decrease the spot size), the working distance (the larger it is, the larger the spot size becomes), and the objective lens aperture (smaller apertures create spots of a smaller diameter).



The four major parameters of the electron beam in a SEM: Accelerating voltage, convergence angle, beam current, and spot size.

However, the size of the final electron probe is a parameter that is far more complex to control and predict, as it depends on many (and interconnected) factors. The relation that describes the spot size has terms that depend on the Gaussian diameter of the gun, the diffraction effect of the final aperture, the chromatic aberration, and the spherical aberrations of the beam-forming lens.

It may seem trivial that in order to have a small probe and sufficient current on the sample, the user simply needs to increase the convergence angle of the probe. This will, however, increase the aberrations of the optical components in the microscope and therefore broaden the beam. It is therefore evident that in order to perform an experiment with accuracy, it is important to understand how different parameters influence the characteristics of the electron beam and identify the trade-offs between them.

High-resolution imaging versus high beam current

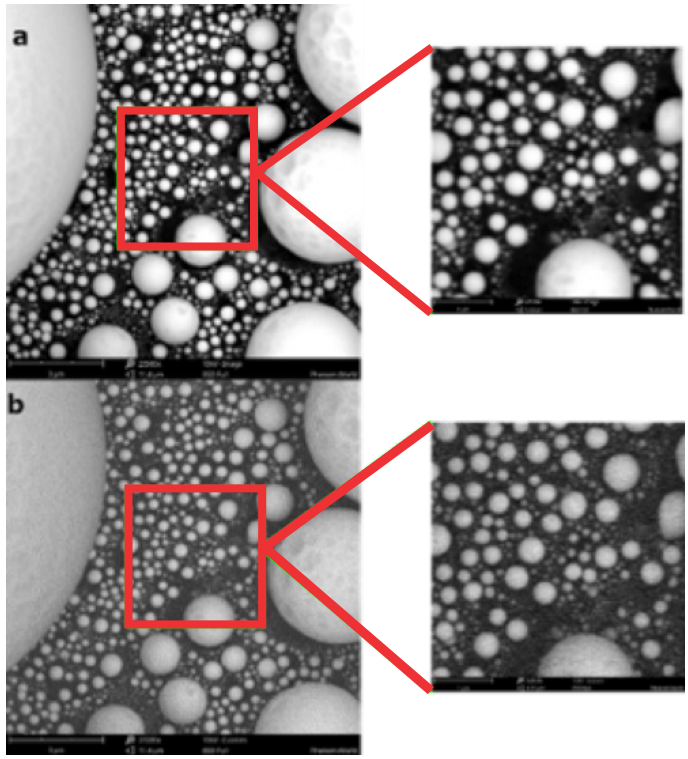
The major factor that affects resolution is spot size. To acquire a high-resolution image, the spot size should be kept as small as possible in order to be able to resolve and describe even the smaller features of the specimen sufficiently.

On the other hand, it is also important that the beam carries enough beam current for sufficient signal-to-noise (S/N) ratio and contrast resolution. Since reducing the spot size also decreases the beam current, users need to identify and select the settings that will best fit their goal.

In general, if high magnification images are needed, the spot size should be kept minimal. If the user only requires low magnification imaging, then it is recommended that the spot size is increased so that the images have more “electron juice” and look sharper.

In the following image you can observe that images acquired at low magnification but with a larger spot size seem brighter and smoother. However, as the magnification increases, the user should switch to the smaller spot size, which gives better results when highresolution imaging is required.

Also, broader spot sizes — and consequently higher beam currents — increase damage to the sample, something that should be taken into account, especially when beam-sensitive samples are to be imaged.



These SEM images are of tin. At low magnification, a high beam current (a) is preferred. At high magnification, using a smaller spot size (b) allows the user to achieve better spatial resolution.

A scanning electron microscope is a fascinating tool with countless applications. However, it is very important that the user has a clear idea of what type of analysis is required — and of how the different spot sizes, beam currents, and accelerating voltages will influence the SEM imaging quality. Selecting the best parameters for any given experiment is crucial.

1.6 Customizability

Scanning electron microscopes can be equipped with many different detectors or accessories to perform different kinds of analysis or to image non-ideal samples. For example:

- Freezing the sample enables the user to work with samples that have a high moisture content.
- An EDS detector can provide the chemical composition.
- Tensile testing provides information on the behavior of the sample when stressed with a big load.
- Motorized tilting systems make it possible to move the sample while it’s in the vacuum.

Endless examples can be made and different technologies prove to be more helpful with specific kinds of samples.

1.7 User experience and time to image

As electron microscopy has become economically accessible, the user experience around these devices has been redesigned to be operated by any user.

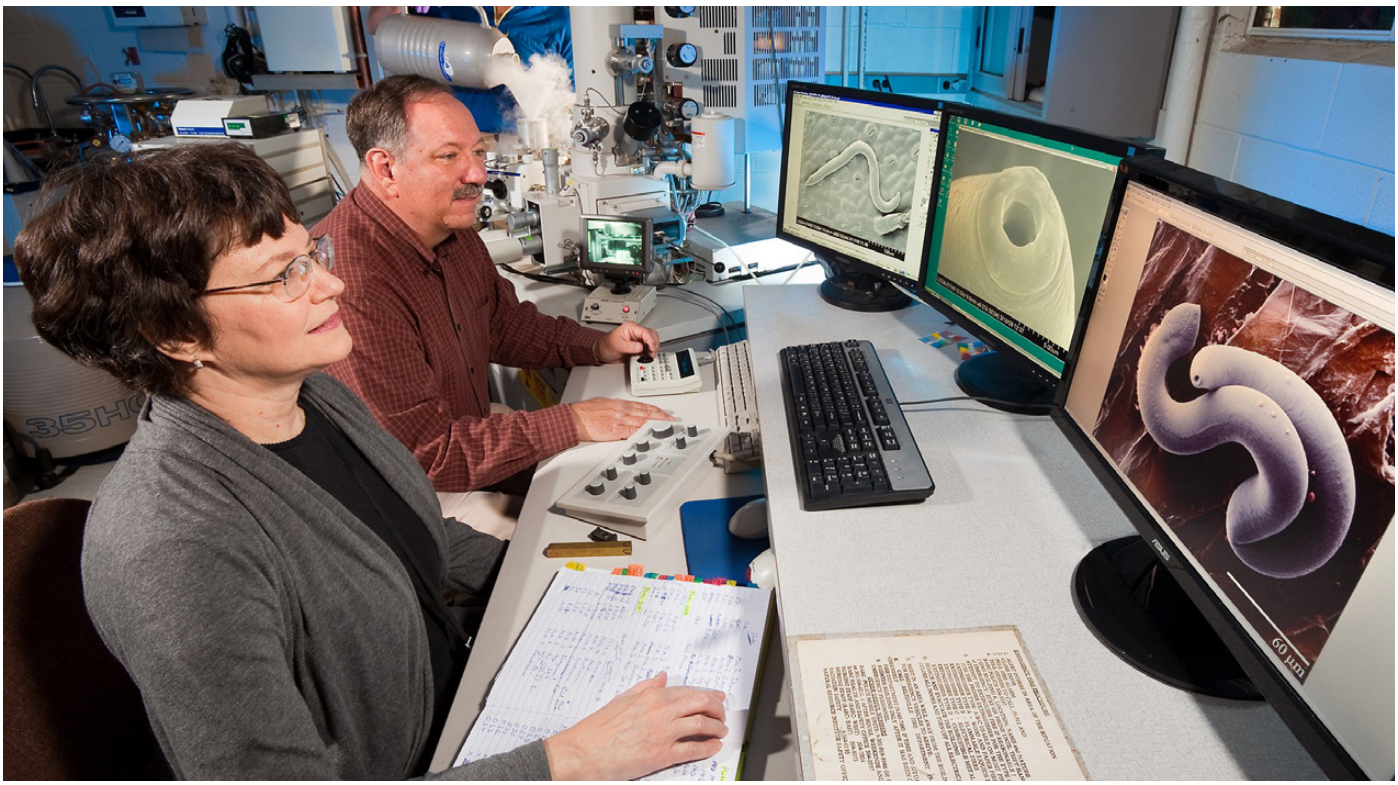
Scanning electron microscopes contain delicate electronic components and they are highly susceptible to contamination from exposure to polluted environments. Reducing the time that the inside of the system is exposed to external environments (for example with an electron source that lasts longer and therefore does not constantly require the system to be opened to replace it) helps in keeping the device in optimal conditions.

