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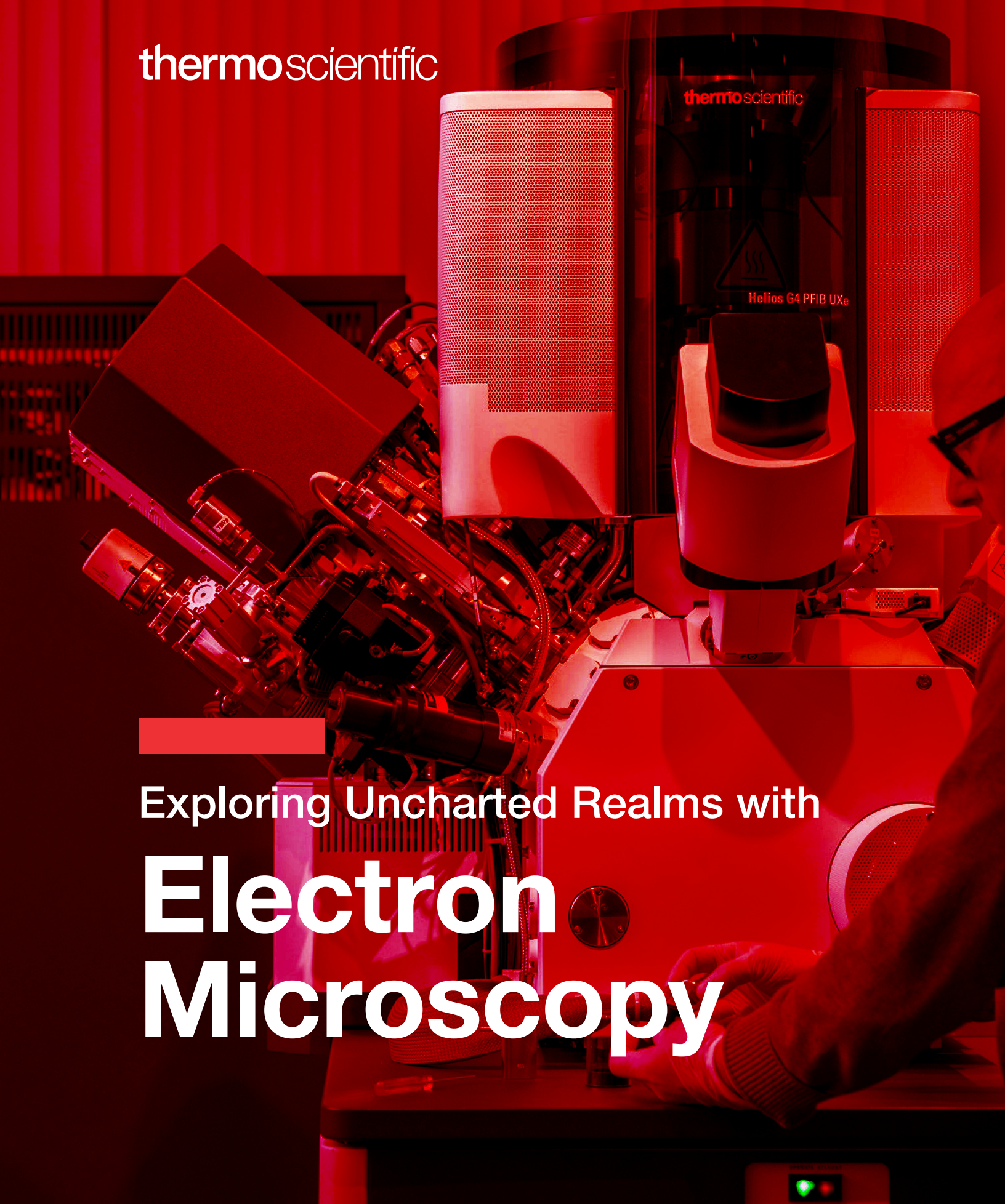


Exploring Uncharted Realms with

Electron Microscopy

You do not need to take a trip into outer space to discover an unknown world. An uncharted realm exists right here on our planet; it is an empire full of strange creatures, exotic landscapes and unusual structures.

The dimension of micrometers and nanometers is a mysterious and almost magical domain that has captured the human imagination for decades. For nearly 60 years, humankind has been exploring the micro and nano realms with the help of electron microscopes.

Table of contents

CHAPTER 1

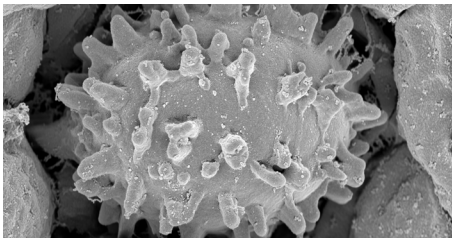
An introduction to electron microscopy

Magnifying ten million times

Electron microscopes reveal hidden wonders that are smaller than the human eye can see. They fire electrons and create images, magnifying micrometer and nanometer structures by up to ten million times, providing a spectacular level of detail that allows us to view single atoms. Observing the world through electron microscopes can make the invisible visible, expand our horizons, transform our perceptions and open our minds to new possibilities.

Electron microscopy has the power to answer long-standing questions, like “Why is spider silk so strong?” What’s more, it will answer questions you never even thought to ask, such as “What does the mouth of a caterpillar look like?”

Thousands of scientific discoveries have been fueled by electron microscopy, creating millions of unique images that not only offer great scientific value, but are art forms in their own right. When you first look at images from an electronmicroscope, you may not believe your eyes; the scenes can easily resemble illustrations of fantasy or science fiction.



Beautiful ornamentation of a spore hidden in the hymenophorous tissue of a Russula emetica mushroom.

Courtesy of Mrs. Anna Siudzinska , PORT Polish Center for Technology Development
Original data collected at a horizontal field width of 11 µm.

Folds of graphite.

Original data collected at a horizontal field width of 59.5 µm.

The Nanometer

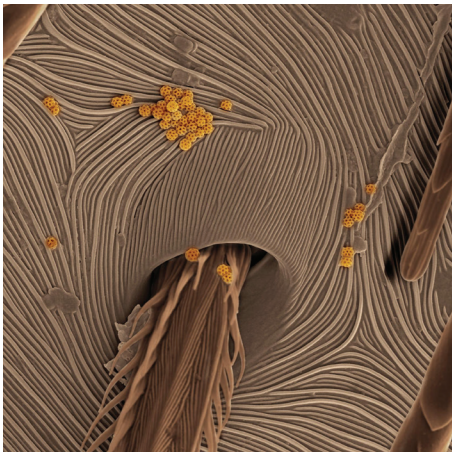
As distances become shorter, the number of zeros after the decimal point becomes larger, so microscopists use the nanometer (abbreviated to nm) as a convenient unit of length. One nanometer is a billionth (10^{-9}) of a meter.

An intermediate unit is the micrometer (abbreviated to µm), which is a millionth (10^{-6}) of a meter or 1,000 nm. Some literature refers to the Ångström unit (Å), which is 0.1 nm and uses micron for micrometer. A picometer is a trillionth (10^{-12})

Electron microscopy in the life sciences—life at microscale

We are surrounded by things that are too small for us to see. The world at microscale is full of tiny, alien-like creatures, from the mites that crawl along our eyelashes at night to tardigrades, who have been proclaimed the most resilient creatures on earth, despite being only 0.5 mm in size. What's more, there may be millions of tiny critters out there who are yet to be discovered.

From the bacteria trapped underneath our nails to the hidden world inside our own bodies, electron microscopy has revealed epic battles between our immune system and diseases. It has lain bare the tangled mass of fibers that constitutes blood clots, how diseased cells and tissues wreak havoc on our health, and how our bodies are vibrant ecosystems in their own right, playing host to millions of bacteria.



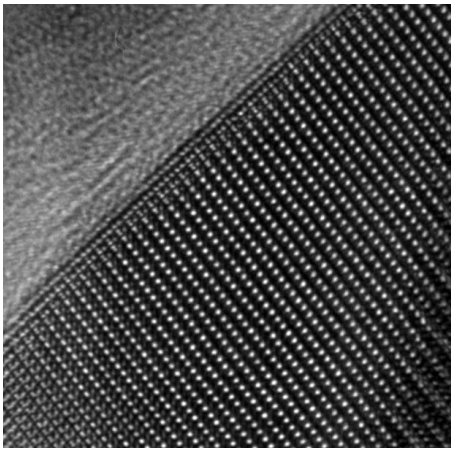
Spider skin, with a hair root and adhered pollen grains.

Courtesy of Maria Carbajo, Universidad de Extremadura.
Original data collected at a horizontal field width of 24.9 µm.

Electron microscopy for nanomaterials and devices

When we zoom in from microscale to nanoscale, we can observe the building blocks of the materials that comprise our world. Electron microscopy is essential for the development of nanotechnology and nanodevices, transforming a material's atoms from an abstract concept to objects we can see with our own eyes, enabling us to engineer materials atom by atom.

By showing us the structure of materials at the nanoscale, electron microscopy provides a way to understand the link between material composition, structure and performance. This has led to technological advances, including smaller, faster computers, chemical sensors, targeted drug delivery, high-performance materials, water filters and many more.



Hematite nanoparticle with individual atoms visible.

Courtesy of Enrique Díaz Barriga Castro.
Original data collected at a horizontal field width of 14.385 nm.

New developments in electron microscopy

In 1959, physicist Richard Feynman proclaimed "There's plenty of room at the bottom," and called for electron microscopes to be improved and increased in power by 100 times to resolve features as small as one nanometer. This would allow scientists to truly explore the features of the nano world.

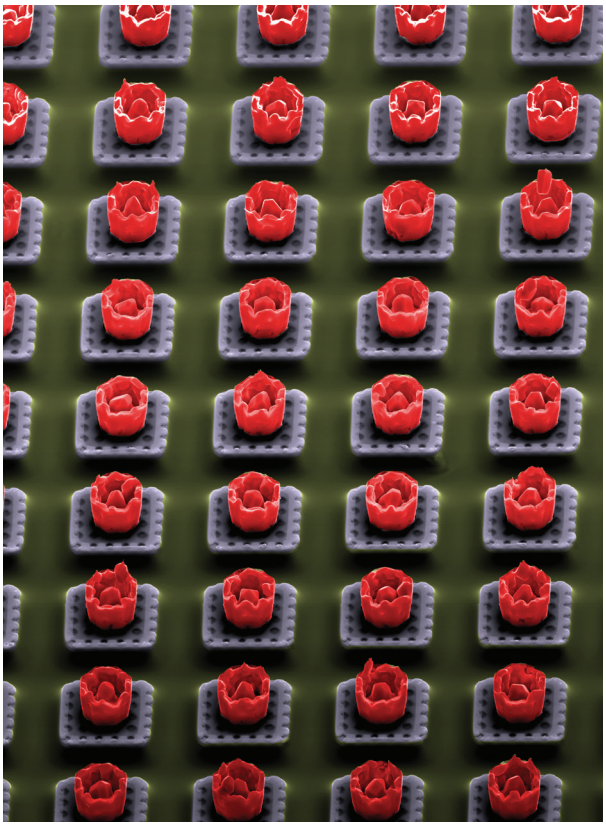
Since then, we have met Feynman's demand, and the possibilities for electron microscopy have increased exponentially. In 2017, Jacques Dubochet, Joachim Frank and Richard Henderson received the Nobel Prize in Chemistry for their contributions to the development of the latest evolution in electron microscopy: cryo-electron microscopy, which enables us to resolve the structures of biomolecules frozen in solution. Each new development in electron microscopy has opened new doors and allowed scientists to explore new features of the world at nanoscale.

Once we understand this hidden realm through exploration, we can use that knowledge to change our own world.

Knowing how the anatomy of tardigrades makes them so durable would allow us to create stronger, more resilient materials. Understanding how our immune system tackles disease in exacting detail would let us know what to do when things go wrong. Examining the nanoscale structures of materials, sensors and devices can help us understand structure/performance relationships and ultimately design new or improved materials and technologies.

Determining the structures of biomolecules in their native environment can allow us to create drugs that are better at targeting specific proteins or receptors.

Electron microscopy has initiated a new era of scientific discoveries that extend far beyond simply observing small things, but there are still many more discoveries out there, just waiting for someone to seek them out. We are on the threshold of extraordinary advances, driven by our exploration of a reality that is just too small for us to see.



Contact side of an infrared detector array.

Courtesy of Sedat Canli.
Original data collected at a horizontal field width of 200 µm.

CHAPTER 2

What is microscopy?

The world of microscopes

Most microscopes can be classified as one of three basic types: optical, scanning probe or charged particle (electron and ion).

Optical microscopes are the ones that are most familiar to everyone and are used in countless places, from the doctor's office to the high school science lab. They use visible light and transparent lenses in order to see objects as small as one micrometer (one-millionth of a meter), such as a red blood cell (7 µm) or a human hair (100 µm).

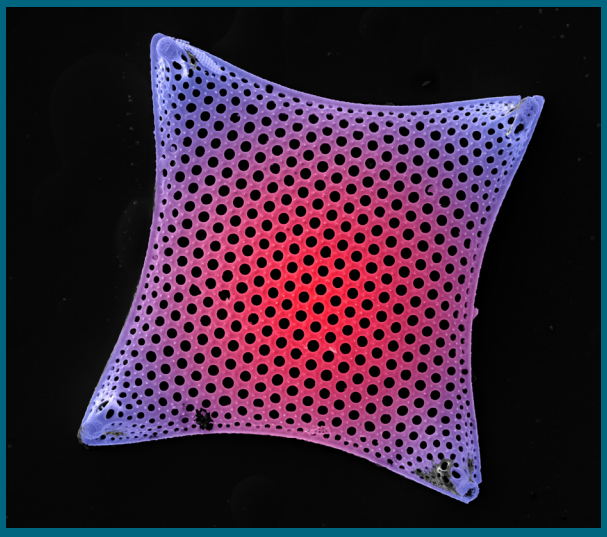
Electron and ion microscopes use a beam of charged particles instead of light and use electrostatic or electromagnetic lenses to focus the particles. They can observe features as small as 0.1 nm (one ten-billionth of a meter), such as individual atoms. Scanning probe microscopes use a physical probe (a very small, very sharp needle) that scans over the sample in contact or near-contact with the surface. They map different forces and interactions that occur between the probe and the sample to create an image. These instruments are also capable of atomic-scale resolution.

A modern light microscope (often abbreviated to LM) has a maximum magnification of about 1,000× and allows the eye to resolve objects separated by 200 nm. As inventors and

The first microscopes

Nobody knows for certain who invented the microscope. The light microscope probably developed from the Galilean telescope during the 17th century. One of the earliest instruments for seeing very small objects was made by the Dutchman Antony van Leeuwenhoek (1632–1723) and consisted of a powerful convex lens and an adjustable holder for the object being studied.

