thermoscientific

How to choose a Scanning Electron Microscope (SEM)

Guidelines for selecting the best microscope for your research

ThermoFisher SCIENTIFIC

Contents

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 >30 kV) 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.

How to select a scanning electron microscope

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.

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

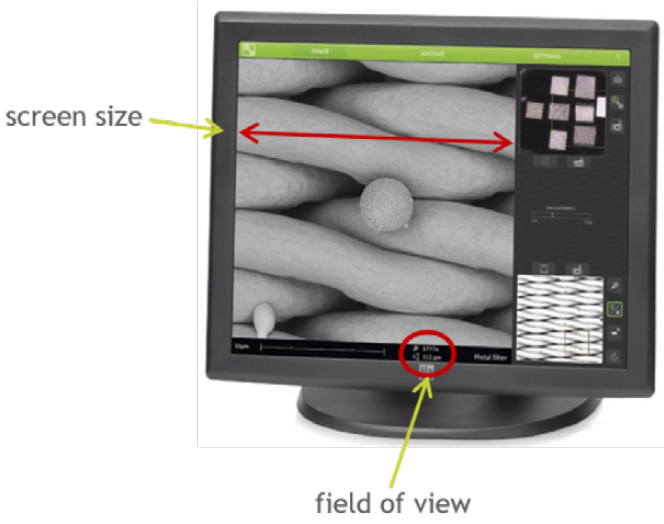
This means that printing a picture of an apple that fits on a standard printer sheet and printing it again to fit on a poster that will be used to cover a building, will change the magnification value dramatically (much larger in the second case).

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.