Defining the sample height is also an operation of crucial importance when doing SEM analysis: If the sample is too low, the signal will not be strong enough and the resolution and image quality will be lower. On the other hand, if the sample is loaded too high, there is a risk of hitting the detectors.

Smart loading systems have been designed to prevent damage to the devices and make it easy to position the sample at the ideal working distance.

The alignment of the columns used to be a sine qua non condition for proper imaging. Now electron columns can be pre-aligned to further save user's time.

The ease of use will define how much time is needed to collect the desired results. A system which is easy to use and with a short loading time can ensure results within 1-2 minutes, saving time so the operator that can focus on more important tasks.



Chapter 2: Anatomy and dissection of a scanning electron microscope

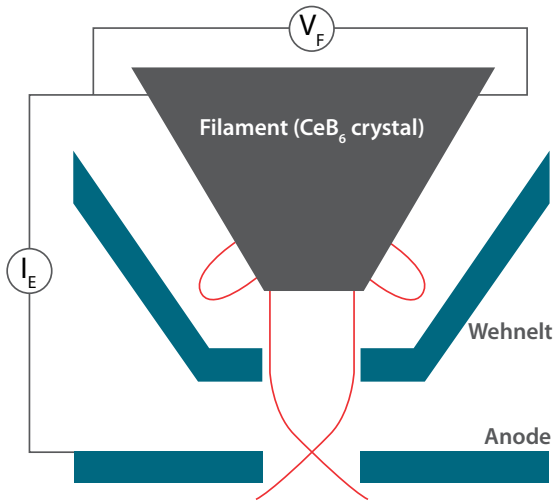
Every scanning electron microscope is made of different main components. The engineering behind these components will determine the final quality of the image and the result of the analysis. This section will analyze, one by one, the main components of a SEM and provide some interesting insights on how they work and what their function is in the microscope.

2.1 The electron source

CeB_6 and tungsten are both thermionic sources with a filament called a cathode, from which electrons are emitted. The emission starts when the electrons are provided with enough energy to cross the potential barrier, given by the work function of the cathode material, which can be either tungsten or CeB_6.

The energy is provided by heating up the cathode, which in turn is done by letting current flow through it. A Wehnelt electrode that is negatively charged with respect to the cathode pushes the unwanted electrons back into the filament, effectively determining the size of the emitting area.

Below the cathode and the Wehnelt electrode, an anode provides a strong electric field, or a strong lens that makes the electron beam converge into a crossover between the Wehnelt and the anode. The following image shows the schematics of the CeB_6 source, consisting of a filament, a Wehnelt electrode and an anode. The filament is at high potential, as well as the Wehnelt, whereas the anode is grounded. The circuitry positioned in between the filament and the anode measures the emission current.



This is a schematics of thermionic source consisting of a CeB_6 crystal (the filament), a Wehnelt electrode. and the anode. The red trajectories indicate that the electrons are pushed back in the filament, due to the Wehnelt voltage and the trajectories of the emitted electrons, forming the primary beam.

Comparing thermionic electron sources: CeB_6, LaB_6 and W

CeB_6 is not the only cathode for thermionic sources, LaB_6 and tungsten are also used. Tungsten cathodes are hair-pin filaments that are bent to reduce the size of the emitting surface. They are typically warmed up to a temperature of 2500-3000 K to achieve high current density, being the work function of tungsten 4.5 eV. At 2800 K, a practical value of current density is 3 A/cm^2.

The lifetime of tungsten cathodes, which can vary between 40 and 200 hours, is limited by the evaporation of the cathode material, resulting in the wire breaking when it becomes too thin. To prevent too much oxidation, a vacuum of 10-3 Pa is kept at the source.

Hexaboride crystals (CeB_6 and LaB_6) cathodes are rods with a flat tip, and are typically heated up to 1400-2000 K, as the work function is lower than the tungsten (2.7 eV for LaB_6 and 2.5 eV for CeB_6). A low work function and low temperatures yield a higher current density than tungsten cathodes, in the range of 20-50 A/cm^2.

Typically, hexaboride cathodes are 10 times brighter than tungsten cathodes, meaning they provide higher beam current in a smaller spot size at the sample. Also, the lifetime of hexaboride cathodes is higher, typically 10 times that of tungsten cathodes.

However, hexaboride cathodes need a vacuum of better than 10^{-4} Pa to prevent oxidation. The performance of hexaboride cathodes strongly depends on vacuum and temperature. Studies suggest that CeB_6 cathodes are less likely to be affected by carbon contamination than LaB_6 cathodes. Also, CeB_6 cathodes have a lower evaporation rate at a working temperature of 1800 K compared to LaB_6. Therefore, the shape of a CeB_6 cathode tip lasts longer.

The following table summarizes the physical properties of the three thermionic sources:

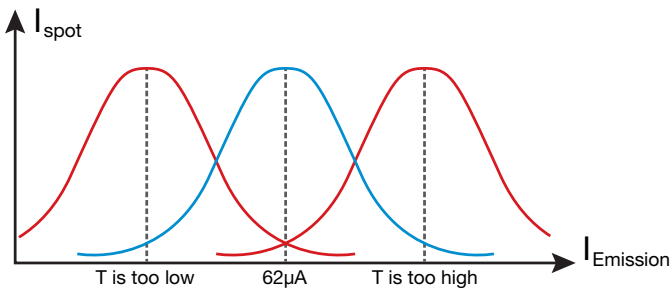
	CeB_6	LaB_6	Tungsten
Work function [eV]	2.6	2.7	4.5
Temperature [K]	1800	1800	2800
Pressure [Pa]	10^{-4}	10^{-4}	10^{-3}
Current density [A/cm^2]	20-50	20-50	3 (@2800 K)
Lifetime [hours]	1500+	1000+	100

Emission current stability in the Phenom CeB_6 electron source

The stability of the emission current is a key requirement for thermionic sources. During the operation of the microscope, the emission current is kept stable by adjusting the Wehnelt voltage in a constant control loop. The emission current is measured in the source, by a circuitry between the filament and the anode, as shown in Figure 10. The Wehnelt voltage is then adjusted according to the read out of the emission.