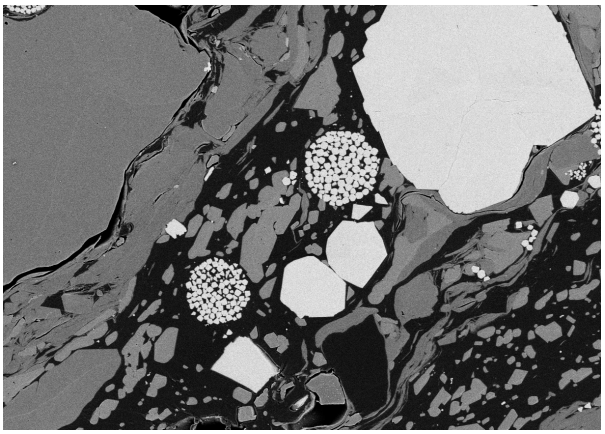
With this remarkably simple microscope, Van Leeuwenhoek may well have been able to magnify objects up to 400×. With it, he discovered protozoa, spermatozoa and bacteria, and he was able to classify red blood cells by shape.



Phytoplankton sample from Atlantic Ocean

Courtesy of Mr. Daniel Mathys, Universität Basel.
Original data collected at a horizontal field width of 170 µm.

scientists worked to attain better resolution, they soon realized that the resolving power of the microscope was not only limited by the number and quality of the lenses, but also by the wavelength of the light used for illumination. With visible light, it was impossible to resolve points in the object that were closer together than a few hundred nanometers.



Shale sample.

Original data collected at a horizontal field width of 25.4 µm.

Using light with a shorter wavelength (ultraviolet or blue) gave a small improvement. Immersing the specimen and the front of the objective lens in a medium with a high refractive index (such as oil) offered another small improvement, but these measures together only brought the resolving power of the microscope to just under 100 nm.

In the 1920s, it was discovered that accelerated electrons act in a vacuum much like light. They pass in straight lines and have wavelike properties, with a wavelength that is about 100,000 times shorter than that of visible light. Additionally, it was found that electric and magnetic fields could be used for shaping the paths followed by electrons, similar to the way glass lenses are used to bend and focus visible light.

Ernst Ruska at the University of Berlin combined these characteristics and built the first transmission electron microscope (TEM) in 1931. For this and following work on the subject, he was awarded the Nobel Prize for Physics in 1986. The first electron microscope used two magnetic lenses, and three years later, Ruska added a third lens and demonstrated a resolution of 100 nm, twice as good as a typical light microscope. Today, electron microscopes have reached resolutions greater than 0.05 nm, more than 4,000 times better than a typical light microscope and 4,000,000 times better than the unaided eye.

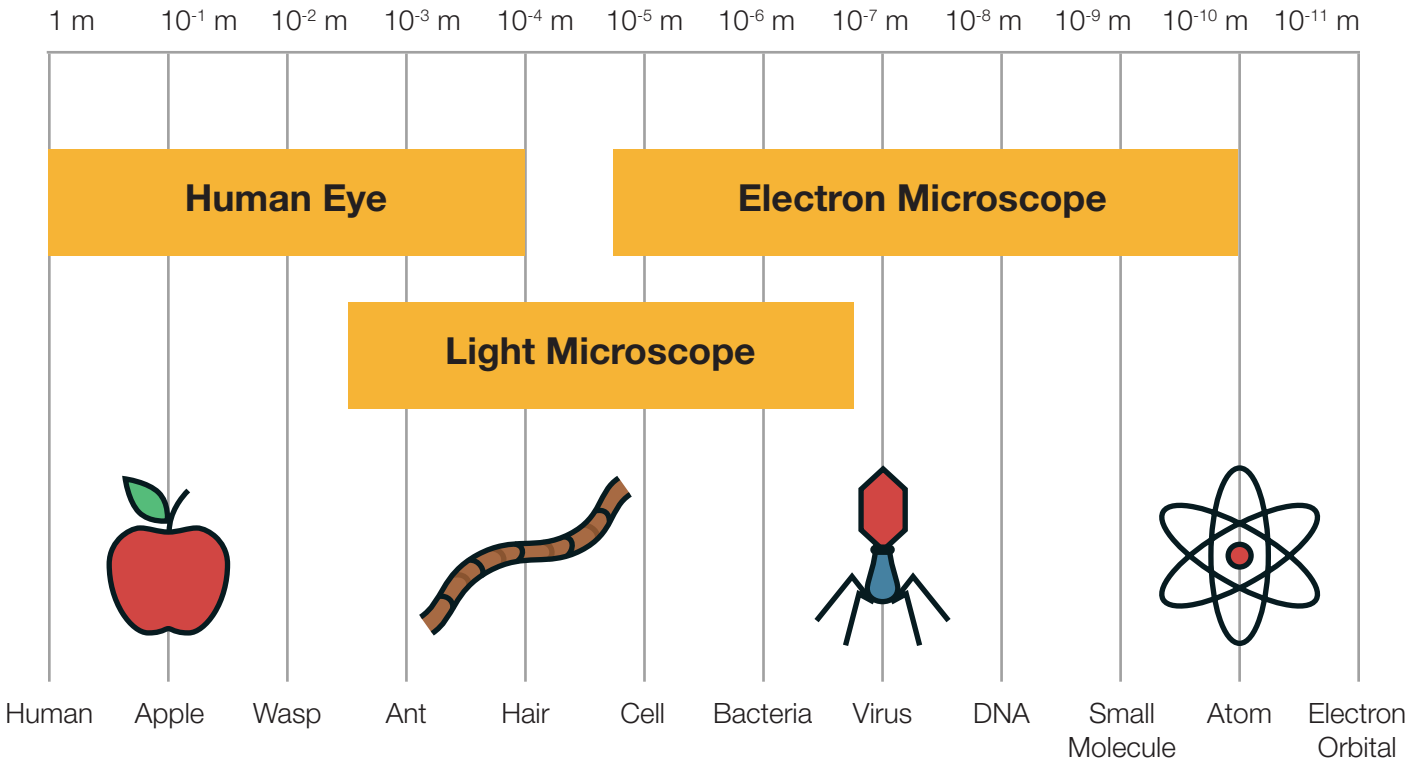
Resolution of the human eye

Given sufficient light, the unaided human eye can distinguish two points 0.2 mm apart. If the points are closer together, they will appear as a single point. This distance is called the resolving power, or resolution of the eye.

A lens or an assembly of lenses (as in a microscope) can be used to magnify this distance and enable the eye to see points even closer together than 0.2 mm. For example, try looking at a newspaper picture, or one in a magazine, through a magnifying glass. You will see that the image is actually made up of dots too small and too close together to be separately resolved by your eye alone.

The same phenomenon will be observed on an LCD computer display or flat screen TV when magnified to reveal the individual “pixels” that make up the image.

Comparing the scale of different microscopes



Resolution and wavelength

When a wave passes through an opening in a barrier, such as an aperture in a lens, it is diffracted by the edges of the aperture. Even a perfectly shaped lens will be limited in its resolving power by diffraction. This is why a high-quality optical lens may be referred to as a diffraction-limited lens—it is as good as it can be, and any further effort to improve the quality of the lens surface will not improve its resolution.

The amount of diffraction is a function of the size of the aperture and the wavelength of the light, with larger apertures and/or shorter wavelengths permitting better resolution. The wavelength of an electron in a TEM may be only a few picometers (1 pm = 10^{-12} m), more than 100,000 times shorter than the wavelength of visible light (400–700 nm). Unfortunately, the magnetic lenses used in electron microscopes do not approach diffraction-limited performance, so electron microscopes have been unable to take full advantage of the shorter wavelength of the electron.

Ultimately, the resolving power of an electron microscope is determined by a combination of beam voltage, aperture size and lens aberrations.

CHAPTER 3

Key components of electron microscopes

Before discussing the different types of electron microscopes and the resulting images that they can produce, it makes sense to understand the different components of an electron microscope.

In general, an electron microscope has four key components:

- Electron source
- Column
- Electronics
- Control software

Electron sources

Three key types of electron sources are employed in electron microscopes: tungsten guns, lanthanum hexaboride guns (LaB_6 - frequently known as “lab six”), and field emission guns (FEG). Each represents a different combination of benefits and costs. The choice of source type is a significant part of the instrument selection process.

Perhaps the single most important characteristic of the source is brightness, which characterizes the electron current density of the beam and the angle into which the current is emitted (current density per steradian solid angle); this eventually determines the resolution, contrast and signal-to-noise capabilities of the imaging system. FEG sources provide brightness up to 1,000 times greater than tungsten emitters, but they are also much more expensive. In some high-current applications, tungsten or LaB_6 may in fact function better than FEG.

The electron

An atom is made up of three kinds of particles: protons, neutrons and electrons. The positively charged protons and neutral neutrons are held tightly together in a central nucleus. Negatively charged electrons surround the nucleus. Normally, the number of protons equals the number of electrons so that the atom as a whole is neutral. When an atom deviates from this normal configuration by losing or gaining electrons, it acquires a net positive or negative charge and is referred to as an ion.

The electrons, which are about 1,800 times lighter than the nuclear particles, occupy distinct orbits, each of which can accommodate a fixed maximum number of electrons. When electrons are liberated from the atom, however, they behave in a manner analogous to light. It is this behavior that is used in the electron microscope.

A tungsten gun is comprised of a filament, an anode and a Wehnelt cylinder. These three together compose a triode gun, which is an extremely stable source of electrons. The tungsten filament is hairpin-shaped and heated to about 2,700 K. By applying a high positive potential difference between the filament and the anode, electrons are extracted near the filament and then accelerated towards the anode.

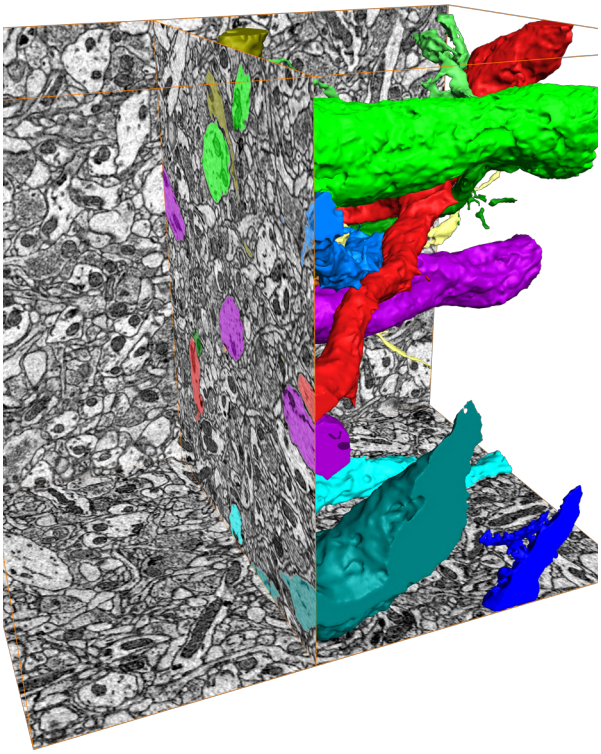
The anode has a hole in it so that an electron beam, in which the electrons could travel faster than 2,000 km/s, emerges and is directed down the column. Tungsten sources are the least expensive, but have limited lifetimes and offer lower brightness. The brightness of a tungsten source can be increased, but only at the cost of a shorter lifetime, as brightness ≈ current/diameter^2.

Like tungsten, LaB_6 guns and CeB_6 guns rely on thermionic emission of electrons from a heated source, a lanthanum (or cerium) hexaboride crystal. LaB_6 sources can offer up to 10 times more brightness than tungsten and have considerably longer lifetimes, but need higher vacuum levels, which increases the cost of the microscope. The emitting area of LaB_6 is smaller than tungsten, increasing brightness but decreasing total beam current capability.

Field emission guns, in which the electrons are extracted from a sharply pointed tungsten tip by an extremely high electric field, are the most expensive type of source, but usually provide the highest imaging and analytical performance. This performance increase is due to electromagnetic electron generation (rather than thermionic), which results in a higher beam current despite having a smaller emitting area than tungsten and LaB_6 sources.

The electron microscope column

The electron column is comprised of elements analogous to those of a light microscope. The light source of the light microscope is replaced by an electron gun. The glass lenses are replaced by electromagnetic or electrostatic lenses. Unlike glass lenses, the power (focal length) of magnetic lenses can be changed by modifying the current through the lens coil.

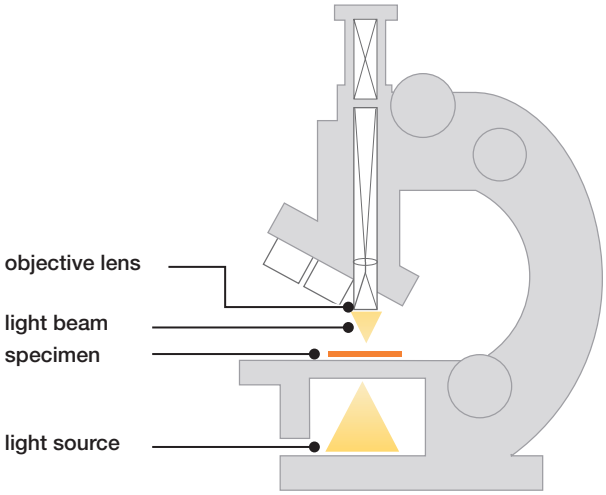


Mouse brain sample 3D.

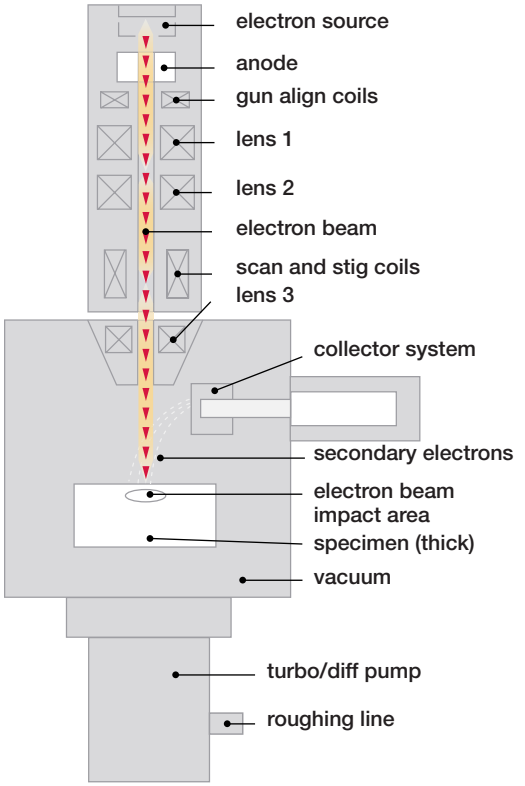
(In the light microscope, deviation in magnification is attained by changing the lens or by mechanically moving the lens). The ocular, or eyepiece, is replaced by a fluorescent screen and/or a digital camera.

The electron beam is generated by the electron gun (generally at the top of the column) and is condensed into a nearly parallel beam at the specimen by the condenser lenses. For TEM, the specimen must be adequately thin such that it can transmit the electrons, usually 0.5 µm or less; however, for SEM this is not important. In TEM, higher energy electrons (that is, higher accelerating voltages) can penetrate thicker samples.

