Scanning Electron Microscopy
Welcome

MyScope was developed by Microscopy Australia to provide an online learning environment for those who want to learn about microscopy. The platform provides insights into the fundamental science behind different microscopes, explores what can and cannot be measured by different systems and provides a realistic operating experience on high end microscopes.

We sincerely hope you find our website: www.myscope.training an enjoyable environment. In there you can explore the microscopy space and leave ready to undertake your own exciting experiments.

Train for advanced research
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A Scanning Electron Microscope (SEM) uses a focussed beam of electrons to create a magnified image of a sample. The electron beam is scanned in a regular pattern across the surface of the sample and the electrons that come out of the sample are used to create the image.

Essentially, the way the scanning electron microscope "looks" at the surface of a sample can be compared to a person alone in a dark room using a fine beamed torch to scan for objects on a wall. By scanning the torch systematically side-to-side and gradually moving down the wall, the person can build up an image of the objects in their memory. The SEM uses an electron beam instead of a torch, an electron detector instead of eyes, and a viewing screen and camera as memory.
Electrons are negatively charged particles within the atom. In a light microscope light photons are focussed by glass lenses. In an electron microscope electromagnets are used to focus the electrons. The interaction of the electron beam with the surface of the sample affects the images that we achieve.

The SEM is a tool for creating images of the otherwise invisible worlds of microspace ($1$ micrometre = $10^{-6}$ m) and nanospace ($1$ nanometre = $10^{-9}$ m). SEMs can magnify an object from about $10$ times up to $300,000$ times. A scale bar is usually provided on an SEM image. The scale bar is used to calculate the sizes of features in the image. Microscopists still often use the term ‘micron’. A micron is the old (non SI) word for a micrometre.

SEM images have no colour (but may be artificially coloured), they may look quite three dimensional (due to depth of field) and they show only the surface of the sample (due to minimal penetration of the electron beam into the sample).

Detectors on SEMs can routinely capture two different types of SEM image: a secondary electron image or a backscattered electron image. The shades of grey in a secondary electron image are created by the topography of the sample. The shades of grey in a backscattered electron image stem from the atomic weight of the constituent elements in the sample and can be used to visualise this information. Detectors on SEMs can routinely capture two different types of SEM image: a secondary electron image or a backscattered electron image. The shades of grey in a secondary electron image are created by the topography of the sample. The shades of grey in a backscattered electron image stem from the atomic weight of the constituent elements in the sample and can be used to visualise this information.
Applications and uses of SEM

Scanning electron microscopy is a technique that is widely used in science and engineering. Some of the most common applications are in materials science, biological science, geology, medical science and forensic science. The technique may also be applied to create digital art works.

Scanning electron microscopy is a remarkably versatile technique. There are many different types of SEM available. With the right SEM one can:
- Image morphology of samples (e.g. view bulk material, coatings, sectioned material).
- Image compositional and some bonding differences through contrast and by using backscattered electrons.
- Image molecular probes by using metal and fluorescent probes in biological samples.
- Undertake micro and nano-lithography (remove material from samples; cut pieces out or remove progressive slices from samples [e.g. using a focussed ion beam]).
- Heat or cool samples while viewing them (this requires a specific type of stage).
- Wet and dry samples while viewing them (only in an Environmental SEM)
- View frozen material using a cryostage
- Analyse X-rays from samples for microanalysis (requires a EDS or WDS detector)
- Study optoelectronic behaviour of semiconductors (requires a cathodoluminescence [CL] detector)
- View/map grain orientation/crystallographic orientation and study related information like heterogeneity and microstrain in flat samples (requires an EBSD detector).

Materials science:

SEM is a key tool used for basic research, quality control and failure analysis. It is a technique suitable for examining metals, alloys, ceramics, polymers and biological materials. SEM plays a key role in many topics of current interest including nanotubes and nanofibres, high temperature superconductors, mesoporous architectures, alloy strength and so on. Many aspects of high technology development - aerospace, electronics, energy, catalysis, environmental, photonics, chemistry - would be impossible without the data provided by SEM.

The image below is of a silicon wafer with copper track and particles on its surface.

Silicon wafer with copper track and particles on its surface.
Biological science:
In biological science, large objects such as insects and animal tissues through to small objects such as bacteria are studied by SEM. SEM can be used in entomology, archaeology, plant science, cell research, and taxonomy among other topics. The first image is of a tick. The second image is Bilbergia pollen.