It is of utmost importance that the current at the sample is kept constant, for given settings. An automated function measures the sample current as a function of the emission current. The emission current is adjusted by varying the voltage on the Wehnelt, thereby regulating the amount of electrons pushed back into the filament, for a constant filament temperature. The current at the sample can be measured indirectly from the signal of an image taken with the BSD detector on a reference material.

The optimal case, shown in blue in the figure below, is when the current at the sample has a maximum at 62 µA. If the peak is before or after this value, it means that the temperature of the filament is either too low or too high and needs to be adjusted. The automated function sets the new temperature of the filament and measures the current at the sample for different emission currents again, until the peak falls at the ideal emission current of 62 µA.

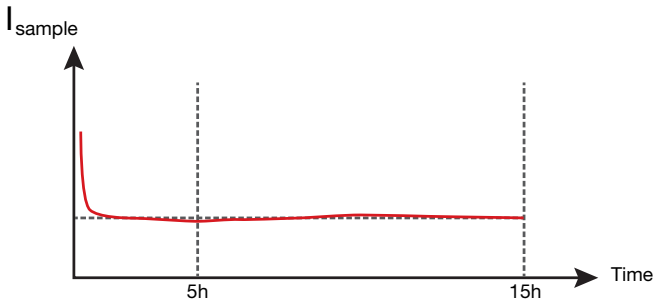


The current at the sample (I spot) is a function of the emission current for different filament temperatures.

Once the temperature is adjusted through the automated function, the voltage on the Wehnelt is set for an emission current of 40 µA, thus on the left of the peak of the blue curve. An emission of 40 µA is chosen as the optimum between resolution and beam current, which translates into image quality.

The current stability of thermionic sources is typically better than 1% RMS. Measurements on sample current stability of a CeB_6 source in a Phenom microscope show that the fluctuation of the beam current is about 0.3 % in the first 5 hours from switching on the source and is 0.2 % from 5 hours up to 15 hours after switching on the source, as shown in the following image. The drop in the first hour is measured to be approximately 10% and is caused by the stabilization of the temperature in the source unit.

Moreover, the vacuum stability at the source does not affect the emission current of the CeB_6 source in a relevant way.



Representation of the measured current at the sample over a period of 15 hours.

2.2 Electron column

The electron beam travels through the electron column, which consists of a set of lenses that focus the beam onto the sample surface. Electron microscope lenses can be electrostatic or magnetic, depending on whether they use an electrostatic field or a magnetic field to focus the electron beam. To better understand how these lenses work, let's take a step back and look at how electrons can be deflected in an electrostatic field.

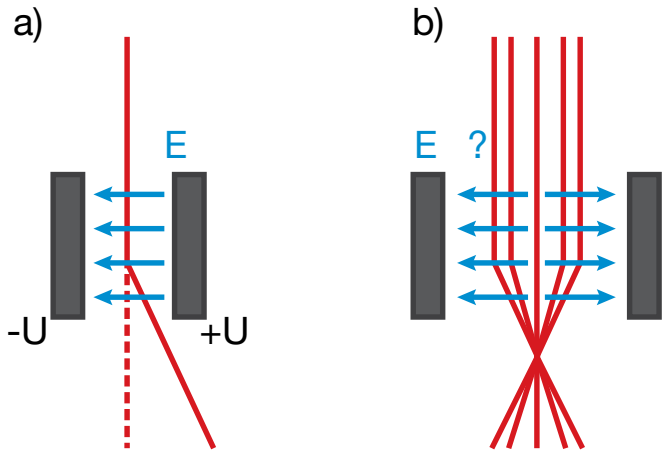
Deflectors

Electrons are negatively charged particles and travel through the electron column at high energy and high speed. One way to deflect these particles is to let them travel through an electric field generated by two plates at potential +U and -U, as shown in Figure 13a. Under the influence of the electric field, the electron is deflected at an angle that depends on the electron energy, the electric field applied in between the plates, and the length of the plates.

The faster, or the more energetic the electron, the smaller the deflection angle. The higher the electric field and the longer the plates, the bigger the deflection angle. A device consisting of two plates at different potential is called a deflector.

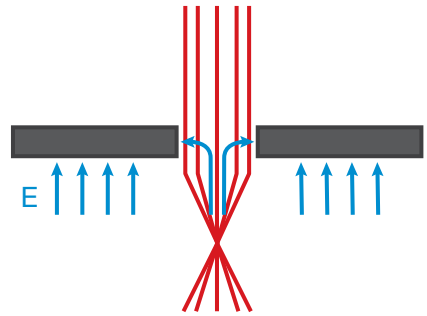
To get an electrostatic lens, one could think of mirroring the effect of a deflector, such that the outer electrons traveling off the optical axis can be focused on the same point, as shown in the following image.

Starting from the fact that electric fields can only start and end on electron charges, how can we get a lens effect as depicted in the following image? The answer to this question lies in the fact that whenever there is a lens effect, the energy of the beam changes, meaning that the electrons either accelerate or decelerate. This can be done simply by having an aperture on different potential around the beam.

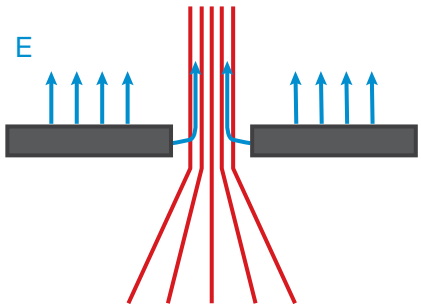


Schematic of an electron beam deflector (a) and electrostatic lens (b).

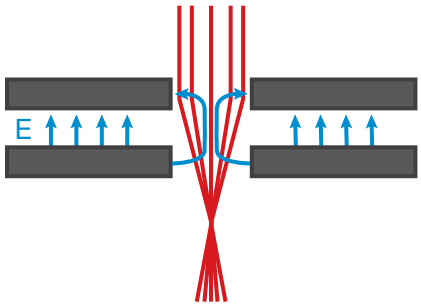
a) Single-aperture lens (positive)



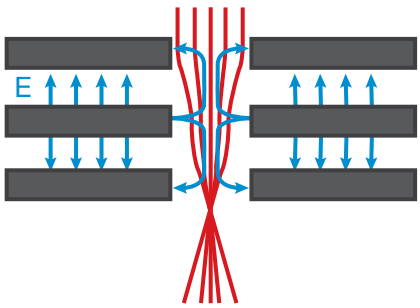
b) Single-aperture lens (negative)



c) Two-aperture lens



d) Three-aperture Einzel lens



These schematics show different kinds of electrostatic lenses: Single-aperture positive (a), single-aperture negative (b), two-aperture (c), and three-aperture Einzel (d).

Electrostatic lenses

Electrostatic lenses consist of metallic plates connected to high voltage with an aperture that the electron beam travels through. Single-aperture lenses consist of a single metallic plate at high voltage and can often be found in electron sources.

The single-aperture lenses can either terminate an accelerating field or be followed by an accelerating field. In the first instance (a), the lens is positive, meaning that the beam converges into a crossover. In the second instance (b), the lens is negative, meaning that the beam diverges.