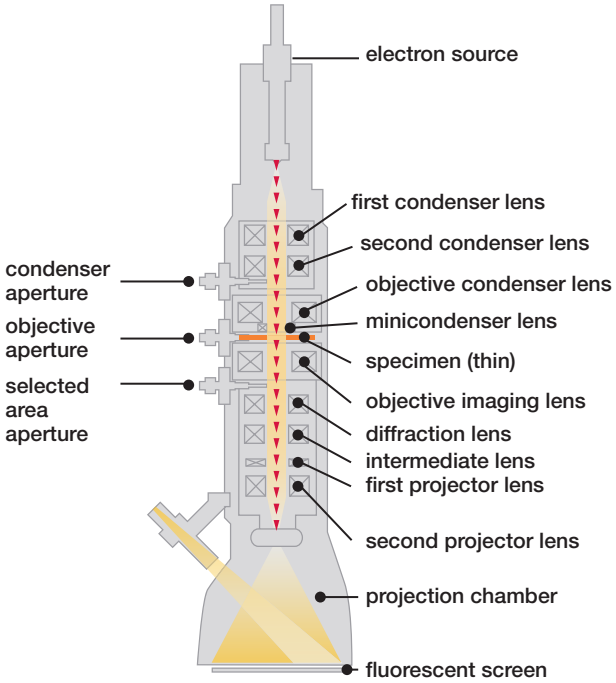
light microscope



SEM



TEM

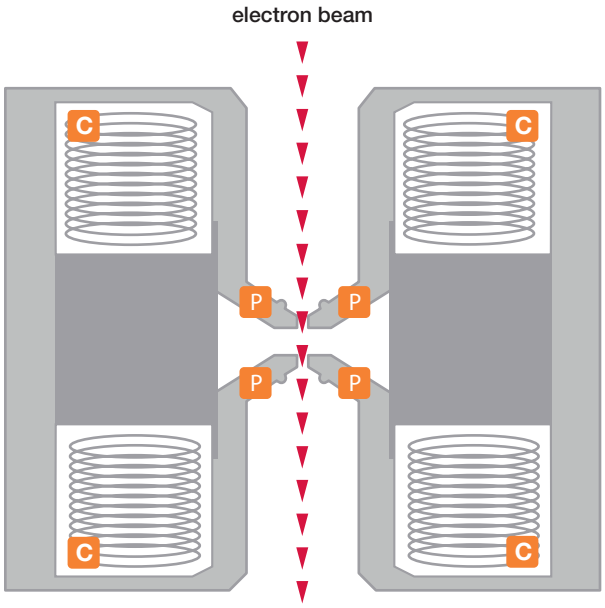


Comparison of the light microscope with TEM, SEM and FIB microscopes.

Electromagnetic lenses

The figure on the right presents a cross-section of an electromagnetic lens. When an electric current is passed via the coils (C), an electromagnetic field is developed between the pole pieces (P), which produces a gap in the magnetic circuit. By changing the current through the coils, the strength of the field, and thus the power of the lens, can be varied.

This is the essential difference between the magnetic lens and the glass lens. Otherwise, they act similarly and have the same types of aberration: spherical aberration (C_s – the power in the center of the lens differs from that at the edges), chromatic aberration (C_c – the power of the lens varies with the energy of the electrons in the beam), and astigmatism (a circle in the specimen becomes an ellipse in the image).



Cross-section of an electromagnetic lens.
C is an electrical coil and P is the soft iron pole piece.
The electron trajectory is from top to bottom.

How do electrons interact with matter?

In the modern view of matter, an atom consists of a heavy charged nucleus surrounded by a number of orbiting electrons. The number of electrons is equal to the number of protons in the nucleus and is known as the atomic number of the atom.The incoming beam electron can interact with the nucleus and be backscattered with virtually undiminished energy (just as a space probe is deviated by the gravity of a planet during a fly-by). Or it can interact with the orbiting electrons of sample atoms in a variety of ways, giving up some of its energy in the process.

Each type of interaction potentially constitutes a signal that carries information about the sample.

For instance, the most frequent interaction is the ejection of an electron from the atom with relatively low energy, a few eV. If this occurs near the sample surface, the liberated electron may escape and be detected as a secondary electron. Other signals include characteristic X-rays, cathodoluminescence, absorbed current and more, each carrying a specific type of information.

What happens during electron bombardment?

Contrary to what could be expected, most specimens are not adversely affected by the electron bombardment as long as beam conditions are carefully controlled. When electrons impinge on the specimen, they can cause any of the following:

- In crystalline specimens, the electrons are scattered in extremely distinct directions that are a function of the crystal structure.
- Other electrons are scattered over small angles, based on the composition and structure of the specimen.
- Some of the electrons are absorbed as a function of the thickness and composition of the specimen.
- Some of the impinging electrons are deflected through large angles or reflected (backscattered) by sample nuclei.
- The impinging electrons can knock electrons from sample atoms that then escape as low-energy secondary electrons.
- The impinging electrons could cause specimen atoms to emit X-rays whose energy and wavelength are related to the specimen's elemental composition; these are called characteristic X-rays.
- The impinging electrons cause the specimen to emit photons (or light); this is known as cathodoluminescence.
- Transmitted electrons lose energy as they pass through the sample.

Colored electrons

We see a world full of color. The color we see comes from our eyes' ability to distinguish among various wavelengths of light. However, most electron detectors see in black and white, or more accurately, shades of gray. What then of the beautiful color images that we see in this publication and elsewhere attributed to electron microscopes? In most cases, color has been added post-imaging for purely aesthetic reasons.

There are exceptions. Energy-filtered TEM (EFTEM) creates images from electrons that have been selected for a specific level of energy loss during their passage through the sample.

Since energy can be equated to wavelength, color EFTEM images, usually made by combining multiple images acquired at different energy loss settings, are perhaps the closest we can come to color electron images. But even EFTEM images are false color images in the sense that the correspondence between energy loss and color is an arbitrary assignment made by the creator of the image.

Color is also used to enhance X-ray maps, where a particular color may be assigned to a particular element to show its distribution in the specimen.

Electron velocity

The higher the accelerating voltage, the faster the electrons. 80 kV electrons have a velocity of 150,000 km/second (1.5×10^8 m/s), which is half the speed of light. This rises to 230,000 km/second for 300 kV electrons (2.3×10^8 m/s, more than three-quarters the speed of light). The wave particle duality concept of quantum physics asserts that all matter exhibits both wave-like and particle-like properties. The wavelength λ of an electron is given by

$$\lambda = \frac{h}{p}$$

where h is Planck's constant and p is the relativistic momentum of the electron. Knowing the rest mass of an electron m_0, and its charge e, we can calculate the velocity v imparted by an electric potential U as

$$v = \sqrt{\frac{2eU}{m_0}}$$

and wavelength at that velocity as

$$\lambda = \frac{h}{p} = \frac{h}{m_0 v} = \frac{h}{\sqrt{2m_0 eU}}$$

Finally, since the velocities attained are a significant fraction of the speed of light c, we add a relativistic correction to get

$$\lambda = \frac{h}{\sqrt{2m_0 eU}} \frac{1}{\sqrt{1 + \frac{eU}{2m_0 c^2}}}$$

The wavelength of the electrons in a 10 kV SEM is then 12.3×10^{-12} m (12.3 pm), while in a 200 kV TEM the wavelength is 2.5 pm.

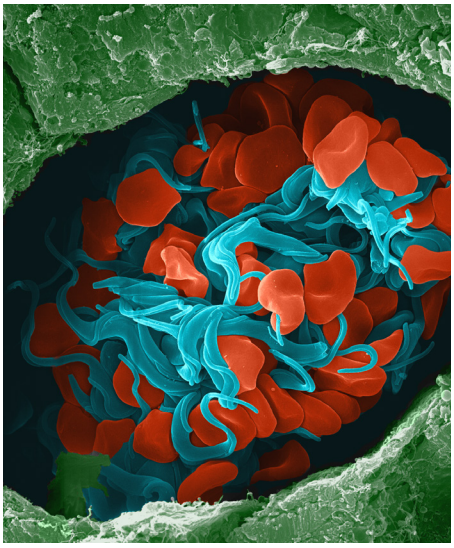
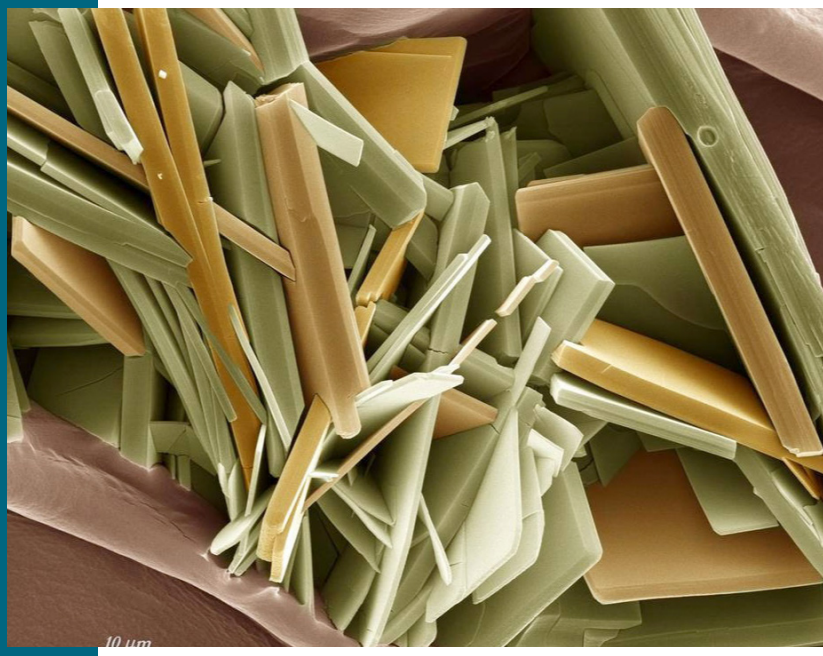


Image of Trypanosoma brucei gambiense parasite (blue) intertwined with red blood cells (red), in a blood capillary (green).

Courtesy of David Pérez-Morga, Université Libre de Bruxelles.
Original data collected at a horizontal field width of 45.76 µm.



SEM image of alginic acid crystals.

Courtesy of Dr. Maria Carbajo, Universidad de Extremadura.
Original data collected at a horizontal field width of 37µm.

CHAPTER 4

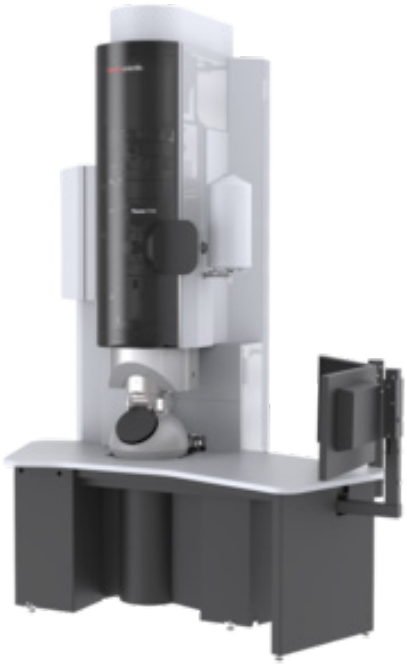
Transmission electron microscopy (TEM)

An overview of TEM

It is possible to compare a transmission electron microscope with a slide projector. In a slide projector, light from a light source is made into a parallel beam by the condenser lens; this travels through the slide (object) and is then focused as an enlarged image onto the screen by the objective lens.

In the electron microscope, the glass lenses are replaced by magnetic lenses, the light source is replaced by an electron source, and the projection screen is replaced by a fluorescent screen, or, more often in modern instruments, an electronic imaging device such as a charge-coupled device (CCD) camera.

The whole trajectory from source to screen is under vacuum, and the specimen (object) has to be extremely thin to allow the electrons to pass through it. All specimens cannot be made thin enough for the TEM. If individuals want to look at the surface of the specimen, rather than a projection through it, they use a scanning electron or ion microscope.



A modern transmission electron microscope - the Thermo Scientific™ Themis™ ETEM.

The electron gun

High-resolution TEM, based on phase contrast, requires the high spatial coherence of a field emission source, i.e., the field emission source should create waves of regular phase and shape. The greater current density and higher brightness provided by these sources produce smaller beams with higher currents for better spatial resolution and faster, more precise X-ray analysis.

Field emission sources are available in two types, cold field emission and Schottky (thermally assisted) field emission. Cold field emission offers extremely high brightness but varying beam currents. It also needs frequent flashing in order to clean contaminants from the tip. Schottky field emission offers high brightness and high, stable current with no flashing. The newest generation of Schottky field emitters (XFEG) retains its present stability while attaining brightness levels close to cold field emission.

As a rule of thumb, if the application demands imaging at magnifications up to 40,000–50,000× in TEM mode, a tungsten source is usually not only adequate but the best source for the application. When the TEM imaging magnification is between 50,000–100,000×, the brightest image on the screen will be produced with the help of a LaB_6 source. If magnifications greater than 100,000× are needed, a field emission source provides the better signal. In the case of small probe experiments such as scanning or analytical techniques, a field emission gun is always preferred.

Electron penetration

Electrons are easily stopped or deflected by matter. (An electron is nearly 2,000× smaller and lighter than the smallest atom.) That is why the microscope has to be evacuated and why specimens—for the transmission microscope—have to be very thin. Typically, for electron microscopy studies, a TEM specimen must be no thicker than a few hundred nanometers. Different thicknesses provide different types of information.

For present day electron microscopy studies, thinner is almost always better. Specimens as thin as a few tenths of a nanometer can be created from some materials using modern preparation techniques. While thickness is a primary consideration, it is equally important that the preparation preserves the specimen's bulk properties and not alter its atomic structure—not a trivial task.

Finally, transmitted beam electrons can be counted and then sorted by an energy loss spectrometer according to the amount of energy they have lost in interactions with the specimen. The energy loss carries information about the chemical, elemental and electronic states of the sample atoms. In a standard TEM, the mass thickness is the key contrast mechanism for non-crystalline (biological) specimens, while phase contrast and diffraction contrast are the most vital factors in image formation for crystalline specimens (most non-biological materials).

The electromagnetic lenses

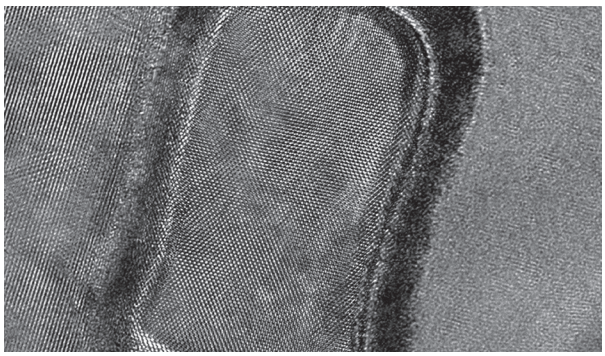
In a standard TEM, spherical aberration, which is primarily determined by the lens design and manufacture, is the main limitation to enhanced image resolution. Chromatic aberration can be decreased by keeping the accelerating voltage as stable as possible and employing extremely thin specimens. Astigmatism can be corrected by using variable electromagnetic compensation coils.