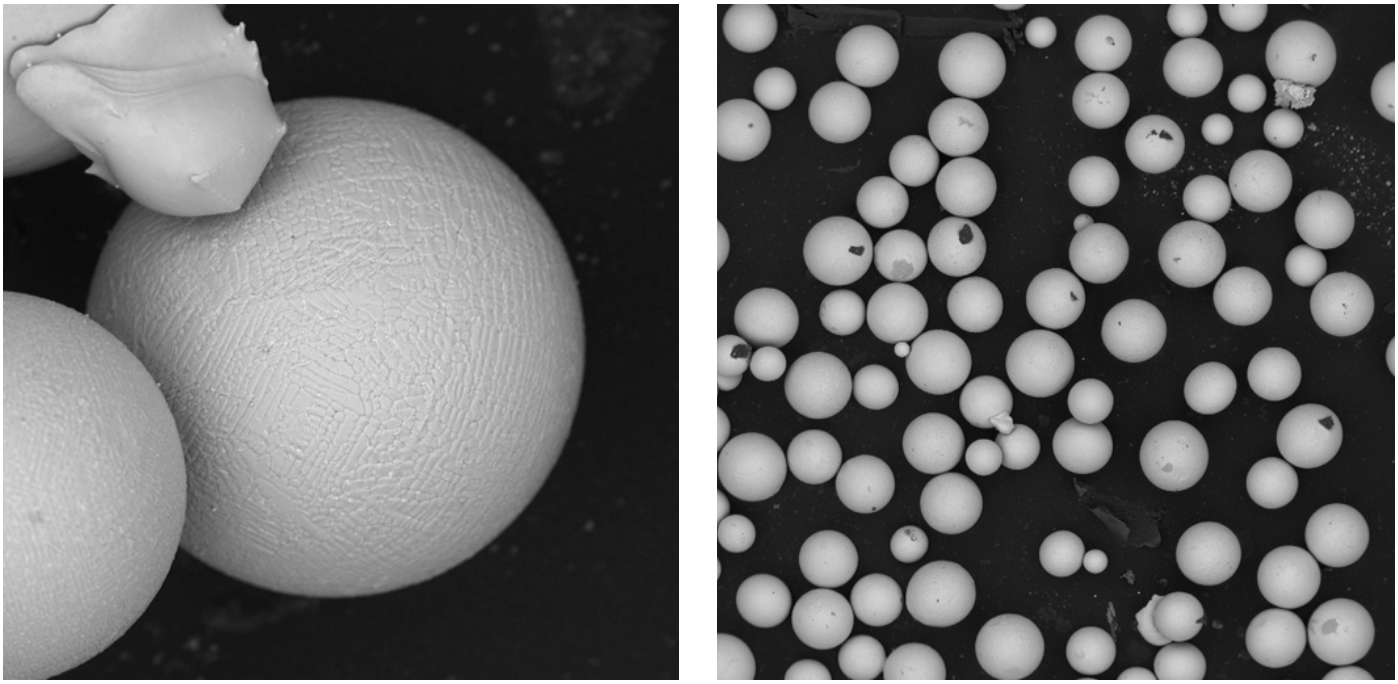


The field of view defines the size of the feature to be imaged. This value typically ranges between some millimeters (a bug) to few microns (the hair of a bug) and a couple of nanometers (the molecular macrostructure of the exoskeleton). With modern instruments, objects in the range of few hundred picometers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

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.

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.

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.

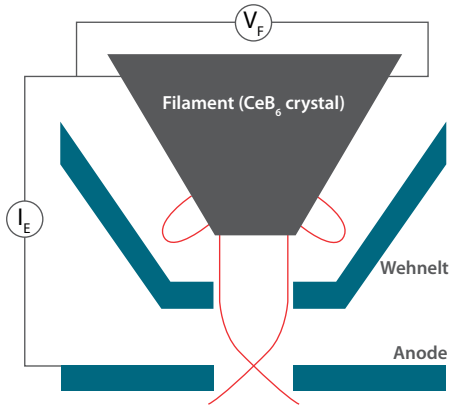
The electron beam that is projected onto your sample is created by the emitted electrons being accelerated from the high negative potential of the source to ground potential at the anode inside the electron column. This process can, of course, only happen inside the vacuum of an electron column and by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



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

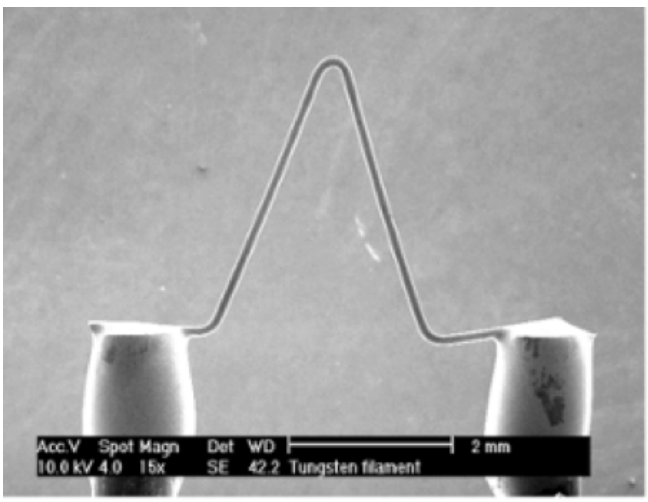
2. **Source size**
   As mentioned before, a small spot size contributes to good image resolution and therefore high-quality images. The lenses (mainly facilitated by the condenser lens) inside the electron column are responsible for demagnifying the beam diameter (or spot size). You can imagine that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

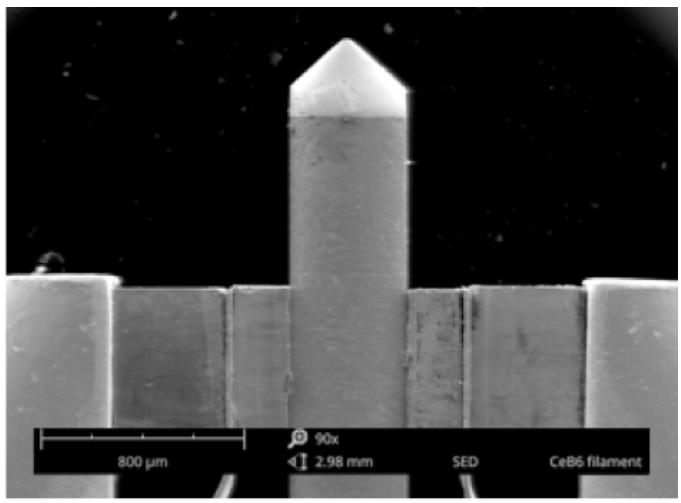
   3a. Electron beam energy spread
   The electron beam energy spread is the spread in electron energies leaving the source. Chromatic aberration becomes the dominant aberration at low acceleration voltage when the energy spread of the source is large. Chromatic aberration is an effect that causes a less 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 filament.