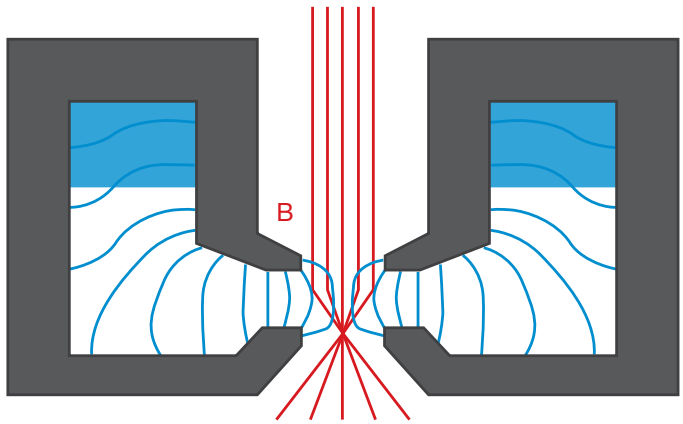
A two-aperture lens consists of two metallic plates at different potential with aligned apertures. This instance (c) shows an accelerating two-aperture lens, where the electric field in between the two plates points at the top plate. The electrons that enter this lens will feel a strong field that pushes them closer to the optical axis. As they travel through the second plate, the electrons feel an opposite force that pushes them towards the aperture. As a total effect, this is a positive lens and the beam is focused in a plane below the second plate.

A three-aperture Einzel lens consists of three plates with aligned apertures, that can either have the same diameter, or a different one. Einzel lenses are commonly used in electron optics because they have the advantage of having an equal beam potential at the entrance and exit of the lens. In an accelerating Einzel lens (d), the three electrodes generate three lenses: The first and third lenses are positive, where the electric field lines point to the plates. The second lens is negative.

Magnetic lenses

Magnetic lenses use the Lorentz force, which is proportional to the electron charge and velocity, to deflect electrons. Magnetic lenses consist of a metallic body (called the ferromagnetic circuit) that ends with two pole pieces.

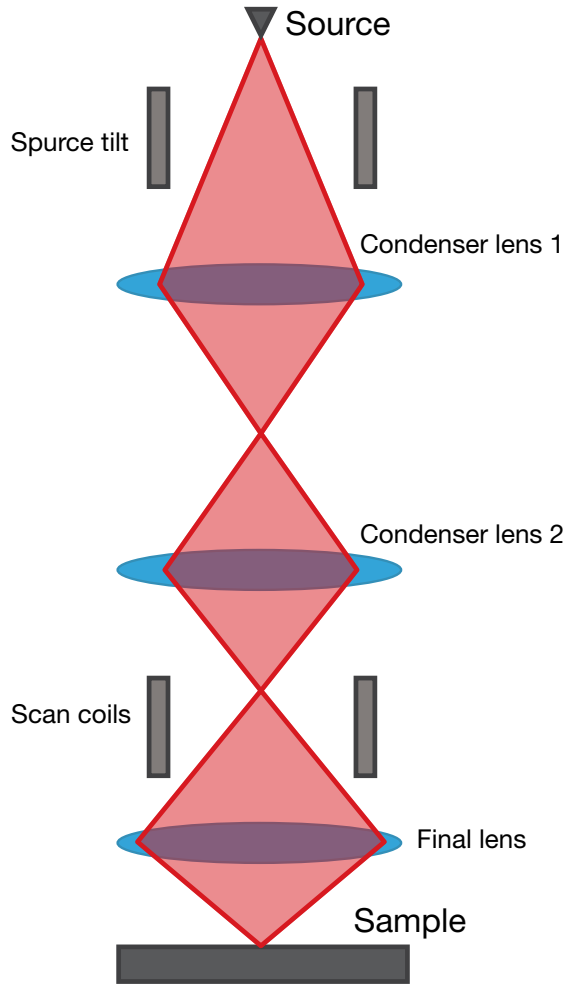
The magnetic field is given by a coil positioned at the top of the ferromagnetic circuit, as shown in the following schematic. The strength of the lens can be altered by varying the magnetic field B. This is done by modifying the geometry of the pole piece, namely the distance between the pole pieces, and the current flowing into the coils (excitation).



Schematic of a magnetic lens.

2.3 SEM electron column

The electron column consists of the electron source, where the electrons are emitted, and a set of lenses. The electrons are condensed into a beam by the condenser lenses and then focused onto the sample surface by the final lens, also called the objective lens, as shown in the schematic below. The source tilt and the scanning of the beam at the sample is done by coils at the source and right above the final lens.

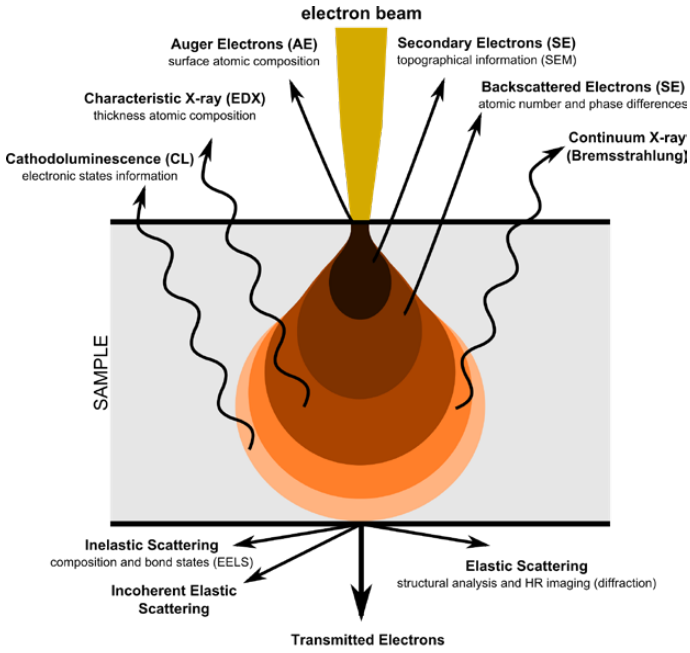


Schematic of an electron column.

The signals

In the following image, you can see the various products that are possible as a result of the interaction between electrons and matter. All these different types of signals carry different useful information about the sample and it is the choice of the microscope's operator which signal to capture.

For example, in transmission electron microscopy (TEM), as the name suggests, signals such as the transmitted electrons are detected that will give information on the sample's inner structure. In the case of a scanning electron microscope (SEM), two types of signal are typically detected; the backscattered electrons (BSE) and the secondary electron (SE).