Geology:
SEM is commonplace in the investigation of soils and geological samples. Analysis of morphology can inform about weathering processes. Compositional differences can be seen with backscattered electron imaging. Microanalysis can provide details on the specific elemental composition of a sample. Because of this the SEM is a very useful tool in the mining industry. The image below is of a mineral sample. The bright areas indicate the presence of high atomic number elements. The dark areas indicate the presence of low atomic number elements.
Medical science:
The SEM can be used by medical researchers to compare blood cells and tissue samples to determine the cause of illness. Some other uses of the SEM include the study of medicine and its effect on patients as well as for researching and developing new treatments. SEM is also extensively used in research and development of medical implants and tools.
Forensic science:
In forensic science police laboratories use SEM to examine and compare evidence, such as metal fragments, paint, inks, hair and fibres to provide evidence of a person's guilt or innocence. Through careful examination detectives are able to determine if samples collected from a crime scene have properties that match the scenario developed by detectives. The image below shows human hair and nylon fibres.
Digital art:
Images taken from the SEM are often very beautiful in their own right but they also may be modified as digital art and compelling marketing imagery.
What can it do differently to a light microscope?

The scanning electron microscope (SEM) provides the competent user with an advantage over the light microscope (LM) in three key areas:

**Resolution at high-magnification:** Resolution can be defined as the least distance between two closely opposed points, at which they may be recognised as two separate entities. The best resolution possible in a LM is about 200 nm whereas a typical SEM has a resolution of better than 10 nm (typically 5 nm).

**Depth of field:** This is the height of a specimen that appears in focus in an image - SEM has more than 300 times the depth of field compared to the LM. This means that great topographical detail can be obtained. For many users, the three dimensional (3D) appearance of the specimen image, is the most valuable feature of the SEM. This is because such images, even at low magnifications, can provide much more information about a specimen than is available using the LM.

**Microanalysis:** The analysis of sample composition including information about chemical composition, as well as crystallographic, magnetic and electrical characteristics.

The image below is of a bee's head showing an eye and an antenna. Note that all of the antenna is in focus in the SEM image (right).

*Two images side to side, from Optical and SEM microscopes, same sample.*
What can’t it do?

Image wet samples
The electron beam requires a vacuum. Samples that are not dry may be damaged by the vacuum as the liquid is pulled out of the sample. This can also cause damage to the microscope. In most cases, samples that are wet naturally have to be dried before they can be examined in an SEM. Generally, SEMs are not used for experiments involving liquids, chemical reactions, and air-gas systems. However, some specialised machines and sample chambers do allow for these experiments.

Image non-conductive samples
If the sample is not electrically conductive, images will not be formed due to the interaction of the negatively charged electron beam with the sample (the sample will become negatively charged as the incident electrons reach it and the beam is then repelled by the sample). Most samples that are inherently non-conductive need to be coated with a thin layer of metal or carbon to make them conductive before they can be imaged in an SEM.

Colour images
SEM images are monochromatic (greyscale), not in colour, because electron wavelengths are much smaller than those of visible light. Any coloured images you see from an SEM have been coloured using post-processing techniques. Secondary electron SEM images — the most common form of SEM image — are effectively intensity maps of electrons collected by a detector. The intensity of brightness on the screen is proportional to the number of electrons originally produced. SEM images are displayed as monochrome greyscale digital images in which each pixel carries only intensity information in a shade of grey varying from black at the weakest intensity to white at the strongest.

Accurate height measurement
SEM is not good for quantifying surface roughness at small scale. Atomic force microscopy (scanning probe microscopy) is more useful for this task. Measurements involving height (z-axis) cannot be taken directly in an SEM. This requires two images that have been tilted relative to one another to create a 3D image, and specialised processing software.

Sub-surface imaging
The SEM cannot image below the surface of the sample due to the small interaction volume between the electron beam and the sample. To examine sub-surface structures a cross section of the sample must be cut. There are a variety of methods for doing this (including by using a focussed ion beam – FIB).