The condenser lens system focuses the electron beam onto the specimen under investigation as much as necessary in order to suit the purpose. The objective lens generates an image of the specimen that is then magnified by the remaining imaging lenses and projected onto the viewing device.

If the specimen is crystalline, a diffraction pattern will be developed at a point below the objective lens called the back focal plane. By varying the strengths of the lenses immediately below the objective lens, it is possible to enlarge the diffraction pattern and project it onto the viewing device. The objective lens is then followed by a number of projection lenses used to focus, magnify and project the image or diffraction pattern onto

the viewing device. To promise high stability and to attain the highest possible lens strength/magnification, the lenses in a modern TEM are generally water-cooled to prevent the build up of heat, which would result in more noise and lower quality data.

On the way from the source to the viewing device, the electron beam travels through a series of apertures with varied diameters. These apertures stop those electrons that are not needed for image formation (for example, scattered electrons). Using a special holder carrying a number of differently sized apertures, the objective lens, the diameter of the apertures in the condenser lens, and the diffraction lens can be changed as needed.



An atomic resolution image of an advanced logic semiconductor device is depicted. Some of the layers critical to chip performance are only a few atoms in width.

Original data collected at a horizontal field width of 60 nm.

Aberration-corrected TEM

The latest development of a dedicated commercial aberration-corrected TEM has allowed major advances in both STEM (Scanning Transmission Electron Microscope) and TEM capability. Without correction, TEM resolution is limited primarily by spherical aberration, which causes information from a point on the object to be spread over an area in the image. This results in a general blurring of the image and also in a phenomenon known as delocalization, in which periodic structures appear to extend beyond their actual physical boundaries.

In a light microscope, spherical aberration can be reduced by integrating lens elements that have opposing spherical aberrations. This method cannot be applied in electron microscopes, as the round magnetic lenses they use display only positive spherical aberration. Multi-pole correcting elements (with fundamentally negative aberration) were described by Otto Scherzer in 1947.

However, their effective commercial implementation needed solutions to a range of practical issues. Some are comparatively simple to achieve; for instance, the diameter of the electron column can be increased to provide the mechanical stability needed to see the advantage of enhanced optical performance. Others were very multifaceted, such as designing adequately stable power supplies and developing techniques and software controls that are advanced enough to reliably measure and then rectify the aberrations by autonomously exciting the multi-pole elements.

The ability to correct spherical aberration leaves chromatic aberration effects as the next key challenge in refining TEM performance. Chromatic aberration correctors have been effectively added into modern S/TEM instrumentation, but their design and operation are considerably more complex than spherical aberration correctors. At the same time, substantial progress has been made in decreasing the energy spread of electrons passing through the lenses. (The energy spread establishes the magnitude of chromatic aberration's deleterious effects.)

Variations in electron energy may originate as the beam is created in the electron gun, or they may be introduced in transmitted electrons by interactions with sample atoms. The first of these, beam energy spread, has been addressed by designing extremely stable high-voltage and lens current power supplies, by using specially improved field emission electron sources and by directing the beam through a monochromator, which passes only a very narrow band of energies.

The energy spread among electrons conveyed through the specimen can be reduced by minimizing sample thickness with modern sample preparation methods.

This image displays the formation of planes that are visible when nanoparticles of titanium dioxide are used as a catalyst in the process of photocatalysis.

Courtesy of Dr. Maria Carbajo, Universidad de Extremadura. Original data collected at a horizontal field width of 63 nm.

Image resolution and information limit

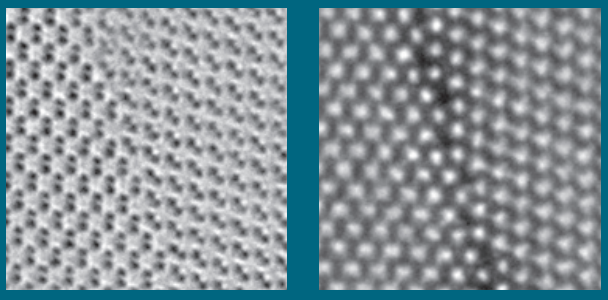
Prior to the development of spherical aberration correctors, scientists knew that a TEM was capable of providing higher spatial resolution than what could be observed directly in the image.

This directly observable resolution, known as point resolution, was limited by spherical aberration of the lenses. However, by appropriately combining data from multiple images in a “throughfocus series” (acquired over a range of defocus values), researchers could reconstruct a model image exhibiting higher resolution information.

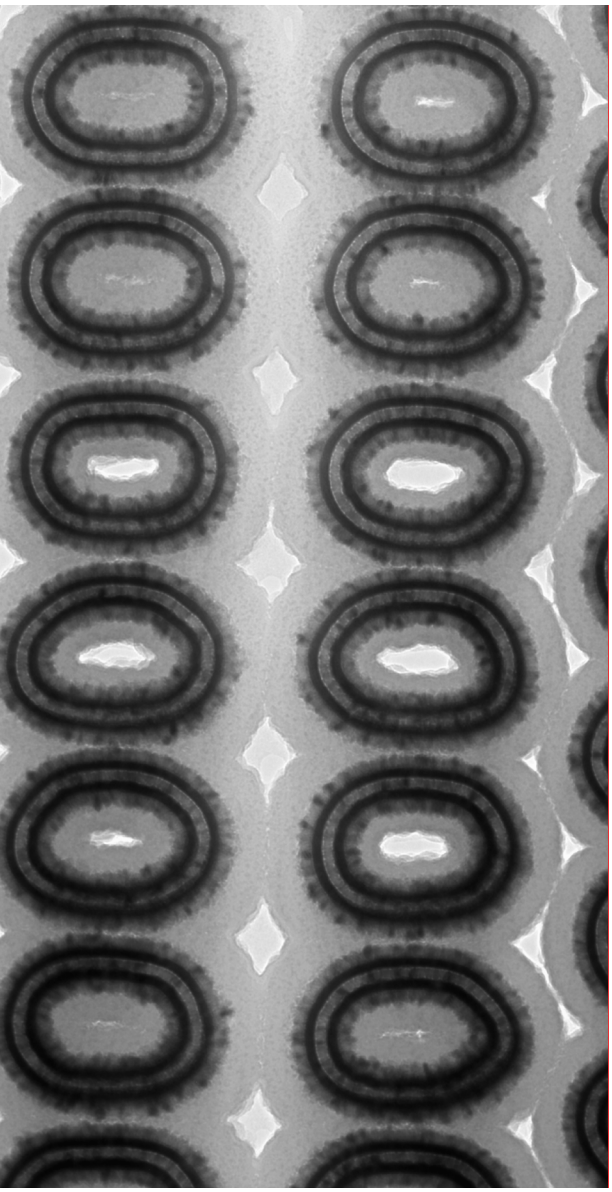
The highest resolution information the instrument is capable of transferring is known as its information limit. With spherical aberration correctors, the point resolution is extended to the information limit, and the distinction disappears for most practical purposes.

Moiré-fringe image extracted from the original TEM image taken on a spherical-aberration corrected S/TEM.

Courtesy of Craig Johnson, CEMES-CNRS.
Original data collected at a horizontal field width of 34 nm.



Comparison of HR-TEMs with (left) and without (right) C_s-correction on the same Si (110) grain boundary at 300 kV.



Dynamic random-access memory (DRAM) capacitors, a type of random-access memory that stores each bit of data in a separate capacitor within an integrated circuit.

Courtesy of Dr. Neerushana Jehanathan, Chipworks
Original data collected at a horizontal field width of 1.5 µm.

Observing and recording the image

Originally, TEMs used a fluorescent screen, which emitted light when influenced by the transmitted electrons, for real-time imaging and alterations. A film camera was used to record permanent, high-resolution images (electrons have the same impact on the photographic material as light). The screen was under vacuum in the projection chamber but could be perceived through a window using a binocular magnifier, if required.

The fluorescent screen typically hinged up to allow the image to be projected on the film below. Advanced instruments rely mainly on solid-state imaging devices, such as a charge-coupled device (CCD) camera, for image capture. They may still include a fluorescent screen, but it can be observed by a video camera. In this article, unless particular aspects of an imaging system are being discussed, the instrument is implied to have a solid-state imaging device.

The subsequent introduction of a direct electron detector promised great improvements in image resolution and contrast, mainly in signal-limited applications. A conventional CCD camera uses a scintillator material over the image detector elements to convert incident electrons to light, which then produces charge in the underlying CCD element.

The scintillator introduces some loss of resolution, and the conversion process reduces the efficiency with which electrons add to image contrast. This can be an issue in applications that are sensitive to damage by the electron beam, such as cryogenically prepared samples of delicate biological materials, where it is vital to extract the highest amount of information from a faint, noisy signal before the sample is damaged.

Eliminating the scintillator with a direct electron detector enhances image resolution and increases detector efficiency by up to three times.

The vacuum

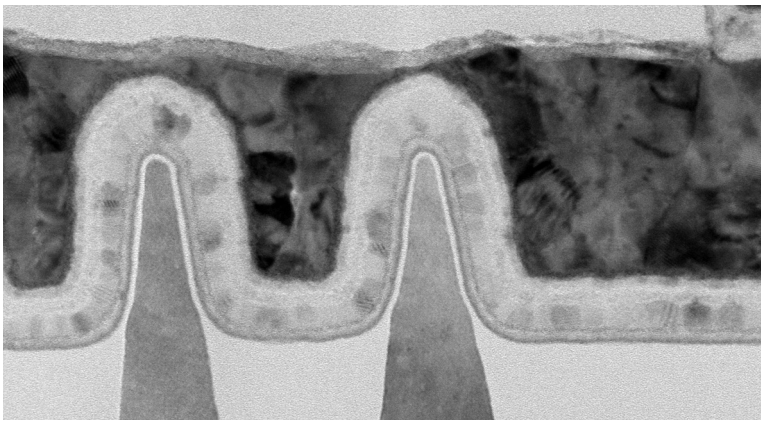
Electrons act like light only when they are controlled in a vacuum. As has already been mentioned, the entire column from source to the fluorescent screen (including the camera) is evacuated. Various levels of vacuum are essential; the maximum vacuum is around the specimen and in the source, while a lower vacuum is found in the projection and camera chambers.

Different vacuum pumps are used to acquire and maintain these levels. The vacuum in a field emission electron gun may be as high as (i.e., "pressure as low as") 10^{-8} Pa. To avoid the need to evacuate the entire column every time a specimen, photographic material or filament is exchanged, many airlocks and separation valves are incorporated. In advanced TEMs, the vacuum system is totally automated, and the vacuum level is continuously scrutinized and fully protected against defective operation.

Environmental TEM

Environmental TEM (ETEM) uses a particularly designed vacuum system to allow researchers to detect specimens in a variety of conditions approaching more "natural" environments, with gas pressures in the sample chamber as high as a few percent of atmospheric pressure. This can be vital for observing interactions between the environment and the sample, as, for example, the action of a solid catalyst particle in a gaseous reaction environment. ETEM depends on pressure-limiting apertures and differential vacuum pumping to allow less restrictive vacuum conditions in the vicinity of the sample while maintaining a high vacuum in the rest of the electron column.

The size of the sample chamber in a TEM is very constrained by the requirements of lens design—the sample is essentially located inside the objective lens. The development of aberration correctors promises to lessen some of these constraints, creating extra flexibility for larger, more complex experimental apparatuses in ETEM.



Bright Field TEM of a 3D transistor used in microprocessors. The gate of the transistor is wrapped around the source drain to increase performance.

Original data collected at a horizontal field width of 190 nm

The electronics

To acquire the very high resolution that advanced TEMs are capable of, the accelerating voltage and the current through the lenses must be very stable. The power supply cabinet contains several power supplies whose output voltage or current does not deviate by more than 1/10,000,000th of the value selected for a specific purpose. Such stabilities necessitate very advanced electronic circuits.

Enhanced electron optical design has made a variety of progressively complicated electron-optical methods possible. This, in turn, has brought on the need to streamline instrument operation, allowing more users with less specialized training to produce data efficiently

and effectively. Digital electronic methods, in general, and microprocessor-based methods, in particular, play a key role in this respect.

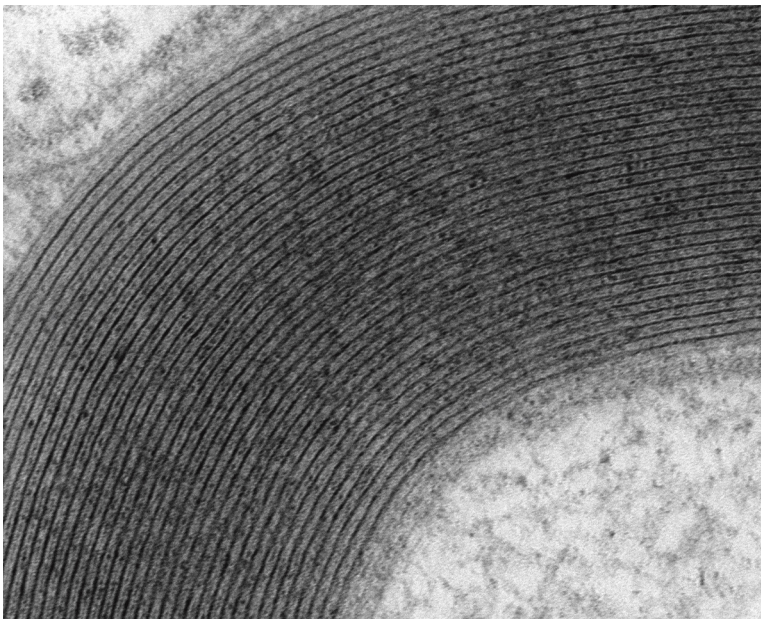
Advanced electron microscopes employ a computer to control, monitor and record the operating conditions of the microscope. This results in a significant reduction in the number of control knobs, compared with previous models, and a microscope that is easier to use, particularly when numerous accessories require simultaneous optimization.

Additionally, it allows special methods and experiments to be embedded in the instrument so that the operator can perform them using the same controls. The computer can be linked to a network to allow automatic backups and data sharing.

Specimen orientation and manipulation

The TEM specimen stage must provide a range of movements to control and orient the sample. Tilt and X, Y and Z translation are used to move the correct region of the sample into the field of view of the microscope. Tilt along a second axis is needed to allow precise orientation of crystalline samples with respect to the beam for diffraction studies and analysis along a specific crystallographic orientation or grain boundary.

Specialized stages may also provide heating, cooling and straining of the specimen for experiments in the microscope. The elementary movements are provided by a goniometer mounted very close to the objective lens. The specimen is usually located in the objective lens field between the pole pieces because it is there that the lens aberrations are smallest and the resolution is highest.