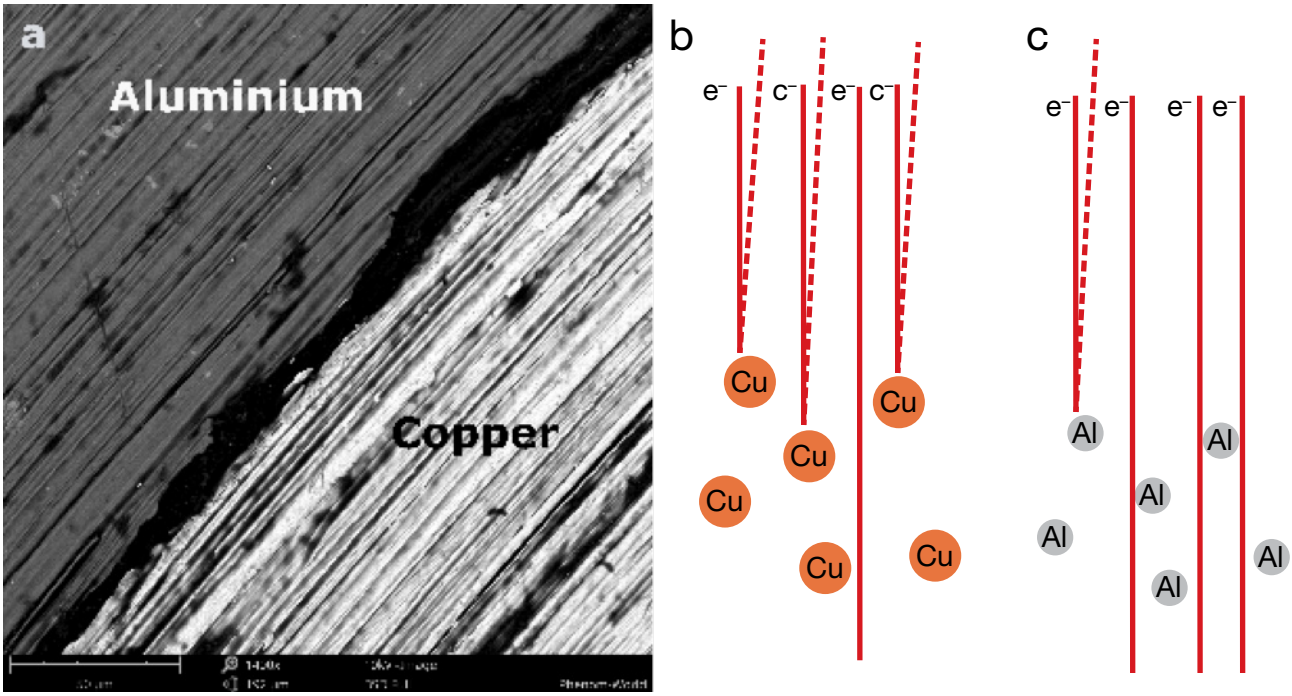


Electron-matter interaction volume: Different types of signals are generated.

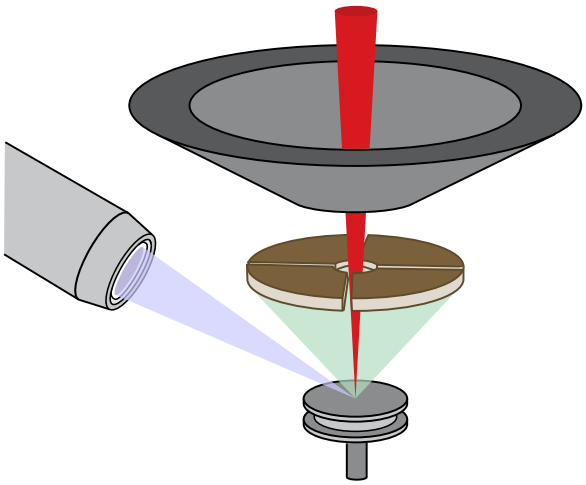
Backscattered electron (BSE) imaging

This type of electrons originate from a broad region within the interaction volume. They are a result of elastic collisions of electrons with atoms, which results in a change in the electrons' trajectory. Think of the electron-atom collision as the so-called "billiard-ball" model, where small particles (electrons) collide with larger particles (atoms). Larger atoms are much stronger than light atoms at scattering electron, so and they produce a higher signal, as shown in the following image. The number of the backscattered electrons reaching the detector is proportional to their Z number. This dependence of the number of BSE on the atomic number helps us differentiate between different phases, providing imaging that carries information on the sample's composition. Moreover, BSE images can also provide valuable information on crystallography, topography and the magnetic field of the sample.

The most common BSE detectors are solid state detectors that typically contain p-n junctions. The working principle is based on the generation of electron-hole pairs by the backscattered electrons, which escape the sample and are absorbed by the detector. The amount of these pairs depends on the energy of the backscattered electrons. The p-n junction is connected to two electrodes, one of which attracts the electrons and the other the holes, thereby generating an electrical current, which also depends on the amount of the absorbed backscattered electrons.



The SEM image (a) is of an Al/Cu sample. A simplified illustration of the interaction between an electron beam with aluminum (b) and an electron beam with copper (c) is provided for comparison. Copper atoms (higher Z) scatter more electrons back toward the detector than the lighter aluminum atoms, so they appear brighter in the SEM image.

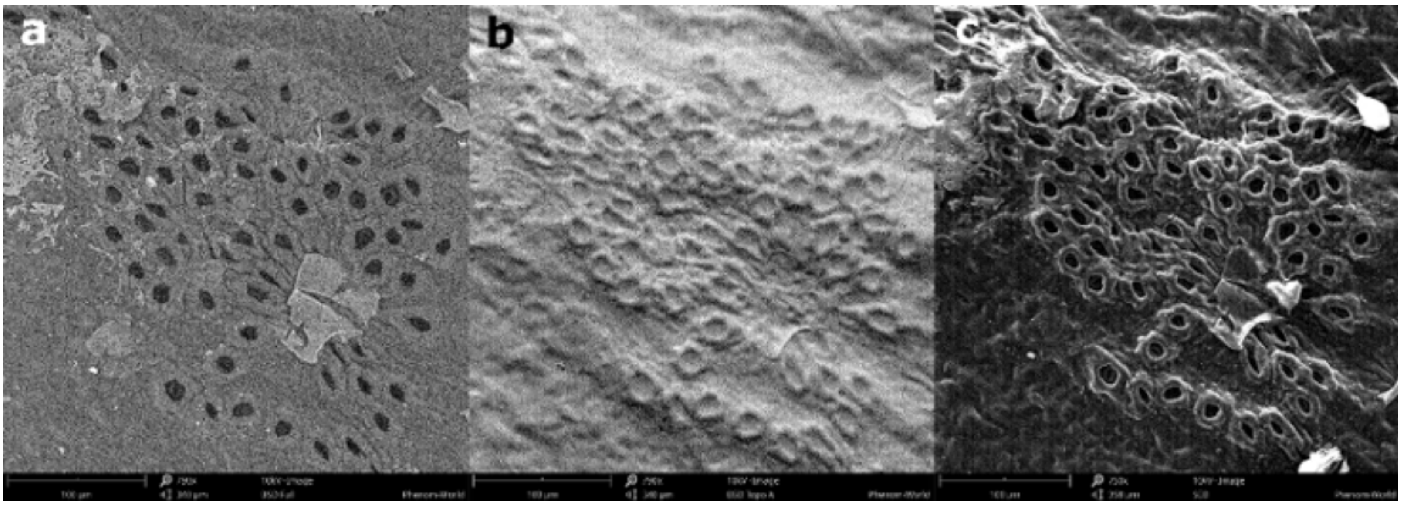


Typical position of the backscattered and secondary electron detectors.