CeB_6 filament.

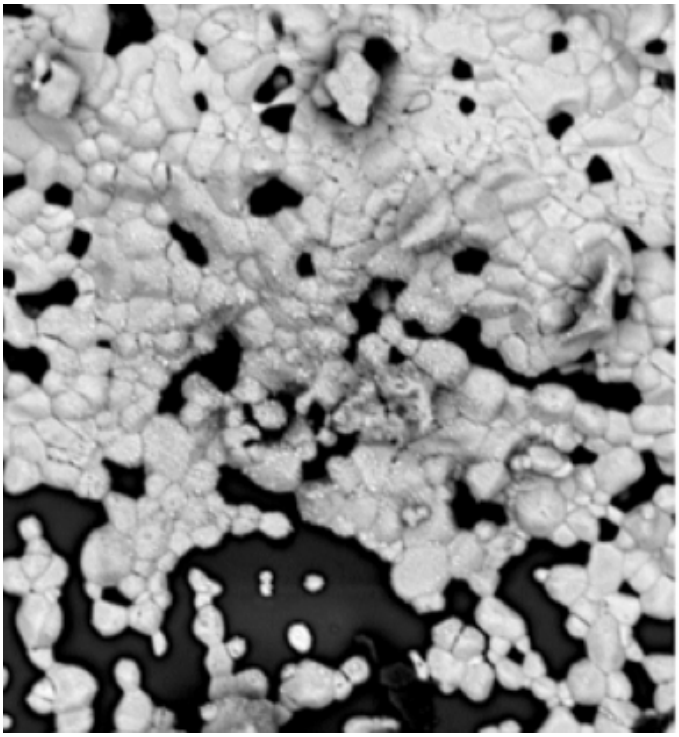


Image from TiO_2 powder made with CeB_6 system.

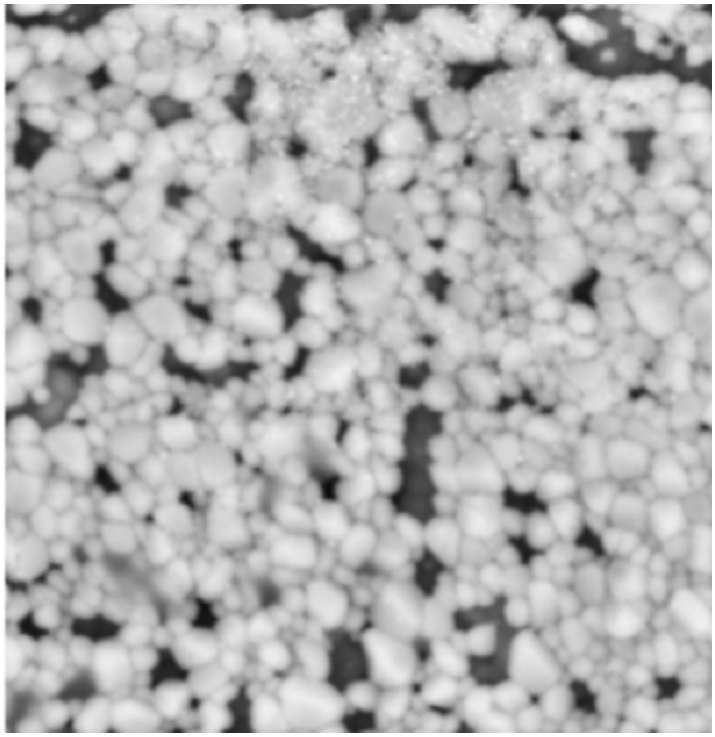


Image from TiO2 powder made with tungsten system.