Cross-section view of the myelin sheath of an axon. Myelin is a fatty substance that electrically insulates the axon of a nerve cell, speeding up the transmission of impulses between neurons.

Courtesy of Professor Juan Carlos Leon-Contreras, INCMNSZ. Original data collected at a horizontal field width of 900 nm.

The goniometer itself offers motorized X, Y and Z movement and tilt along one axis.The specimen is mounted near the tip of a rod-shaped holder, which in turn is added into the goniometer through an airlock. It is the specimen holder rod that provides the additional tilt axis, rotation, heating, cooling or straining, with a special holder required for each purpose.

Specimen preparation

A TEM can be used in any branch of science and technology where it is informative to examine the internal structure of specimens down to the atomic level. The sample must be stable and small enough (about 3 millimeters in diameter) to allow its introduction into the evacuated microscope column and thin enough to allow the transmission of electrons.

Different thicknesses are required for various applications. For critical high-resolution materials studies, the sample cannot be thicker than 20 nm or so; for bio-research, the film can be 300–500 nm thick.

Every branch of research has its own specific sample preparation techniques for electron microscopy. In biology, for instance, there may first be a chemical treatment that removes water and preserves the tissue (as much as possible) in its original state, followed by embedding in a hardening resin. After the resin has hardened, slices (sections) with an average thickness of 0.5 µm are cut with an instrument called an ultramicrotome, which contains a glass or diamond knife. The minute sections created this way are placed on a specimen carrier, typically a 3 mm diameter copper specimen grid that has been coated with a 0.1 µm thick structureless carbon film.

Cryogenic freezing and vitrification

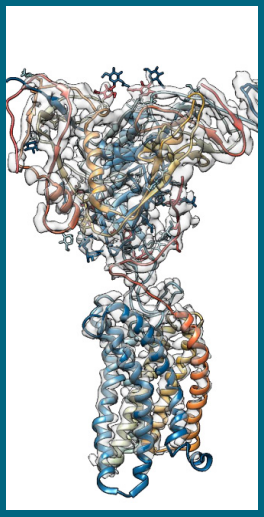
Cryo (freezing) methods avoid the sample damage inevitably caused by conventional drying, fixing and sectioning preparations. However, traditional freezing methods, while avoiding the introduction of foreign materials, can still damage the sample, as the formation of expanding ice crystals may disrupt delicate biological structures.

Vitrification is a fast freezing process that occurs so rapidly that water molecules do not have time to crystallize, instead forming a vitreous (amorphous) solid that does little or no damage to the sample structure.The low temperature of the vitrified sample also decreases the damage caused by beam electrons during observations, allowing more (or longer) exposures at higher beam currents, producing better quality images.

Cryo-TEM allows biological molecules to be tested in their natural context, in connection with other molecules that are frequently vital to their form and function. Moreover, vitrified samples are, quite plainly, frozen in time, allowing researchers to examine time-based phenomena such as the structural dynamics of flexible proteins or the aggregation and dissociation of protein complexes.

What is cryo-electron microscopy?

Cryo-electron microscopy is a form of transmission electron microscopy that uses cryogenic temperatures to enable proteins, viruses and sub-cellular structures to be observed in aqueous environments. Recent technological advancements, including improved detectors and computer algorithms, have allowed scientists to use cryo-electron microscopy to determine 3D structures of biological macromolecules with atomic resolution, taking structural biology and biochemistry into a new era.



HIV spike protein structure solved by single particle analysis, visualized by Chimera. PDB ID 6MEO, Shaik et al, Nature 565, 318-323 (2019)

By measuring the variability within a set of images, each capturing the shape of a molecule at an instant in time, researchers can calculate the range of motion and the intramolecular forces operating in flexible proteins. Similarly, a collection of images might offer a freeze frame sequence of the assembly of a protein complex or conformational changes during antigen binding. Automated vitrification tools allow precise control of the process, ensuring reliable, repeatable results.

Focused ion beam milling

In metallurgy, a 3 mm diameter sample disc (~0.3 mm thick) is chemically treated in such a way that the material in the center of the disc is completely etched away. Around this hole, there will typically be areas that are appropriately thin (approximately 0.1 µm) to allow electrons to pass through. For studies in aberration corrected systems, this thickness cannot be over a few dozen nanometers.The use of a focused ion beam to mill and thin a sample from a bulk specimen is more and more important, mainly in semiconductor and other nanoscience applications where the sample site must be precisely situated.

Automated systems, like the Thermo Scientific™ Vitrobot™ System, provide fast, easy and reproducible sample preparation.

Challenges of determining the 3D structure of macromolecules

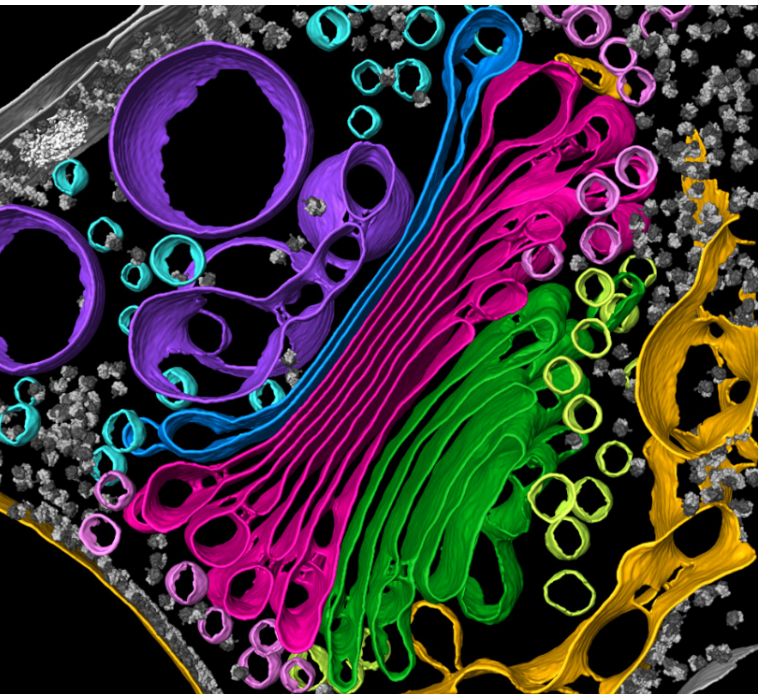
Macromolecules, like proteins, are too small to see with visible light. For decades, researchers have been using X-ray diffraction (XRD) to determine the structures of biological macromolecules. However, to conduct XRD, samples must be crystallized, which is often challenging for proteins and macromolecules. Nuclear magnetic resonance (NMR) is also widely used to study the structure of biological molecules. However, NMR works best for small biomolecules and is less effective for characterizing larger biological structures such as proteins and viruses.

It is not possible to use traditional electron microscopy techniques to image proteins and macromolecules, as interactions between the sample and the electrons can cause extensive damage to the sample. Lower electron doses can be used to protect the sample and reduce damage, but this often results in poor signal-to-noise ratios.

Furthermore, electron microscopes operate in a vacuum, which damages biological samples and does not allow for an aqueous environment. As biomolecules exist largely in water, the true structure of these samples can only be characterized in their native, aqueous states.

3D imaging techniques

Comprehending the organization of matter in three dimensions has become increasingly important. Semiconductor manufacturers regularly create nanometer-scale structures that they must observe and measure in order to regulate their manufacturing processes. Perhaps the most vital application of 3D microscopy is in the sciences, where investigators are separating the multifaceted molecular interactions that are the basis of life. Most of these rely directly on the intricate 3D shapes of the interacting molecules.



In situ cryo-electron tomogram of the native Chlamydomonas Golgi. Image courtesy of Ben Engel.

Electron tomography is in some ways similar to larger scale medical imaging technologies such as CAT scans or MRIs. Tomography obtains a series of projected images from different perspectives as the sample is rotated incrementally about an axis perpendicular to the viewing direction. A computer then combines these images into a 3D model of the sample. It is similar to the way someone would turn an object about in their hand while looking at it to appreciate its 3D shape. Electron tomography has been limited by the technique's inability to acquire information from perspectives that lie close to the plane of the thin sample. Here, the trajectory of the beam through the sample becomes extremely long, resulting in a region known as the missing wedge.

The development of dual-axis tomography, in which the sample is also rotated about a second axis perpendicular to the first, has enhanced results—decreasing the missing wedge to a missing pyramid. Tomography looks at a single instance of the subject structure, which allows it to examine differences within a population of such structures, but also restricts the analysis to the data that can be attained from that single sample (frequently a biological entity quite vulnerable to beam damage). Presently, the best spatial resolution available from the tomographic analysis is a few nanometers.

Single particle analysis (SPA, a slightly misleading name) obtains images of a large number of arbitrarily oriented, nominally identical particles and uses a computer to categorize them into groups of similar orientation, creates composite projected images representative of each orientation and integrates the composited images into a 3D model.

By combining numerous images, SPA builds contrast and enhances the signal-to-noise ratio of the resulting model. In theory, it could continue refining by merely increasing the number of images, though the diminishing returns from incremental increases impose a practical limit. SPA results have been reported with a spatial resolution of a few tenths of a nanometer.

Automation plays a key role in both approaches to 3D imaging. In the tomographic analysis, the entire series acquisition can be automated. Automation is virtually indispensable in SPA, which may require the analysis of tens of thousands of particles. In both cases, automation can also help to decrease sample damage by guaranteeing consistent use of low-dose methodologies. (Low-dose imaging refers to methods that reduce the exposure of the sample to damaging radiation from the electron beam.) It is vital in 3D analysis (mainly of biological materials) to guarantee that the highest amount of information is acquired before the sample is damaged or destroyed.

Scanning transmission electron microscopy (STEM)

TEM can also be combined with SEM to give scanning transmission electron microscopy (STEM). The first commercial instrument in which the transmission and scanning techniques were incorporated was a Philips EM200 equipped with a STEM unit, produced in 1969 by Ong Sing Poen of Philips Electronic Instruments in the U.S. It had a resolving power of 25 nm. Modern TEM systems equipped with STEM capability can attain resolutions down to 0.05 nm in STEM mode.

Scanning transmission electron microscopy incorporates the principles of TEM and SEM and can be carried out on either type of instrument. Like TEM, STEM needs extremely thin samples and looks primarily at beam electrons transmitted by the sample. One of its principle benefits over TEM is in enabling the use of other signals that cannot be spatially correlated in TEM, including scattered beam electrons, secondary electrons, characteristic X-rays and electron energy loss.

While the technique can be used in both an SEM and TEM, the higher accelerating voltages available in a TEM allow the use of thicker samples, and the additional lenses below the sample greatly expand the number of possibilities for gathering information. TEM-based STEM, using a condenser lens aberration corrector, has attained a resolution of 0.05 nm.

Similar to SEM, the STEM technique is capable of scanning a very finely focused beam of electrons across the sample in a raster pattern. Interactions between the beam electrons and sample atoms produce a serial signal stream, which is correlated with beam position. This builds a virtual image in which the signal level at any location in the sample is represented by the gray level at the corresponding location in the image.

Its main advantage over conventional SEM imaging is the enhancement in spatial resolution, which results from eradication of the electron scattering that occurs in bulk specimens as the beam electrons penetrate into the sample.

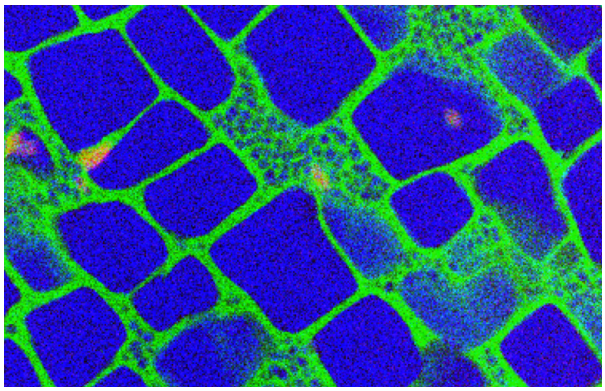
Secondary electrons (SE) are electrons from sample atoms that have been scattered by beam electrons. They have extremely low energies and are capable of escaping from the sample only if they originate extremely close to the surface. SE is the primary imaging signal in SEM, where they offer good spatial resolution and high topographic sensitivity. SE are not often used in STEM mode but are mentioned here for completeness.

X-ray microanalysis

Electrons bombarding the specimen lead it to emit X-rays whose energy is characteristic of the elemental composition of the sample. X-ray microanalysis uses an energy dispersive X-ray (EDX) spectrometer to count and then sort characteristic X-rays according to their energy.

The resulting energy spectrum displays distinctive peaks for the elements present, with the peak heights indicating the elements' concentrations. Analysis of the spectrum can help determine accurate elemental concentration with a spatial resolution down to the 100 nm scale in bulk SEM specimens and the 10–20 nm scale in thin specimens for SEM-based STEM.

Sub-angstrom (10^{-10} m) spatial resolution has been reported for X-ray microanalysis in the TEM-based STEM. Due to the extremely small volume analyzed at any given instant, X-ray microanalysis can detect extremely small quantities of elements (down to one-thousandth of a picogram (10^{-15} g) or less). It is mainly useful for detecting locally concentrated occurrences of elements that are present at extremely low bulk concentrations, such as grains of precious metal ores.



Energy-dispersive X-ray map of a superalloy.

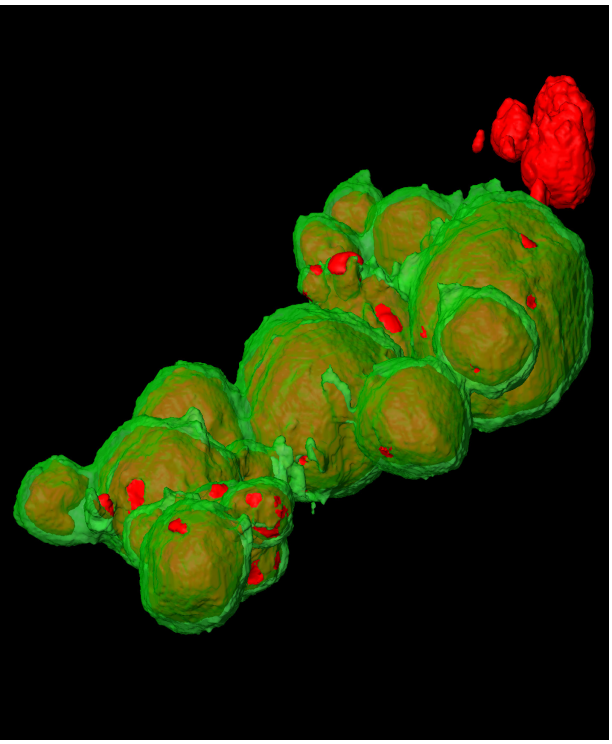
Original data captured at 3.6 µm.

The primary restrictions on the speed and precision of X-ray analysis are the fraction of outgoing X-rays that can be gathered, the energy resolution of the detector and the speed with which X-rays can be detected and measured. That rate, at which a single detector can examine X-rays, has been increased considerably by the development of silicon drift detectors.