The BSE detectors are placed above the sample, concentrically to the electron beam in a 'doughnut' arrangement, in order to maximize the collection of the backscattered electrons and they consist of symmetrically divided parts. When all parts are enabled, the contrast of the image depicts the atomic number Z of the number. On the other hand, by enabling only specific quadrants of the detector, topographical information from the image can be retrieved.

Secondary electrons

In contrast, secondary electrons originate from the surface or the near-surface regions of the sample. They are a result of inelastic interactions between the primary electron beam and the sample and have lower energy than the backscattered electrons. Secondary electrons are very useful for the inspection of the topography of the sample's surface, as shown in the following images.



Full BSD (a), topography BSD (b) and SED image of a leaf (c).

The Everhart-Thornley detector is the most frequently used device for the detection of SE. It consists of a scintillator inside a Faraday cage, which is positively charged and attracts the SE. The scintillator is then used to accelerate the electrons and convert them into light before reaching a photomultiplier for amplification. The SE detector is placed at the side of the electron chamber, at an angle, in order to increase the efficiency of detecting secondary electrons.

2.4 Chemical composition analysis

The electron beam-matter interaction generates a variety of signals that carry different information about the sample, as shown below. For example, backscattered electrons produce images with contrast that carries information on the differences in atomic number; secondary electrons give topographic information (you can read more about it here); cathodoluminescence can give information on the electronic structure and the chemical composition of materials; and transmitted electrons can describe the sample's inner structure and crystallography. Another type of signal that is widely used in SEMs is X-rays.

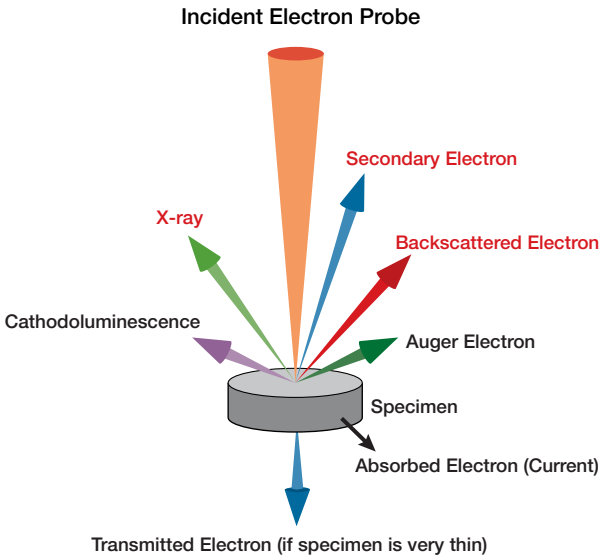
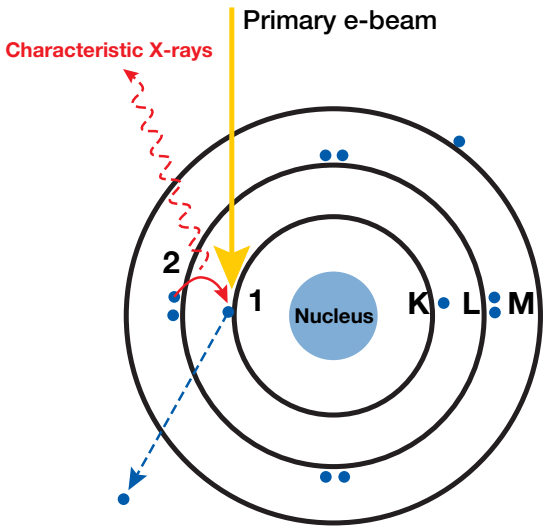


Illustration of the electron-matter interaction depicting its different products.

EDX in SEM: The principle explained

Every atom has a unique number of electrons that reside under normal conditions in specific positions, as shown below. These positions belong to certain shells, which have different, discrete energies.



In this X-ray generation process, the energy transferred to the atomic electron knocks it off, leaving behind a hole (1), its position is filled by another electron from a higher energy shell (2), and then the characteristic X-ray is released.

The generation of the X-rays in a SEM is a two-step process. In the first step, the electron beam hits the sample and transfers part of its energy to the atoms of the sample. This energy can be used by the electrons of the atoms to "jump" to an energy shell with higher energy or be knocked-off from the atom. If such a transition occurs, the electron leaves behind a hole. Holes have a positive charge and, in the second step of the process, attract the negatively-charged electrons from higher-energy shells. When an electron from such a higher-energy shell fills the hole of the lower-energy shell, the energy difference of this transition can be released in the form of an X-ray.

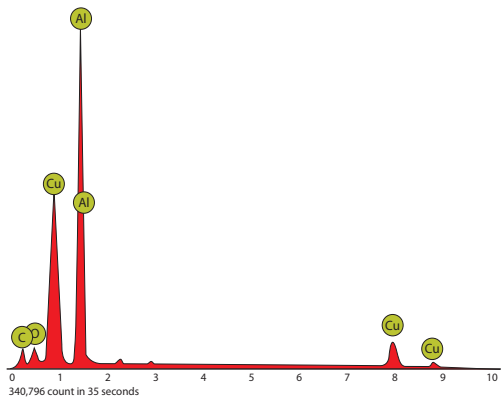
This X-ray has energy which is characteristic of the energy difference between these two shells. It depends on the atomic number, which is a unique property of every element. In this way, X-rays are a "fingerprint" of each element and can be used to identify the type of elements that exist in a sample.

EDX analysis: How X-ray detection works

Unlike BSE, SE and TE, X-rays are electromagnetic radiation, just like light, and consist of photons. To detect them, the latest systems use the so-called silicon-drift detectors (SDDs). These are superior to the conventional Si(Li) detectors due to higher count rates, better resolution, and faster analytical capabilities. These detectors are placed under an angle, very close to the sample and have the ability to measure the energy of the incoming photons that belong to the X-rays. The higher the solid angle between the detector and the sample, the higher the X-rays' detection probability, and therefore the likelihood of acquiring the best results.

The data that is generated by EDX analysis consists of spectra with peaks corresponding to all the different elements that are present in the sample. Every element has characteristic peaks of unique energy, which can be found in bibliography.

Furthermore, EDX can be used for qualitative (the type of elements) as well as quantitative (the percentage of the concentration of each element of the sample) analysis. In most SEMs, dedicated software enables auto-identification of the peaks and the calculation of the atomic percentage of each element that is detected. One more advantage of the EDX technique is that it is a non-destructive characterization technique which requires little or no sample preparation.



In this typical EDX spectrum, the y-axis depicts the number of counts and the x-axis depicts the energy of the X-rays. The position of the peaks leads to the identification of the elements. And the height of the peaks leads to quantifying each element's concentration in the sample.

Chapter 3:
Techniques for ideal 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 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.