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.

2. **Source size**
   The source size 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 CeB_6 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 Acceleration voltage

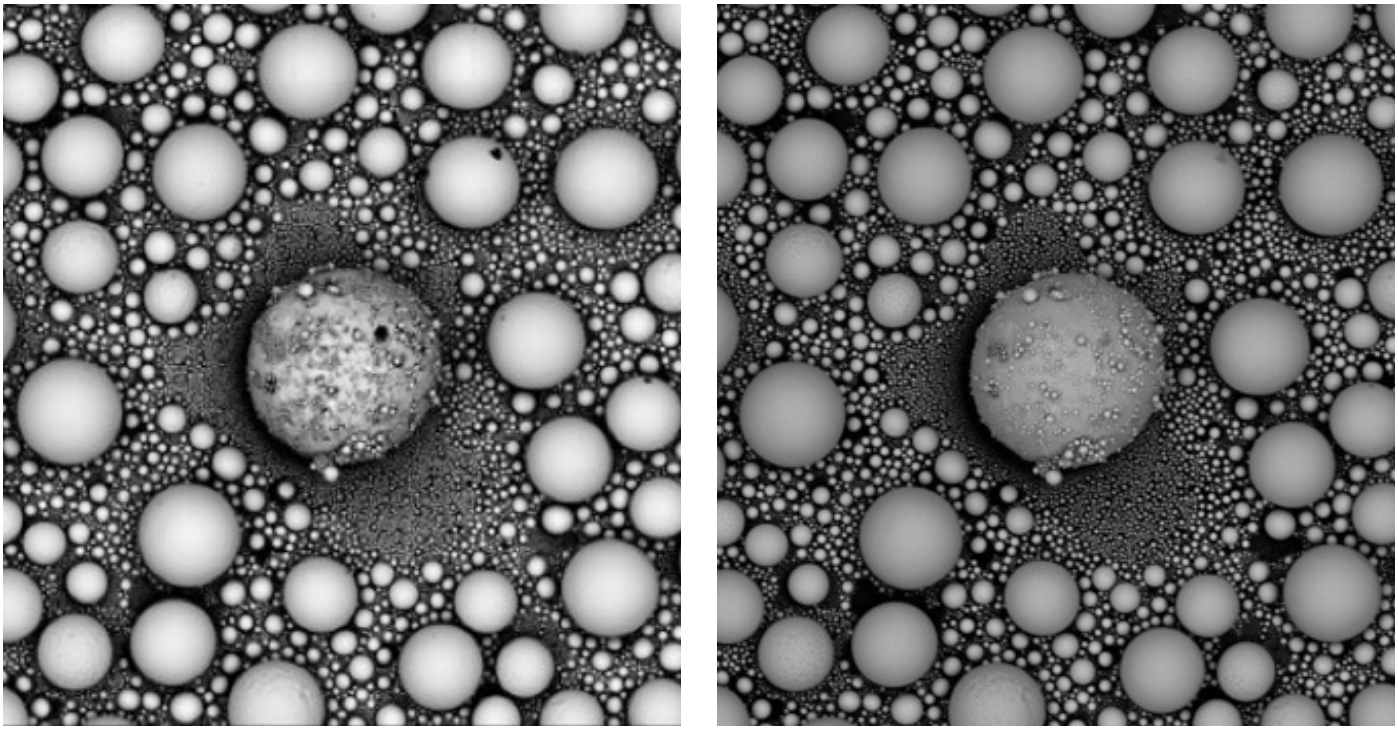
The voltage is an indication of the electrons' energy content: this will therefore determine what kind of interaction the beam will have with the sample. As a general guideline, a high voltage corresponds with a higher penetration beneath the surface of the sample — also known as bigger interaction volume.