Imaging through fluid
The SEM cannot image through water or other fluids. Note: An ESEM using a wet Scanning Transmission Electron Microscope (STEM) detector can be used to image through thin water films. Generally, SEMs are not used for experiments involving liquids, chemical reactions, and air-gas systems although some specialised machines and sample chambers do allow for these experiments.

Atomic imaging
The resolution of the SEM is not high enough to image individual atoms (use a transmission electron microscope).

Elemental analysis below micrometre scale
Elemental analysis of areas less than 1 micrometre can be very difficult in an SEM. This is due to the interaction volume between the electron beam and the sample which is often in the micrometre range. The interaction volume can be reduced by reducing the electron beam accelerating voltage. However, the corresponding reduction in signal can make it difficult to acquire useful data.

Image charged molecules
The SEM cannot reliably image charged molecules that are mobile in a matrix. For example, some species (e.g. Na+) are volatile under the electron beam because the negative electron beam exerts a force on charged material.
How does an SEM work? - Structure of an SEM

Electron microscopes are in many ways analogous to light microscopes. This is surprising at first glance, given the contrast between the simple technology of the light microscope and the complex electronics, vacuum equipment, voltage supplies and electron optics system of electron microscopes. In both cases there is a source of illumination (light bulb vs electron source), a condenser lens (glass vs electromagnetic), a specimen and a detector (the eye vs an electron detector). A comparison of these features is used frequently as a starting point for any discussion of SEM.

The SEM uses a beam of high energy electrons generated by an electron gun, processed by magnetic lenses, focused at the specimen surface and systematically scanned (rastered) across the surface of a specimen. Unlike the light in a light microscope, the electrons in an SEM never form a real image of the sample.

The SEM image is a result of the beam illuminating the sample one point at a time in a rectangular scanning pattern (raster), with the strength of the signal generated from each point being a reflection of differences (e.g. topographical or compositional) in the sample. The viewing screen is scanned in synchrony with the beam on the specimen in a one-to-one relationship between points on the specimen and points on the image viewing screen (a point-by-point translation). Increased magnification is produced by decreasing the size of the area scanned on the specimen.
In order to produce contrast in the image the signal intensity from the beam-specimen interaction must be measured from point-to-point across the specimen surface. Signals generated from the specimen are collected by an electron detector, converted to photons via a scintillator, amplified in a photomultiplier, and converted to electrical signals and used to modulate the intensity of the image on the viewing screen.

The major components of an SEM are: the electron gun, the vacuum system, the water chilling system, the column, the specimen chamber, the detectors and the imaging system.
The electron gun - How the gun works

The electron gun refers to the top region of the SEM that generates a beam of electrons. The simplest and cheapest gun uses a heated tungsten wire to produce electrons. Other more expensive types use crystals (lanthanum hexaboride: LaB₆; or tungsten) and are either heated or a large electrical potential is used to pull the electrons out of them, cold. The electron gun produces a source of electrons (comprised of free electrons i.e. detached from the atom) and accelerates these electrons in an energy range typically 1-40kV. The conventional electron gun (triode) has three components, a hot wire (called the filament or cathode [- ve] or electron emitter), a Wehnelt (grid) cap [-ve], and an anode [+ ve]. In a thermal emission (thermionic) filament the tungsten filament is heated white hot by a filament current. This results in the emission of thermal electrons. The emitted electrons are those that have overcome the work function energy of the material. In the diagram the filament (also called the emitter) is surrounded by the Wehnelt cylinder that closes over the filament assembly and has a small hole in the centre through which electrons exit. The electrode pins run to the filament through an insulator disc, and carry the current flow to the filament. The schematic (a-c) shows three filament types: a) a tungsten (W) wire, b) a lanthanum hexaboride crystal assembly: LaB₆, and c) a tungsten crystal (for field emission guns: FEGs). The tip of a tungsten wire hairpin filament is about 10µm in diameter whereas the tungsten crystal is sharpened to a much narrower tip.

Below the Wehnelt cap sits an anode, which is positively charged and attracts the electrons away from the filament. If the filament is broken, no electrons can be produced. The hole in the anode allows a fraction of the electrons to continue down the column through the lenses to produce a smaller, more cohesive beam. Electrons that strike the anode are returned to the high voltage power supply via ground. The portion of the beam that leaves the anode through the hole is termed the beam current.
Electron sources

Electron guns can be classified into two types: thermionic and field emission. The typical thermionic electron gun consists of three parts, the filament, Wehnelt cap and the anode. Thermoelectrons produced by the filament are accelerated through the application of a voltage between the filament and the anode, thus creating an electron beam that streams down the microscope column.