X-ray analysis

The impinging electrons in the primary beam may eject an electron from a sample atom. If the ejected electron originates from one of the inner orbitals, the resulting vacancy may be filled by an electron from an outer orbital of the same atom with the concurrent emission of an X-ray. The energy of the emitted X-ray is equal to the energy difference between the orbitals and is thus "characteristic"of the elemental identity of the emitting atom.

An X-ray spectrometer counts and measures the energy of emitted X-rays. The relative intensity of the X-ray signal at each energy (the energy spectrum) can be used to calculate the quantitative elemental composition of the sample within the volume of interaction—the region within the sample from which the X-ray signal originates as the beam electrons penetrate and scatter.



3D EDS tomogram of Ag-Pt core-shell nanoparticles, where most of the Ag cores (red) are covered by Pt shells (green). Pores in the Pt partially expose the cores.

Sample courtesy of Professors Yi Ding and Jun Luo of the Center for Electron Microscopy, Tianjin University of Technology, China. Original data collected at a horizontal field width of 550 nm.

Custom-designed systems, optimized for rapid elemental analysis in applications such as SEM-based automated mineralogy, may also employ multiple detectors in order to increase the total area of the detectors, and thus the number of X-rays they intercept.

Adding detectors is a vital design problem since the detectors must be arranged close to the specimen without interfering with other functions of the microscope. TEMs specifically optimized for X-ray analysis have achieved collection of solid angles approaching 1 steradian, significantly enhancing minimum detectable mass performance.

Overall, this allows users to extract more information from the sample within a short time frame, a key factor when looking at samples that may be damaged or changed under the electron beam. This also decreases the time needed to develop elemental maps from samples.

Wavelength dispersive X-ray (WDX) spectrometry measures and then counts X-rays by their wavelength (a correlate of energy). A wavelength spectrometer uses a crystal or grating with known spacing in order to diffract characteristic X-rays. The angle of diffraction is a function of the X-ray wavelength, and the crystal is mechanically scanned over a range of angles while a detector measures varying intensity.

WDX is usually much slower than EDX but offers greater spectral (energy) resolution (which helps to prevent inference among closely spaced spectral peaks) and better sensitivity to light elements. Several WDX spectrometers are needed with different crystals in order to cover the complete range of elements. Their size usually limits their application to SEM or dedicated electron probe instruments. Electron energy loss spectrometry (EELS) examines transmitted electrons to define the amount of energy they have lost in interactions with the sample. It supplies information about the interacting atoms, including chemical bonding, elemental identity, valence and conduction band electronic properties, surface properties and element-specific pair distance distribution functions. EELS is mainly used with the TEM-based STEM.

CHAPTER 5

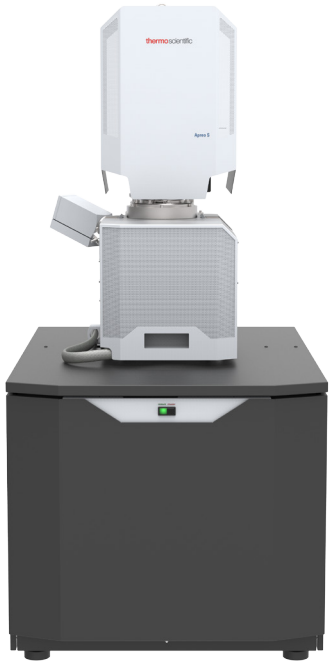
Scanning electron microscopy (SEM)

An overview of SEM

A scanning electron microscope (SEM), like a TEM, uses a vacuum system, an electron optical column, electronics, detectors and software to capture a nanoscale image of a sample.

SEM uses a short column because the only lenses needed are those above the specimen (used to focus the electrons into a fine spot on the specimen surface). The specimen chamber, on the other hand, is large because the SEM method does not impose any limits on specimen size (unlike TEM) other than that set by the size of the chamber itself.

The fine spot produced by the beam can be as small as 1 nm in diameter on the specimen surface. This beam is scanned in a rectangular raster over the sample and the intensities of various signals formed by interactions between the beam electrons and the specimen are measured and recorded.



A modern scanning electron microscope - the Thermo Scientific™ Apreo SEM.

Scanning microscopy

Imagine yourself alone in an unknown darkened room with only a narrowly focused flashlight. You might start exploring the room by scanning the flashlight systematically from side to side, gradually moving down (a raster pattern) so that you could build up a picture of the objects in the room in your memory.

A scanning electron microscope uses an electron beam instead of a flashlight, an electron detector instead of your eyes, and computer memory instead of your brain to build an image of a sample's surface.

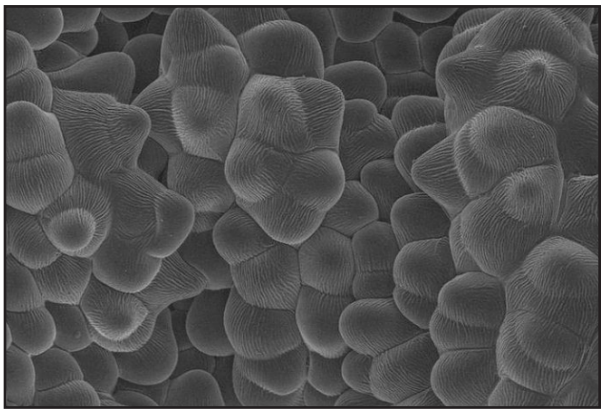
The stored values are then mapped as differences in brightness on the image display. The secondary electron (SE) signal is the most commonly used signal. It differs with the topography of the sample surface much like an aerial photograph: edges are bright, recesses are dark. The ratio of the size of the exhibited image to the size of the area scanned on the specimen gives the magnification. The magnification can be increased by decreasing the size of the area scanned on the specimen.

The most significant differences between TEM and SEM are:

- Instead of the wide static beam used in TEM, the SEM beam is concentrated to a fine point and scanned line by line over the sample surface in a rectangular raster pattern.
- The accelerating voltages in SEM are a lot lower than in TEM because it is no longer essential to penetrate the specimen; in an SEM they range from 50 to 30,000 volts.
- The SEM specimen need not be thin, significantly simplifying specimen preparation.

The interactions between the beam electrons and sample atoms are similar to those defined for TEM:

- The specimen itself produces secondary electrons (SE).
- Some of the key electrons are reflected backscattered electrons (BSE). These backscattered electrons can also cause the emission of secondary electrons as they move through the sample and exit the sample surface.
- Electrons are absorbed by the specimen.
- The specimen releases X-rays.
- The specimen occasionally emits visible light (cathodoluminescence).
- If the sample is thin, the SEM may be worked in scanning electron transmission microscope (STEM) mode with a detector located below the sample to collect transmitted electrons.



SEM image of orchid flower, courtesy of Nurshaiba Md. Nasir.

Original data collected at a horizontal field width of 330 µm.

SEM magnification

SEM magnification is simply the length of one line scanned in the image (usually the width of the image) divided by the length of the line scanned on the sample surface (usually the width of the raster pattern). A high-resolution computer display might be half a meter wide and display 2,000 pixels over that distance (pixel width = .25 mm).

If each pixel represents one square nanometer on the sample surface, then an image that fills the display represents a scanned area 2,000 nm (2 µm) wide, and the magnification of the image on the display is 250,000×.

All these phenomena are interconnected, and all of them rely to a certain extent on the topography, the atomic number, structure and the chemical state of the specimen. The most commonly imaged signals in SEM are SE and BSE. Secondary electrons, because of their very low energies, can escape the sample to be detected only if they originate very close to the sample surface. This gives SEM images high spatial resolution and robust topographic contrast. The BSE signal is used predominantly for its strong atomic number contrast. Characteristic X-rays are also extensively used in SEM for elemental microanalysis.

Electron detection

Detectors for backscattered and secondary electrons are typically either a scintillation detector or a solid-state detector. In the scintillator case, electrons strike a fluorescent screen, which releases light that is amplified and converted into an electrical signal by a photomultiplier tube or diode. The solid state detector works by amplifying the minute signal created by the incoming electrons in a semiconductor device.

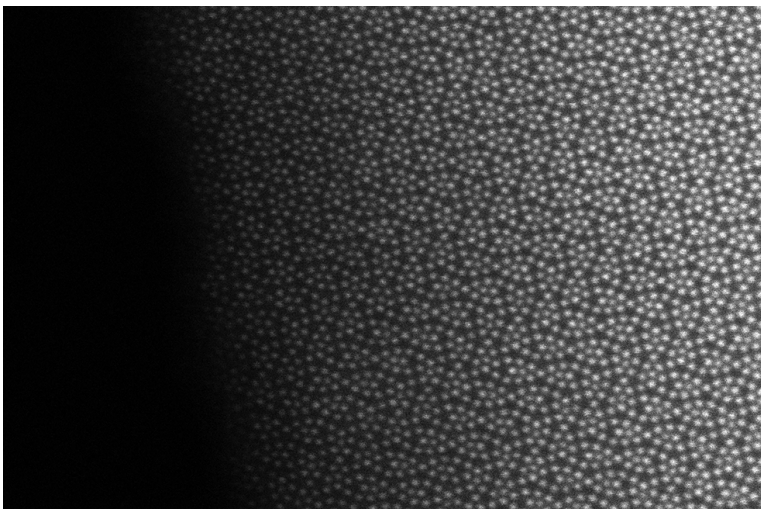
The third type of detector monitors the net current absorbed by the specimen, where net current = beam current - (SE + BSE), or the current induced in a semiconductor junction by the incoming beam electron. These absorbed current and electron beam induced current (EBIC) measurements allow the study of dynamic electrical phenomena in electronic devices.

Resolution

Resolution in an SEM relies on the degree to which the signal, at any instant in time, can be related to the position of the electron beam. Specifically, for a particular beam location, how large is the region within the sample from which the signal originates? This can be influenced by a number of factors, including the type of signal, composition of the sample, the size of the spot formed by the beam, the energy of the beam and more.

Generally, at lower voltages, where the beam electrons do not travel far into the sample, the size of the spot is the main determinant of image resolution. At higher voltages, the volume of interaction, from which the signal originates, may become the main consideration.

Presently, the best SEMs offer resolution below 1 nm with voltages below 1 kV up through the entire range of accelerating voltages, allowing the operator to select a beam energy to suit the requirements of the analysis; for instance, higher energy to provide a broad energy spectrum for X-ray analysis, or lower energy to improve surface specificity, avoid charging and/or beam damage.



Atomic scale HAADF image of potassium tungsten niobate Sample.
Original data collected at a horizontal field width of 23.75 nm.

Beam deceleration

Beam deceleration offers extra flexibility in the choice of accelerating voltage. With beam deceleration, the beam traverses most of the column at high energy to decrease the adverse effects of chromatic aberration and is then decelerated by an opposing electrical potential. The beam electrons thereby land with decreased energy.

Image treatment

Since the image in an SEM is completely electronically produced, it can be subjected to sophisticated analysis and manipulation with the help of modern digital techniques. This is comprised of contrast enhancement, inversion (black becomes white, etc.), filtering, mixing of images from various detectors, subtraction of the image from one detector from that generated by a different detector, and color coding. The application of these techniques must be guided by the key goal of extracting the best possible information from the specimen.

Resolution and magnification

The resolving power of a microscope determines its maximum useful magnification. For instance, if a microscope has a resolving power of 200 nm (typical of a light microscope), it is only useful to magnify the image by a factor of 1,000 to make all the available information visible.

At that magnification, the smallest details that the optical system can transfer from the object to the image (200 nm) are large enough to be seen by the unaided eye (0.2 mm). Further magnification makes the image larger (and more blurred), but does not reveal additional detail. Magnification in excess of the maximum useful magnification is sometimes referred to as “empty resolution.” Notwithstanding the limiting principle of maximum useful resolution, it is often convenient, for a variety of practical or aesthetic reasons, to use higher magnifications; and commercial instruments typically offer magnification capability well beyond the maximum useful magnification implied by their resolving power. This text will emphasize resolving power as the primary measure of an instrument’s imaging capability, and refer to magnification only to provide a relative sense of scale among various electron microscopy techniques. When a more precise usage of magnification is required, it will be cited explicitly.

Magnification is often quoted for an image because it gives a quick idea of how much the features of the specimen have been enlarged. However, a magnification that was accurate for the original image will be inaccurate when that image is projected on a large screen as part of a presentation or reproduced at a smaller size in a printed publication.

For this reason, most microscopes now routinely include reference scale markers of known length that scale accurately as the image is enlarged or reduced for various uses.

Resolution and accelerating voltage, spot size and volume of interaction

The resolution of an SEM is determined by the size of the region from which the signal originates. Certainly, this will not be smaller than the extent of the spot illuminated by the beam on the sample surface. In conventional SEM, it is easier to form a smaller spot at higher beam energies because the degrading effects of chromatic aberration are relatively less significant. However, at higher beam energies, the beam electrons penetrate deeply and scatter widely within the sample, contributing signal from locations well outside the spot, thereby degrading image resolution.

When beam energy is reduced, spot size increases as the fixed energy spread among electrons in the beam becomes larger relative to the nominal beam energy, and the adverse effects of chromatic aberration increase. At some point, the benefit of reducing penetration is overwhelmed by the cost of increasing spot size.

A monochromator reduces the energy spread of the beam by eliminating beam electrons that fall outside a selected range. Combined with a field emission electron gun, monochromator-equipped SEMs have demonstrated sub-nanometer resolution at accelerating voltages below 1 kV. Monochromator technology avoids restrictions on sample type and size that have limited the utility of other approaches to low-voltage imaging, such as “in-the-lens” configurations and chromatic aberration correctors.

Vacuum

Generally, a sufficiently good vacuum for an SEM is generated by either an oil diffusion pump or a turbomolecular pump (the current standard for most SEMs), in each case backed by a mechanical pre-vacuum pump. These combinations also offer reasonable exchange times for filament, specimen and aperture (less than a few minutes).

Vacuum airlocks could also be used for huge chambers and in high-volume applications when fast sample exchange has great value. Modern SEM vacuum systems are wholly automatically controlled and protected against operating failures. Samples for standard SEM usually have to be dry, clean, vacuum-compatible and, preferably, electrically conductive.

In recent years, the environmental scanning electron microscope (ESEM) has expanded the variety of samples and sample environments that can be accommodated in the SEM chamber. Examples of specimens that pose problems are wool or cotton tissue, cosmetics, fats and emulsions (for example, margarine).

Early attempts to view a specimen containing volatile components (by placing it in an environmental chamber isolated from the main column vacuum by small, differential pumping apertures) were hindered by the inability of standard secondary electron detectors to work in a low-vacuum or non-vacuum environment.

The ESEM’s gaseous secondary electron detector uses gas molecules in the sample environment in a cascade amplification in order to detect and amplify the secondary electron signal while, at the same time, generating positive ions. This effectively suppresses charging artifacts, as

they are attracted by any negative charge accumulating on insulated specimen surfaces.