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 (left) and at 15kV (right). 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

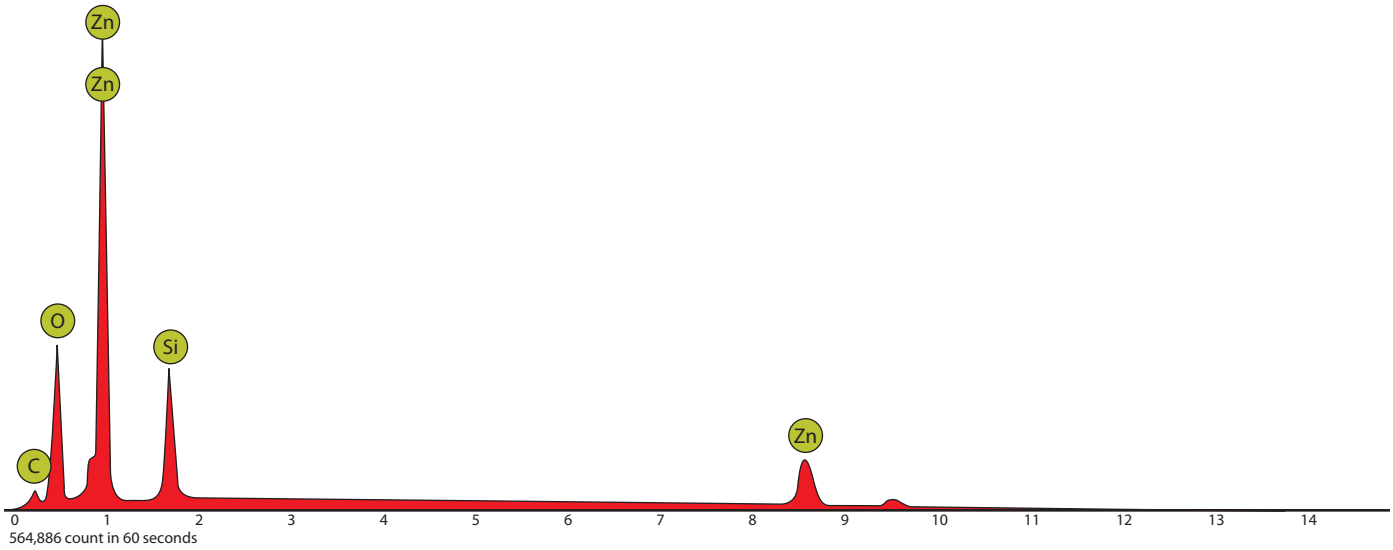
- **Overvoltage:** The ratio between the energy of the incoming beam and the energy necessary to ionize the targeted atom.
- **Interaction volume:** Defines the spatial resolution of the analysis.

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.



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.

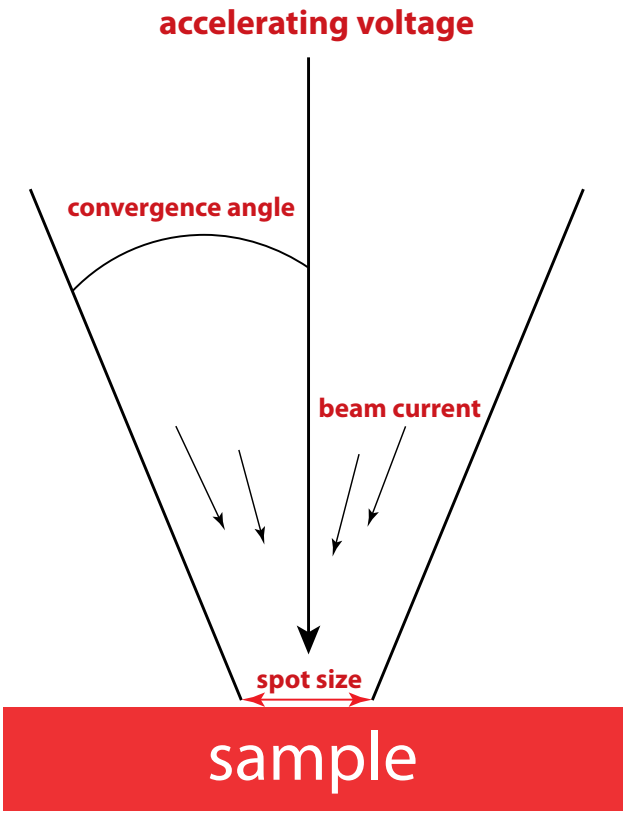
1.5 Current intensity

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).