![Electron beam diagram](image)

The LaB₆ gun is made from a crystal of lanthanum hexaboride in a specialised housing. This material is a refractory ceramic material with a high melting point and is heated to generate electrons. It has the advantage of a longer usable life time than the thermionic W filament.

The field emission gun (FEG) uses a pointed single crystal W wire filament that is not heated by a filament current. Instead, electrons are pulled off the cold filament by a strong electrostatic field called an extraction voltage. FEGs provide significant advantages over thermionic filaments including a much smaller electron virtual source size, high current, high brightness, low energy spread and a long life. These advantages make the FEG SEM a high-resolution machine for high-magnification work. However, while the cold field emission gun provides the most coherent source for high-resolution secondary electron imaging, it is the least appropriate for energy dispersive X-ray analysis.

A Schottky field emission (hot field emission) gun has some advantages compared to cold field emitters. The major advantages are better beam current stability, less stringent vacuum requirements and the fact that there is no need for periodic emitter flashing (heating the cold filament for a short time each day) to restore the emission current.

Increasingly the majority of high-resolution FEG SEMs use Schottky emitters. One significant advantage of the Schottky emitter is the high beam current (>100nA achievable by most manufacturers nowadays) that can be achieved with little decrease in spatial resolution.
Comparison of lament types.

The lament load current of the thermionic gun has to be set correctly. Too low a current will yield an image of inadequate brightness, whereas excessive current will reduce the working life of the lament. Some samples are especially sensitive to the electron beam and may even melt. If this happens, the electron beam should be turned down and the specimen removed from the microscope.

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### A comparison of SEM filament types

<table>
<thead>
<tr>
<th>Emission (kind of gun)</th>
<th>Field Emission</th>
<th>Schottky</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>W</strong> (tungsten hairpin)</td>
<td><strong>LaB6</strong> (single/multicrystal)</td>
<td><strong>FE</strong> (tungsten)</td>
</tr>
<tr>
<td>Diameter of Electron Source (nm)</td>
<td>30,000</td>
<td>10,000</td>
</tr>
<tr>
<td>Brightness (A/cm².sr)</td>
<td>106</td>
<td>107</td>
</tr>
<tr>
<td>Energy spread (eV)</td>
<td>1.5 (~2)</td>
<td>0.5-3.0 (~1.5)</td>
</tr>
<tr>
<td>Operating Lifetime (hrs/months)</td>
<td>~50 hrs</td>
<td>~1000 hrs</td>
</tr>
<tr>
<td>Vacuum (torr)</td>
<td>10-4-10-5</td>
<td>10-6-10-7</td>
</tr>
<tr>
<td>Temperature of cathode (°C)</td>
<td>−2330</td>
<td>−1530</td>
</tr>
</tbody>
</table>

Comparison of filament types.

The filament load current of the thermionic gun has to be set correctly. Too low a current will yield an image of inadequate brightness, whereas excessive current will reduce the working life of the filament. Some samples are especially sensitive to the electron beam and may even melt. If this happens, the electron beam should be turned down and the specimen removed from the microscope.
Filament saturation

Two important parameters for any electron gun are the amount of current produced and the current stability. The beam is most stable at the saturation point. A constant beam current is required to create a good quality image because all image information is recorded as a function of time. The electron micrograph is a scanned image of intensity values, projected as a function of the position on the specimen. For high quality micrographs it is best to use a slow scan rate (which can be up to a few minutes). Any changes in the filament emission during the image acquisition will affect the image intensity at that point in the scan. This will produce a poor quality image because the brightness will vary across the image. A constant beam current is dependent on saturating the filament properly.

An important factor in using an SEM with a thermionic gun is to understand saturation of the filament. The more current that is put through the filament, the greater the emission of electrons. However a point is reached where the emission is at its maximum. This is called saturation. Putting more current through the filament after this point does not increase electron emission. It simply shortens the life of the filament, or may even break it prematurely. The relationship can be seen in a graph of filament current against electron emission (or brightness). In many SEMs, filament saturation is an important task for the user. There are several important considerations in regard to achieving filament saturation as a machine operator.