3.1 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

3.2 Non-conductive samples

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

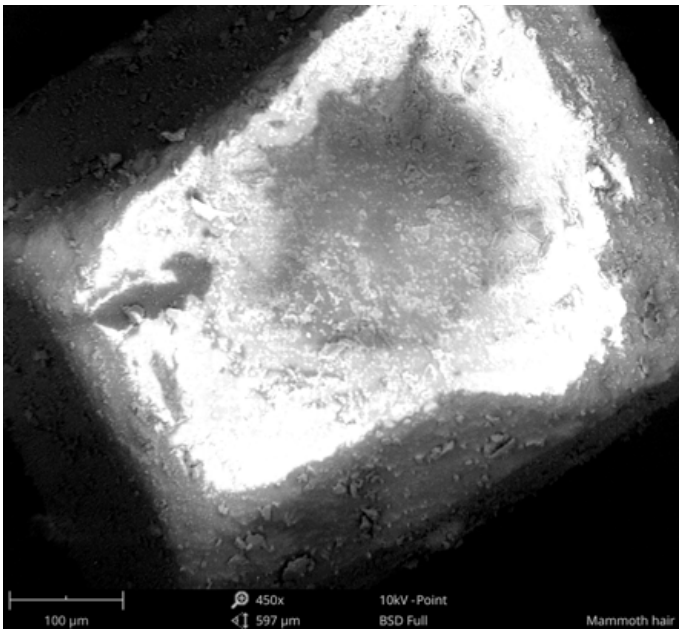
By covering part of the sample with a piece of conductive tape (*e.g.* copper tape) or some conductive paint, a bridge to the surface of the aluminum stub is created.

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.

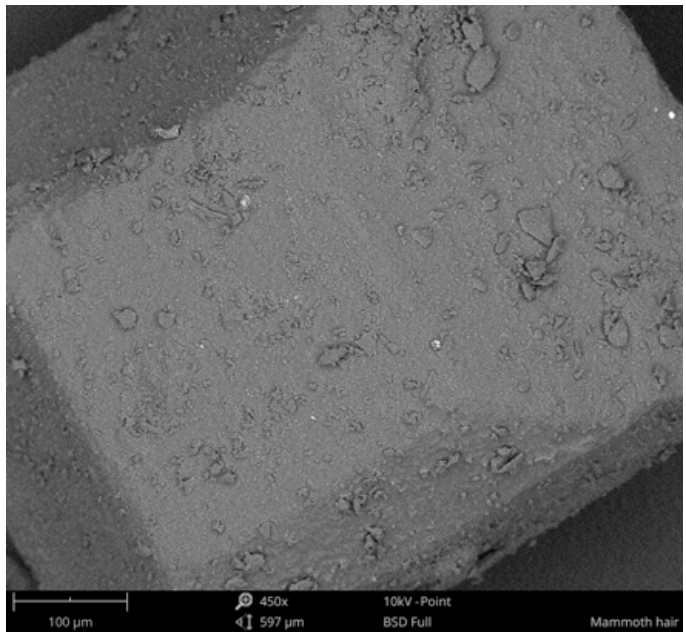
Low vacuum

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.

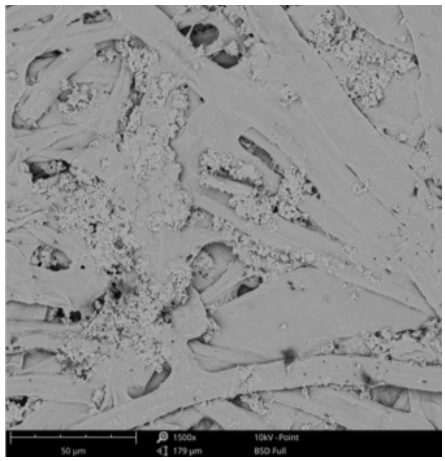


SEM image of sugar cube charging.

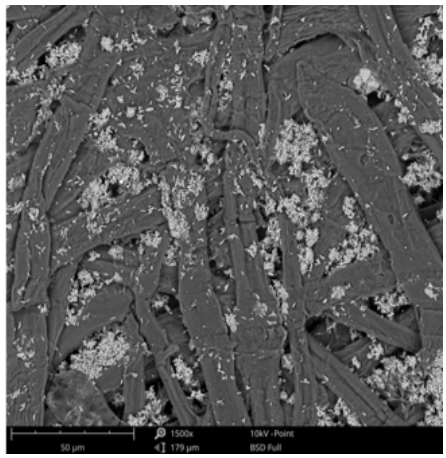


SEM image of sugar cane in low vacuum.

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



SEM image of paper imaged with gold coating.



SEM image of paper in low-vacuum mode.

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.

3.3 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.

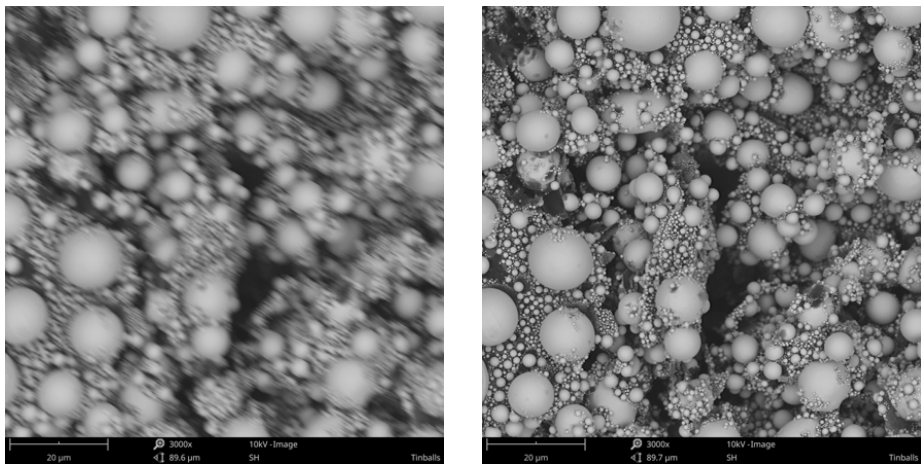


Image of tin particles embedded in a carbon matrix before and after correcting the beam astigmatism. The focus between the two images has not changed, but correcting the shape of the beam results in a sharp image.

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.

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.