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.



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

High-resolution imaging versus high-beam current

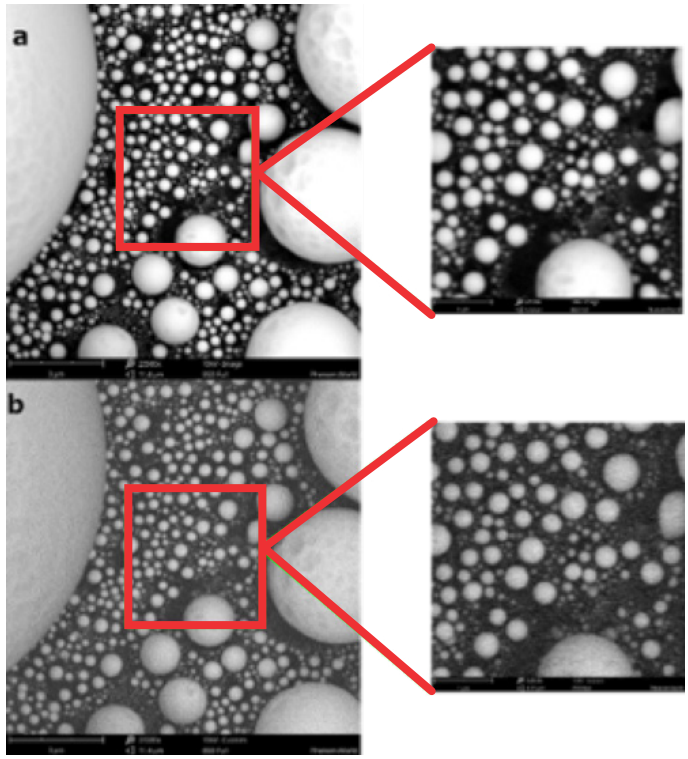
The major factor that affects resolution is spot size. To acquire a high-resolution image, the spot size should be kept as small as possible in order to be able to resolve and describe even the smaller features of the specimen sufficiently.

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 increasing the spot size is recommended, 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.



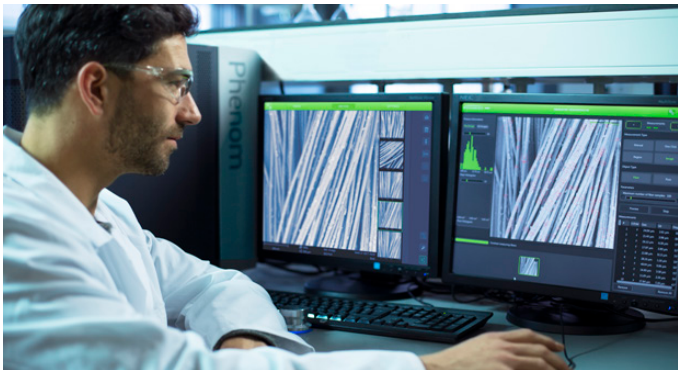
These SEM images are of tin. At low magnification, a high beam current (a) is preferred. At high magnification, using a smaller spot size (b) allows the user to achieve better spatial resolution.

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.

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.

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Thank you for your interest

We hope with this guide has provided you with enough information about the most relevant features of an electron microscope.

If you are still curious about electron microscopy and how this can improve your analysis and products, do not hesitate to contact us. We will be happy to **give you a live demonstration of how the device works** and work together with you to discover what kind of information electron microscopy can provide on the materials you work with.

See Phenom SEMs in action and anaylze your own samples

Get a personal demo at a location nearby

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