Thermionic filament saturation is set at a position just above the second peak i.e. at beginning of platform

The filament must not be turned up too quickly or else it will “blow” (burn out). A well-aligned gun will usually show a “false peak” or “knee” that is observed as the filament current is increased (see graph). This is a result of some part of the filament surface reaching emission temperature before the tip. As the filament current is increased the false peak collapses and a small, tight and more stable beam is ultimately achieved. To the user this false peak can be observed as an increase in the probe current (brightness) followed by a drop in emission and a further increase in probe current as the filament current is increased up to the saturation point.

With a badly aligned gun there is no false peak and only a single maximum emission peak is observed. As the filament current is increased beyond this peak (because the operator is looking for the next peak) the beam current (brightness) continues to drop, instead of rising. If not recognised, this can easily lead to the filament being blown by using too much filament current. In some SEMs, particularly those with expensive filaments (such as LaB6 and field emission guns), the saturation may occur automatically to avoid the possibility of a careless user turning them up too quickly.
The vacuum system - Vacuum system overview

Most SEMs use at least two types of vacuum pump to achieve the level of vacuum required to produce a consistent electron beam. The first pump in the sequence (often a rotary pump) is used for rough evacuation and the next level of pump (often a diffusion pump) achieves higher vacuums. A fail-safe circuit controls the vacuum sequence and maintains the appropriate vacuum in the optical column and specimen chamber. (Note: The second pump will not work until the first pump vacuum is adequate. Attempting to operate a diffusion pump without a good backing vacuum will lead to ‘backstreaming’ and oil contamination of the specimen chamber.) Pump-down times will be longer if a specimen is moist or degassing. It is important to dry the sample before introduction into the microscope, unless the microscope is specifically designed to operate with wet samples. To get a specimen into the microscope, vent the chamber (let air into the chamber), insert the sample onto the stage then evacuate the chamber (pump the air out of the chamber).

Types of pumps

Rotary pumps
The rotary pump is sometimes known as a roughing or backing pump - it is usually the first in a series of pumps used to achieve the vacuum levels required for effective operation of the SEM. The pump consists of a rotating vane housed in a cylindrical chamber in an oil bath. The rotation of the vane changes the pressure of the gas in the pump. Alternate high and low pressure sequences within a single rotation suck gas from the inlet of the pump and expel the gas from the pump's exhaust. (The gas coming out of the exhaust will contain molecules of oil, so these pumps should have a suitable filter installed).

Diffusion pumps
The diffusion pump consists of a cylinder containing a series of vanes. At the base of the cylinder, there is a heater. There are water cooling pipes running around the sides of the cylinder. Inside the cylinder there is fluid (diffusion pump oil). When the oil is heated, vapour rises in the cylinder and is directed by the vanes towards the walls of the cylinder. As this happens the vapour collects gas molecules in the pump. When the vapour hits the water-cooled walls of the cylinder, the vapour condenses and releases the gases that are then extracted by the backing pump (rotary pump).

Scroll pumps
A scroll pump uses two interleaving scrolls to compress gases. Usually, one of the scrolls is fixed and the other orbits eccentrically. This extracts the gases from the system. One advantage of this type of pump is that they are usually oil-free and relatively quiet. This reduces the chance of contamination of the microscope by pump oil.

Turbo-molecular pumps
The turbomolecular pump (TMP) can be used in place of a diffusion pump. It is made up of a series of pairs of rotor fan blades that are mounted close together. In each pair one rotor spins (turbine blade) and the other is stationary (stator blade). The spinning rotors draw gas molecules down through the pump to the extraction point (usually a backing pump).

Ion getter pumps
Ion getter pumps are used are used in cases where very high vacuum levels are required. This will be the last pump in a series of pumps as the vacuum has to be very high before the ion getter pump can be effective. The ion getter pump works by ionising the gases in the chamber. A strong electrical potential is applied and the ions are accelerated into and captured by a solid electrode. The ion getter pump contains no moving parts or oil so they are clean and produce no vibrations. This makes them ideal for high-resolution instruments.
Vacuum requirements

High vacuum mode is the normal mode of operation for the SEM. A high vacuum minimises scattering of the electron beam before it reaches the specimen. This is important as scattering or attenuation of the electron beam will increase the probe size and reduce resolution, especially in the SE mode. The high-vacuum condition also optimises collection efficiency, especially of the secondary electrons.