Variable pressure and low pressure are terms used to describe SEMs that work in an intermediate vacuum range between high-vacuum SEM and ESEM. These instruments offer some of the sample flexibility of ESEM, though they are not usually capable of providing pressure/temperature conditions that will sustain liquid water.

Application and specimen preparation

An SEM can be used whenever information is needed about the surface or near-surface region of a specimen. It finds application in almost every branch of technology, science and industry. The only requirement is that the specimen must be capable of withstanding the vacuum of the chamber and bombardment by the electron beam. Since there is no need for a thin sample, SEM sample preparation is significantly simpler than the preparation of specimens for TEM.

A number of samples can be brought into the chamber without preparation of any kind. If the specimen contains any volatile components, such as water, these must be removed by a drying process. In some circumstances, the sample can be frozen solid prior to being used in a high-vacuum system. Non-conducting specimens will accumulate charge under electron bombardment and may need to be coated with a conducting layer.

Iridium provides a fine-grained coating and is effortlessly applied in a sputter coater. It offers a good yield of secondary electrons and, consequently, a good quality image of the surface. Other metals, such as platinum, chromium and gold, are options as well. Carbon is considered to be an alternative when the X-ray emissions from iridium might interfere with elemental analysis. The layer itself must be thick enough to provide a continuous conductive film, but also not so thick that it obscures surface details of interest. Typical thicknesses are in the range of 1–10 nm based on the sample and application.

The Vacuum in an SEM

A vacuum is a body of space not occupied by matter. Creating a perfect vacuum is a near-impossible task, though SEM columns are close. Gaseous matter exerts a pressure on the vessel inside which it is contained, and therefore pressure is used as a measure of vacuum strength, where a perfect vacuum exerts no pressure whatsoever.

Physicists use the Pascal (Pa) as the SI unit of pressure, but microscopists frequently use torr, millimeters of mercury (mm Hg) and millibar as well.

Normal atmospheric air pressure at sea level is represented as 1 atmosphere (atm), which is approximately equivalent to 1 bar.

Normal air pressure = 1 bar = 1,000 mbar = 100, 000 Pa = 760 torr = 760 mm of Hg. The usual residual pressure of the vacuum in an electron microscope = 2.5×10^{-5} Pa.

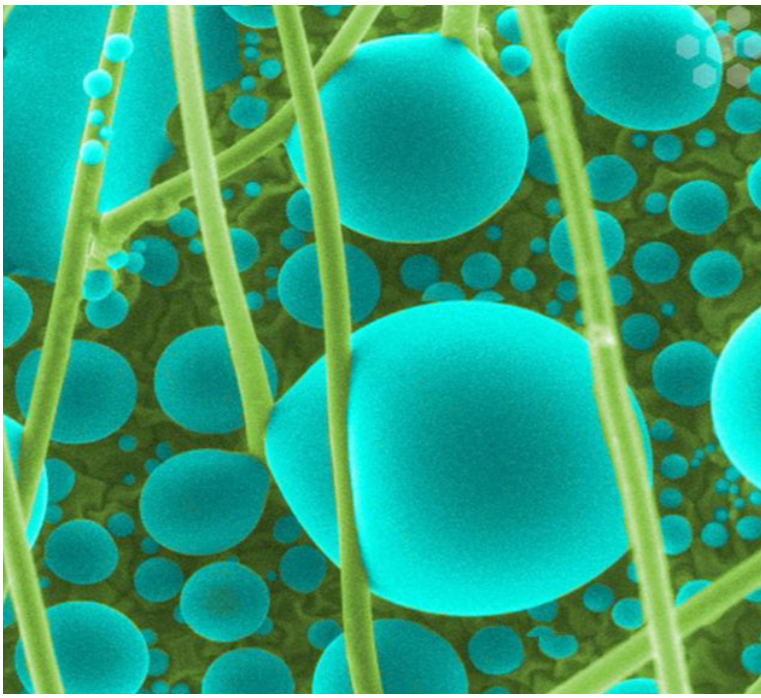
At this pressure, the number of gas molecules per liter is around 7×10^{12}, and the chance of an electron striking a gas molecule while traversing the column is almost zero.

Sometimes, it is extremely important to prevent any alteration of the sample during preparation, as is the case for, forensic specimens or silicon wafers examined during the integrated circuit (IC) manufacturing process as well as the ICs themselves, which will have to be studied while in operation. In such cases, special techniques, such as low-voltage SEM, are employed in order to prevent charging without the use of conductive coatings. Cryo preparations are also used in SEM, mainly in biological applications or with organic materials (polymers).

Specimen orientation and manipulation

The quality of the image in an SEM relies on the orientation and distance of the specimen from the final lens and the detectors.The specimen stage allows it to be shifted in a horizontal plane (X and Y directions), up and down (Z direction), rotated and tilted as needed. These movements are usually motorized and controlled by a computer using a mouse or joystick.

The different SEM models in a range differ in the size of their specimen chambers, allowing samples of different sizes to be introduced and manipulated. The larger the specimen chamber, the larger the stage mechanism needed to move and manipulate the sample and the larger the pumping system needed to attain and maintain a good vacuum. The simplest models accept specimens of a few centimeters in diameter and can move them 50 mm in the X and Y directions. Bigger models can accommodate samples up to 300 mm in diameter. Most models also allow samples to be tilted to high angles and rotated through 360 degrees.



Water droplets on the hydrophobic top side a leaf (SEM).

Courtesy of Dr. Jim Buckman.
Original data collected at a horizontal field width of ~400 µm.

There are special stages or attachments for cooling, heating and straining specimens, but because of the wide range of possible sample sizes, these stages are frequently produced by specialized firms.

If the specimen in an SEM is thin enough to transmit electrons, a detector located below the specimen may be used for collecting these electrons, offering STEM capabilities similar to those described earlier for TEM. The lower accelerating voltages and lack of post-specimen lenses limit the ultimate resolution and flexibility of SEM-based STEM. However, it can be a powerful technique, extending the contrast capabilities and resolution seen in SEM imaging of bulk samples. It can also enhance the spatial resolution of X-ray microanalysis by decreasing the large volume of interaction from which X-rays can originate in bulk specimens.

CHAPTER 6

DualBeam – combining SEM with FIB

An overview of DualBeam technology

Thus far, the focus has been on electron microscopy and the beneficial information that can be obtained using an electron beam. However, electrons are not the only charged particles that can be fast-tracked and focused using electric and magnetic fields. Usually, an atom is neutral since there are an equal number of electrons and protons. However, an atom that has lost one or more of its outermost electrons has a positive charge and can be accelerated, deflected and focused in the same way as a negatively charged particle (electron).

The most vital difference lies in the mass of the ions. The lightest ion has nearly 2,000 times the mass of an electron, and heavier ions can be another 250 times as massive.

In an SEM, the comparatively low-mass electrons interact with a sample non-destructively to produce secondary electrons which, when collected, provide superior quality image resolution, down to the sub-nanometer range. A focused ion beam (FIB) instrument is virtually identical to an SEM but uses a beam of ions instead of electrons. The higher-mass ions dislodge neutral and charged particles (molecules, atoms and multimolecular particles) from the sample surface in a process known as sputtering. Ionized specimen atoms and

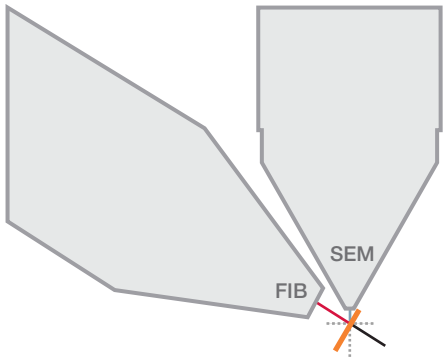


Diagram illustrating the DualBeam system, a combined application of a focused ion beam (FIB) and SEM on a sample (orange).

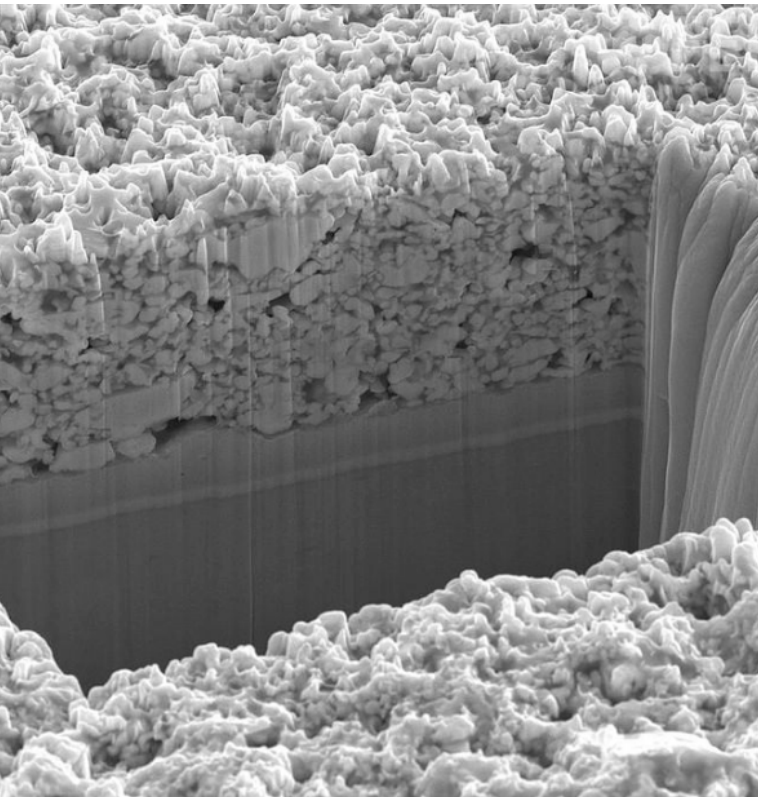
molecules are known as secondary ions, which can be used for imaging and compositional analysis. Ion bombardment also forms secondary electrons that can be used for imaging, just as they are in an SEM.

The ion beam directly alters (mills) the surface (via the sputtering process), and this milling can be regulated with nanometer precision. By carefully regulating the energy and intensity of the ion beam, it is possible to perform highly precise nano-machining to create minute components or to eliminate undesirable material.

Futhermore, ion beam-assisted chemical vapor deposition can be used to deposit material with a level of precision similar to FIB milling. A small quantity of a precisely selected precursor gas is injected into the beam vicinity, where it is

decomposed by the beam, depositing the non-volatile products on the specimen surface, while the volatile products are removed by the vacuum system.

Other reactive gases can be used with the ion beam, which, based on the particular gas and substrate, can enhance the milling rate, increase the milling selectivity for specific materials, or subdue the redeposition of milled material. A FIB becomes even more robust when it is joined with an SEM, as in a Thermo Scientific™ DualBeam™ system.



Cross-section made by FIB milling of a multilayer photovoltaic panel.

Courtesy of Dr. Maria Carbajo, Universidad de Extremadura. Original data collected at a horizontal field width of 29.8 µm.

In a DualBeam instrument, the electron and ion beams intersect at a 52° angle at a coincident point near the sample surface, allowing instant, high-resolution SEM imaging of the FIB-milled surface. Such systems integrate the benefits of both the SEM and FIB and provide complementary imaging and beam chemistry capabilities.

Ion column

FIB columns must deliver a beam of energetic ions for use in all three application categories: imaging, analysis and sample modification. High-resolution imaging demands small spot sizes with low currents. Analysis requires higher currents to produce sufficient signal for precise measurement.

Sample modification requires a variety of beam currents, from the very lowest for precise spatial control to the very highest for high material removal rates. Low-energy final polishing, to eliminate the amorphous and/or ion-implanted damage layer left by high-energy milling, is also a vital capacity. Over the whole variety of applications, higher beam current to spot size ratios generally enhance system performance.

Ion source

Most FIBs use a liquid metal ion source (LMIS) to provide charged ions for the beam. Other types of sources may be used in special applications, such as those necessitating very high beam currents for rapid milling. The LMIS has a sharply pointed tungsten needle coated with a liquid metal. Gallium provides the ideal combination of large atomic number, low vapor pressure and ease of use.

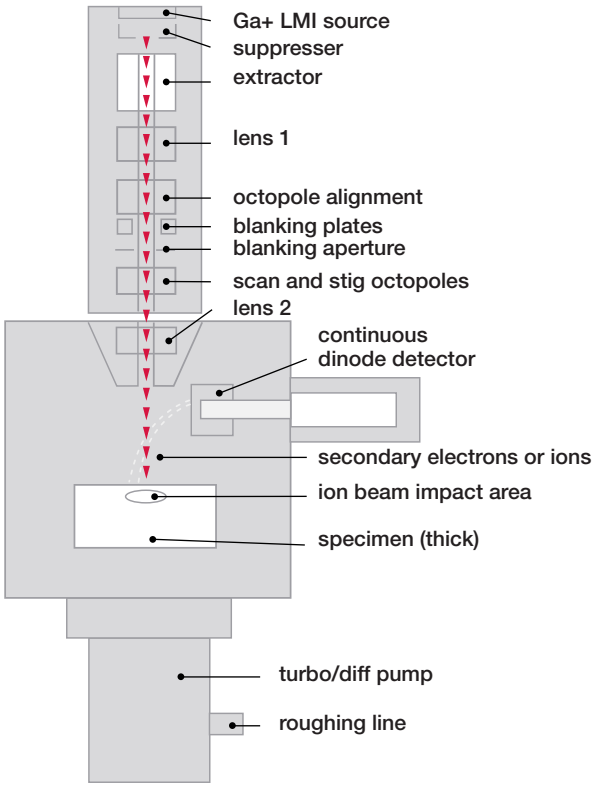
A wire, welded to the needle, holds the needle in position and heats it to burn off contamination. A coiled wire below the needle holds a reservoir of gallium to refill the coating. The needle points in the direction of an aperture in a negatively biased extraction electrode. The field formed by the extraction electrodes accelerates ions from the needle tip through the aperture. The extraction field is extremely strong at the sharply pointed needle tip.

In this field, the liquid gallium coating flows into an even more sharply pointed cone. A balance between electrostatic and surface tension forces establishes the shape of this point, known as a Taylor cone. If the apex of the cone were to become flawlessly sharp, the extraction field would be extremely strong.

The ion density is subsequently very high near the tip, and the ions exert significant Coulomb forces on each other. As they hurry away from the tip in the extraction field, they spread out, and their coulombic interactions reduce. This process eliminates gallium from the tip and decreases its sharpness. Thus, a balance exists at the tip of the cone between the elimination of gallium, through ionization, and the replenishment of gallium, through fluid flow into the tip region.