3.4 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

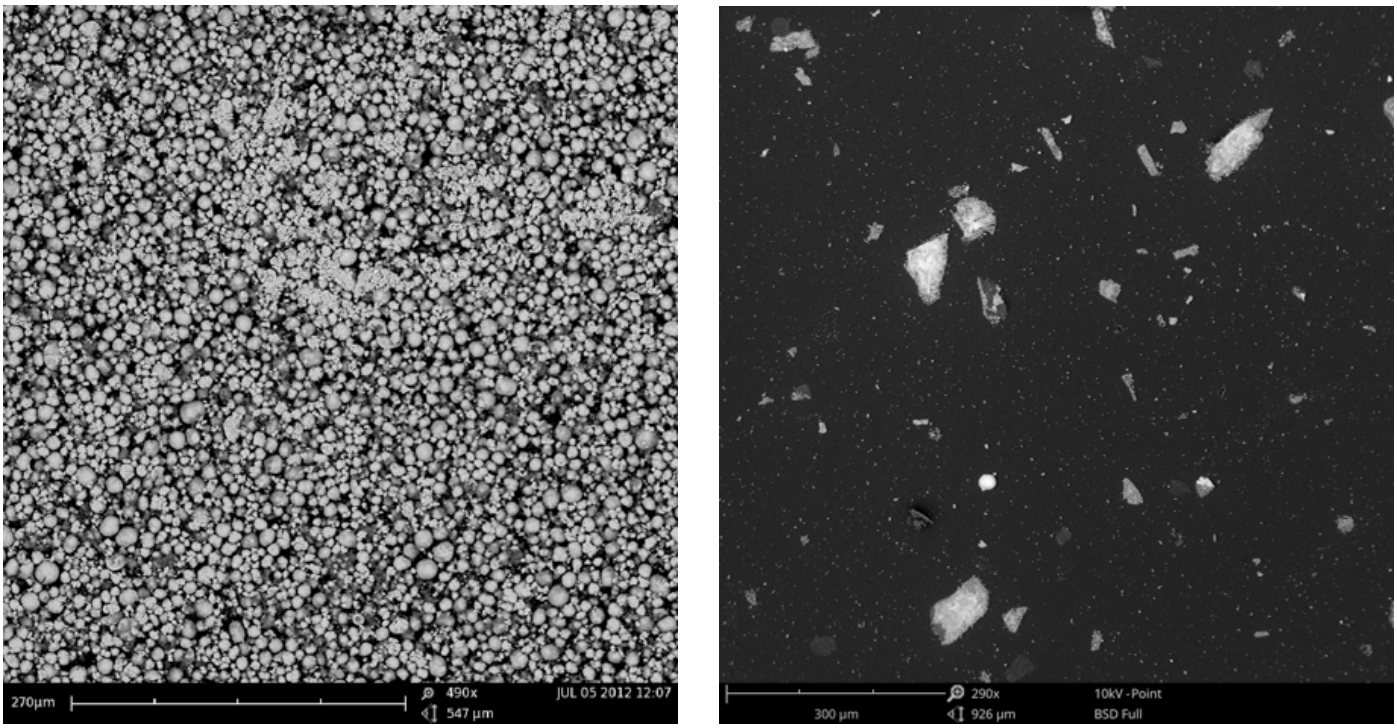
Thermal effects can be reduced by using a temperature controlled device. Removing the heat generated by the beam will protect the sample from thermal-induced surface modifications.

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.



SEM image of powder samples using a spoon (left image)and SEM image of powder sample using a disperser (right image). When using a disperser, the particles are clearly and evenly spread, and can be counted by using software.

3.4 Powders and particles

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.

3.6 Moist or outgassing samples

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.

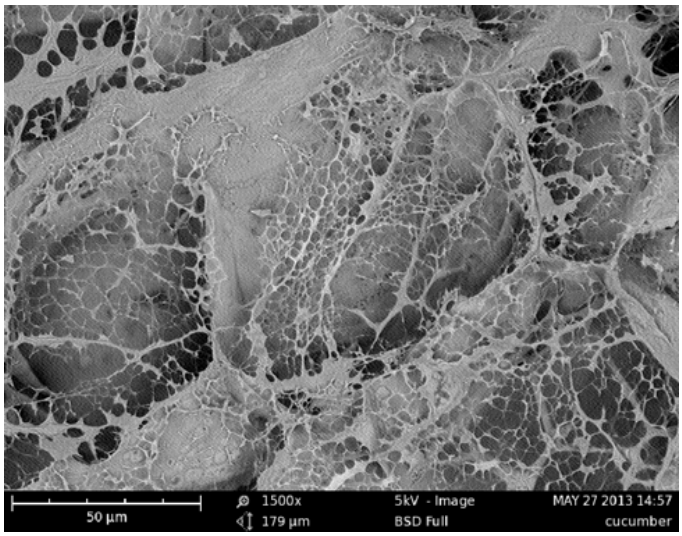
Force drying

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.

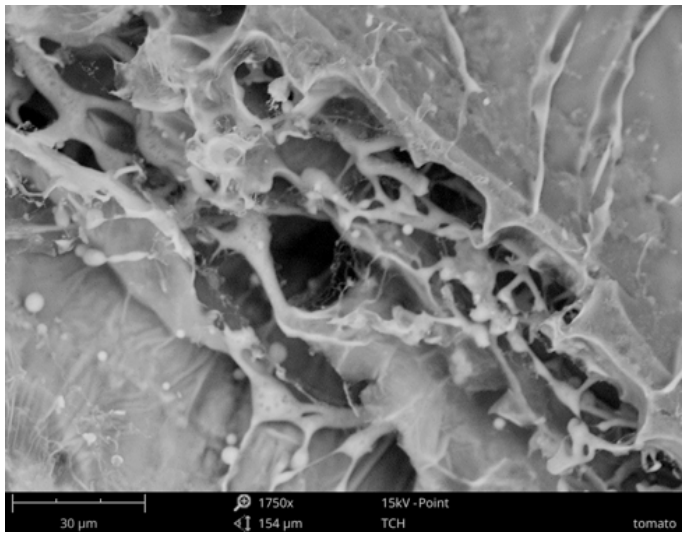
Critical point drying

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 vapour 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 the intact structure of a cucumber.



SEM image of the interior structure of a tomato peel.

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.

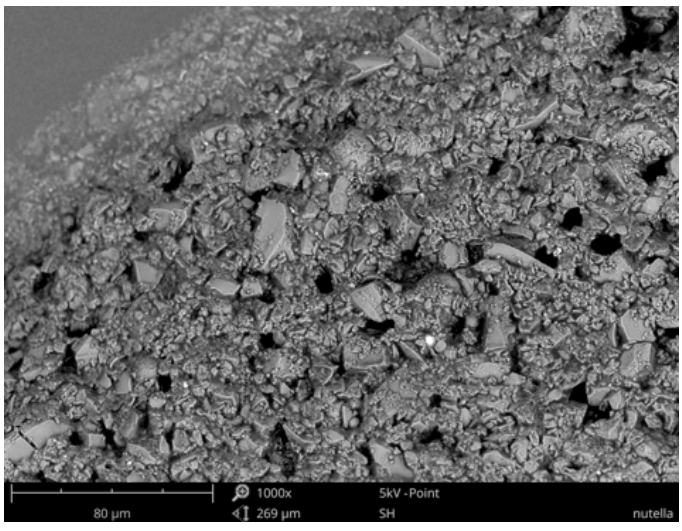
Low vacuum

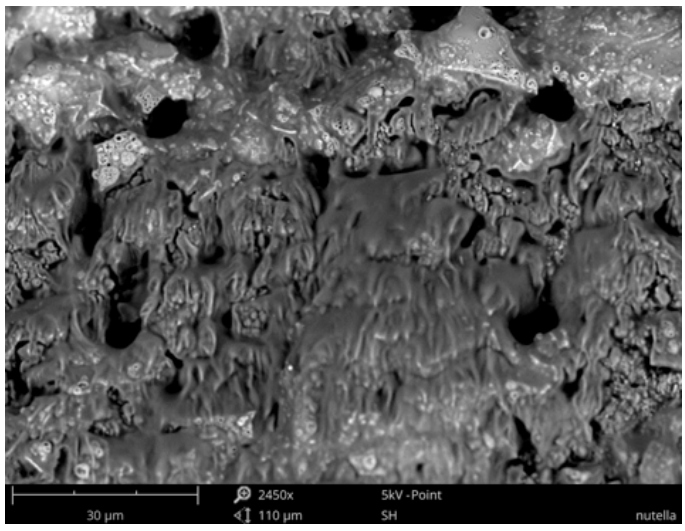
If the sample does not have a particularly high moisture content, using a small amount of sample at a reduced vacuum level can be enough to collect images. The overall image quality will be lower, but the sample can be imaged in its original state.

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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