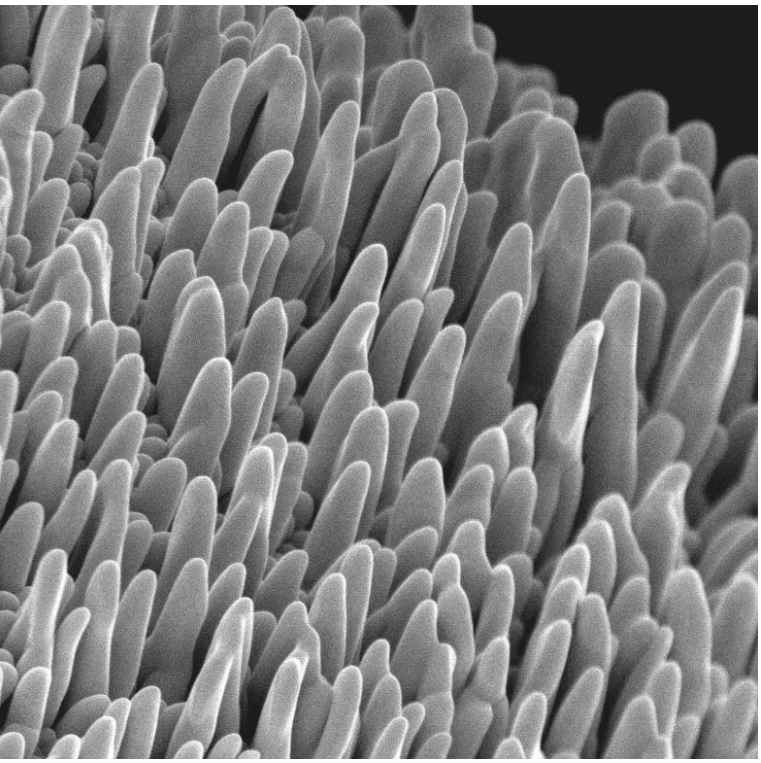
These forces essentially create a protrusion, or jet, at the tip of the cone. The jet is extremely small, having a radius of possibly five nanometers. The ion trajectories out of the jet typically lie within twenty to thirty degrees of the needle axis. Even with a low total emitted current, around one microampere, the small source size and narrow emission angle give the LMIS a brightness of over a million amperes per square centimeter per steradian.

FIB



Components of a FIB microscope.

When the FIB column is improved for image resolution (i.e., low beam current, small spot size and small apertures), the spherical aberrations of the column lenses are significantly reduced and system performance is restricted by certain features of the source, namely, its apparent size and energy distribution. Though the radius of the ion jet is just a few nanometers, its apparent size (i.e., the radius of the region from which the ions seem to originate when their trajectories are plotted backward through the optical system) is larger by a factor of ten, about 50 nm.



Platinum nano-features fabricated by FIB microscopy and imaged using SEM.

Courtesy of Guillaume Audoit.
Original data collected at a horizontal field width of 5 µm.

This apparent source is the signal that the optical system must demagnify onto the sample surface. The enlargement of the apparent source is mostly because of perturbations in particle trajectories caused by coulombic interactions between ions.

These same interactions cause an increase in the energy spread of the ions, which results in increased chromatic aberration in the optical system. Preferably, the beam should have a Gaussian intensity profile. In practice, beam tails extend many times the full-width-half-maximum diameter of the beam. These tails can be credited to the transverse energy spread resulting from coulombic interactions.

Therefore, energy distribution, apparent source size and beam shape are all affected unfavorably by space charge effects in high current density beams. Anything that increases the current density near the emitter tip increases the space charge effects and degrades performance. Therefore, LMISes are always used at the lowest possible total emitted current.

Minimization of space charge effects, through a cautious balance of electrode geometry and field strength, is the main concern in the design of advanced high-intensity LMIS. A higher extraction field and larger extractor-electrode spacing decrease the time the ions spend in the high interaction zone of the ion jet while still keeping a low level of total emission current.

From a practical point of view, the gallium supply of the LMIS is consumed during use, and so the source must be changed periodically. Similarly, the various beam-limiting apertures in the column will be eroded by the ion beam and, therefore, also need periodic replacement. Source lifetime and ease of replacement are key considerations. Lifetime relies on the size of the liquid metal reservoir; however, larger reservoirs increase the total size of the source, making the source harder to integrate into the column design as an easily replaceable module.

Current ion sources have lifetimes in excess of 1,000 hours and exchange times, including system pump-down, of less than four hours. Removable source-end structures that simplify source replacement have also been developed.

CHAPTER 7

Glossary of common electron microscopy language

A

Aberration

The deviation from perfect imaging in an optical system, caused by imperfections in the lens or by non-uniformity of the electron beam.

Accelerating voltage

The potential difference in an electron gun between cathode and anode over which electrons are accelerated. The higher the voltage, the faster the electrons (or ions) and the more penetrating power they have. Voltages may range from a few hundred volts up to several hundred thousand.

Airlock

A chamber within the electron microscope that can be isolated from the rest of the instrument to allow the specimen to be inserted. The airlock is then pumped out, and the specimen moved into the chamber (SEM, FIB) or column (TEM) vacuum. This reduces the amount of air and other contaminants brought into the column and speeds sample exchange.

Amplitude

The maximum value of a periodically varying parameter, as in the height of a wave crest above the mean value.

Amplitude contrast

Image contrast caused by the removal of electrons (or light) from the beam by interactions with the specimen.

Ångström

Unit of length, 1 Å = 0.1 nm = 10^{-10} m.

Anode

In an electron gun, the negatively charged electrons are accelerated towards the anode, which has a positive charge relative to the filament (cathode) from which the electrons emerge. In practice (for ease of construction), the filament has a high negative charge and the anode is at ground potential.

Aperture

A small hole in a metal disc used to stop those electrons that are not required for image formation (e.g., scattered electrons).

Astigmatism

A lens aberration in which the power of the lens is greatest in one direction and least in the perpendicular direction. It causes a round feature in the object to assume an elliptical shape in the image.

Atom

The smallest unit of physical matter that retains its elemental identity. There are many ways of looking at the atom. The most useful one for electron microscopists is to think of it as consisting of a positively charged nucleus (containing positively charged protons and uncharged neutrons) surrounded by negatively charged electrons in discrete orbits.

Atomic number

The number of protons in the atomic nucleus. This number determines the chemical nature of the atom. An atom of iron, for example, has 26 protons, an atom of oxygen 8, and so on.

B

Backscattered electrons

Primary (beam) electrons that have been deflected by the specimen through an angle generally greater than 90° so that they exit the sample with little or no loss of energy.

Binocular magnifier

A light microscope built into a TEM for viewing a fine-grain fluorescent screen for critical focusing and astigmatism correction.

C

Cathodoluminescence

The emission of light photons by a material under electron bombardment.

Chromatic aberration

See aberration. The power of the lens varies with the wavelength of the electrons in the beam.

Column

The physical structure of an electron microscope that accommodates the evacuated electron beam path, the electromagnetic lenses, the aperture mechanisms and the specimen in TEM.

Condenser lens

Part of the illumination system between the gun and the specimen designed to form the electron beam, usually into a parallel configuration as it transits the sample (TEM) or enters the objective lens (SEM). It may also be used to form a finely focused spot on the specimen (STEM).

Crystal

A material in which the atoms are ordered into rows and columns (a lattice). This periodicity causes electrons, whose wavelength is about the same size as the spacing between atoms, to undergo diffraction.

D

Detector

A device for detecting particular electrons or photons in the electron microscope.

Diffraction

Deviation of the direction of light or other wave motion when the wave front passes the edge of an obstacle.

Diffraction contrast

Constructive interference of waves caused by interaction with a periodic structure.

E

EDX

Energy dispersive X-ray analysis or spectrometry (sometimes called EDS). An EDX spectrometer makes a spectrum of X-rays emitted by the specimen on the basis of their energy.

EELS

Electron energy loss spectroscopy (or spectrometry) analyzes transmitted electrons on the basis of energy lost to interactions with sample atoms. Energy loss provides information about the sample atoms' elemental identity, chemical bonding and electronic states.

Electron

Fundamental sub-atomic particle carrying a negative charge and conventionally described as orbiting the nucleus of the atom. Free electrons can easily flow in a conductor and can be extracted into a vacuum by an electric field.

Electron microscope

A microscope in which a beam of electrons is used to form a magnified image of the specimen.

Electrostatic lens

Device used to focus charged particles into a beam. Although it may also be used with electrons, it is most frequently used with ions in a FIB column. The much greater mass of ions requires the stronger optical power available from an electrostatic lens. Lighter electrons can be effectively focused by a weaker magnetic lens.

ESEM

Environmental scanning electron microscope—a scanning electron microscope that can accommodate a wide range of pressures in the sample chamber, up to that required to sustain water in its liquid phase.

ETEM

Environmental transmission electron microscope—a transmission electron microscope that can accommodate a wider range of environmental conditions and apparatuses in the sample space to enable *in situ* examination of materials and processes.

Excitation

The input of energy to matter, which can lead to the emission of radiation.

Excited atom

An atom that has a vacancy in one of its inner electron orbitals (see also, ion) and, therefore, has higher energy. It returns to its ground state when an electron from an outer orbital drops down to fill the vacancy, emitting the excess energy as radiation (typically an X-ray). The energy difference between orbitals and, thus, the energy of the X-ray, is characteristic of the emitting atom's elemental identity.

F

FEG

Field emission gun. An electron source in which electrons are extracted from a sharply pointed tungsten tip by a very strong electric field.

FIB

Focused ion beam. Similar to an SEM but uses an ion beam instead of an electron beam. DualBeam instruments combine FIB and SEM.

Filament

Metal wire, usually in the form of a hairpin, which, when heated in vacuum, releases free electrons. This provides a source of electrons for an electron microscope.

Fluorescent screen

Large plate coated with a material (phosphor) that gives off light (fluoresces) when bombarded by electrons. A TEM may project its electron image onto a fluorescent screen to make it visible in real time.

Focal length of a lens

The distance (measured from the center of the lens in the direction of the beam) at which a parallel incident beam is brought to a focus.

Focusing

The act of making the image as sharp as possible by adjusting the power of the objective lens.

G

Goniometer

Specimen stage allowing linear movement of the specimen in two or more directions and rotation of the specimen in its own plane and tilting about one or more axes that remain fixed with respect to the beam.

Ground state

The lowest energy state of an atom.

I

Ion

An atom or molecule that has lost or gained an electron and, therefore, has a net positive or negative electric charge.

Ion getter pump

Vacuum pump that uses electric and magnetic fields to ionize and trap residual gas molecules by embedding them in the cathode of the pump.

L

Lattice

Regular three-dimensional array of atoms in a crystal.

Lens

In a light microscope, a piece of transparent material with one or more curved surfaces that is used to focus light. In an electron microscope, a similar effect is achieved on a beam of electrons by using a magnetic (or electrostatic) field.

LMIS

Liquid metal ion source. An ion source in which ions are extracted by a strong electric field from a layer of liquid metal Ga^{+} coating a sharply pointed electrode.

M

Micrometer

Unit of length (distance). One micrometer (μm) is a millionth of a meter (10^{-6} m) or 1,000 nm.

Microtome

Instrument for cutting extremely thin sections from a specimen prior to examination in the microscope. In electron microscopy, this is usually referred to as an ultramicrotome.

N

Nanometer

Unit of length (distance). One nanometer (nm) is a billionth of a meter (10^{-9} meter).

O

Oil diffusion pump

Vacuum pump in which the pumping action is produced by the dragging action of a stream of oil vapor though an orifice.

Objective lens

In a TEM, this is the first lens after the specimen. Its function is to focus transmitted electrons into an image. In an SEM, it is the last lens before the specimen, and it produces the extremely fine electron spot with which the specimen is scanned. Its quality largely determines the performance of the microscope.

P

Phase

Relative position in a cyclical or wave motion. It is expressed as an angle, with one cycle or one wavelength corresponding to 360°.

Phase contrast

Image contrast caused by the interference among transmitted electrons with phase shifts caused by interaction with the sample.

Phase diagram

Graph of temperature and pressure showing the range of each under which a given material can exist in the solid, liquid or vapor phase.

Photomultiplier

Electronic tube in which light is amplified to produce an electrical signal with very low noise.

Photons

Discrete packets of electromagnetic radiation. A light beam is made up of a stream of photons.

Primary electrons

Electrons in the beam generated by the microscope.

Q

Quantum

A discrete packet of energy, as a photon of light.

R

Raster

The track of the beam in an SEM or STEM. It is analogous to eye movements when reading a book: left to right, word by word, and down the page line by line.

Refraction

Changes in direction of a beam of light (or electrons) as the beam passes through regions in which its propagation speed changes.

Refractive index

The ratio of the speed of light in a vacuum to that in a given medium such as glass, water or oil.

Resolving power

The ability to make points or lines that are closely adjacent in an object distinguishable in an image.

Resolution

A measure of resolving power.

S

Scanning

Process of investigating a specimen by moving a finely focused probe (electron beam) in a raster pattern over the surface.

Scintillation detector

Electron detector used in SEM or STEM in which electrons are accelerated towards a phosphor, which fluoresces to produce light, which is amplified by means of a photomultiplier to produce an electrical signal.

Secondary electrons

Electrons scattered from sample atoms by interactions with beam (primary) electrons.

SEM

Scanning electron microscope or scanning electron microscopy.

Semiconductor detector

Electron detector used in SEM or STEM in which a high-energy electron is detected by the current it generates as it dissipates its energy in a solid state diode.

Spectrometer

Instrument for obtaining a spectrum.

Spectrum

A display produced by the separation of a complex radiation into its component intensity as a function of energy or wavelength.

Spherical aberration

See aberration. The power of a lens varies with radial distance from its center.

Sputter coater

Instrument for coating a non-conducting specimen with a very thin uniform layer of a conducting element such as gold or iridium to eliminate artifacts caused by accumulating charge.

STEM

Scanning transmission electron microscope or scanning transmission electron microscopy.

Steradian

Standard unit for solid angles, also called square radian. On the surface of a sphere with radius r, 1 steradian results in a region with area = radius squared.

T

TEM

Transmission electron microscope or transmission electron microscopy.

Turbomolecular pump

Vacuum pump in which the molecules are moved against the pressure gradient by collisions with rapidly rotating, angled vanes.

V

Vacuum

A region of reduced (lower than ambient) gas pressure.

W

Wavelength

The distance on a periodic wave between two successive points at which the phase is the same; for example, two crests.

WDX

Wavelength dispersive X-ray analysis or spectrometry. An alternative to energy dispersive spectrometry for X-ray analysis. In WDX, X-rays are dispersed into a spectrum by diffraction from a crystal or grating. The crystal is mechanically scanned through a range of angles while a detector measures changes in signal intensity.

Wehnelt cylinder

An electrode between the cathode (filament) and the anode (ground) in a triode electron gun. Used to form the beam and control its current.

Working distance

In an SEM, the physical distance between the external metal parts of the objective lens and the specimen surface. This is the space available for placing certain electron, X-ray and cathodoluminescence detectors. For highest resolution, the working distance has to be made as small as possible, which leads to compromises.

X

X-rays

Electromagnetic radiation with wavelengths much shorter than visible light, ranging from 10 to 0.01 nm. In the electron microscope, characteristic X-rays are used to analyze elemental composition with high spatial resolution.