Many SEMs may also be operated in "low-vacuum mode". Since backscattered electrons and characteristic X-rays are generally of higher energy than secondary electrons their detection is not critically dependant on a high vacuum being maintained in the specimen chamber. Therefore, BSE and x-ray detectors can be used (but only semi-qualitatively) in low vacuum operational mode. In this mode, a small amount of air is leaked into the chamber, where it ionises and reduces surface charging of insulating samples.

Water chilling system

Many SEMs include a water chilling system. The purpose of the water chiller is to maintain a constant temperature of 20°C for the operation of the magnetic lenses in the microscope. If the chiller fails and the magnetic lenses heat up, the SEM will automatically shut down.
Structure of the column

The electron column focuses and illuminates the specimen using the electron beam generated by the electron gun. As the beam is scanned over the specimen in the X- and Y- directions, secondary and backscattered electrons are produced and detected. An image is produced by amplifying and modulating the brightness of the detected electron signals.

Electromagnetic lenses

A series of electromagnetic lenses and apertures are used to reduce the diameter of the electron source and to place a small, focused beam of electrons (or spot) onto the specimen. The lens system consists of a condenser lens, objective lens and scanning coils. The purpose of a lens in a light microscope is to change the path of the light in a desired direction. Glass or transparent plastic may bend light and so are used in optical lenses. Electrons cannot travel through glass or plastic lenses. Therefore, they are not appropriate for use in an electron microscope.

Magnetic lens

Electrons are charged particles so their pathway can be bent by a magnetic field. Lenses for electrons are constructed with ferromagnetic materials and wound copper wire. These produce a focal length that can be changed by varying the current through the coil. They are called electromagnetic lenses. The magnetic field bends electron paths in a similar way that solid glass lenses bend light rays. Under the influence of a magnetic field, electrons assume a helical path, spiralling down the column. This helical path can easily be demonstrated at low magnification by changing the focus up and down to cause image rotation.
There are two lens sets - the condenser lens and the objective lens. The electron beam travels through the condenser lens first. The condenser lens converges the cone of the electron beam to a spot below it, before the cone flares out and is converged again by the objective lens down onto the sample.

**The condenser lens**
The initial convergence can occur at different heights, that is, close to the lens, or further away. The closer it is to the lens, the smaller the spot diameter at the point of convergence. The further away, the larger the diameter of this point. So the condenser lens current controls this initial spot size and is referred to as the spot size control. The diameter of this initial convergence (also called a cross-over point) affects the final diameter of the spot the beam makes on the sample. The condenser lens controls the intensity of the electron beam reaching the specimen.
The objective lens
The main role of the objective lens is to focus the beam onto the sample. The objective lens also has some influence over the diameter of the spot size of the electron beam on the specimen surface. If the condenser lens is not aligned correctly, the objective lens cannot achieve the best results. A focused beam produces a smaller spot on the surface than an under- or over-focused beam. This means that the resolution will be higher.

Scan coils
The formation of an image requires a scanning system to construct the image point-by-point and line-by-line. The scanning system uses two pairs of electromagnetic deflection coils (scan coils) that scan the beam along a line then displace the line position to the next scan so that a rectangular raster is generated both on the specimen and on the viewing screen. The first pair of scan coils bends the beam off the optical axis of the microscope and the second pair bends the beam back onto the axis at the pivot point of the scan. The scanning coils deflect the electron beam horizontally and vertically over the specimen surface. This is also called rastering.
The specimen chamber

Stage
The specimen holder is fixed to the specimen stage. The stage can be moved manually along the X, Y (in the specimen plane), and Z directions (at right angles to the specimen plane). The Z adjustment is also known as the specimen height. Most specimen stages will also rotate and tilt the specimen.

The detectors
Once the electron beam reaches and interacts with the sample there are several different kinds of signal that can be detected. The most commonly used signals are secondary electrons, backscattered electrons, X-rays, Auger electrons and photons.

Secondary electron detector
Secondary electron imaging (SEI) is ideal for recording topographical information. Secondary electrons (SE) have low energies (~2 to 50 eV). They are ejected only from close to the surface of the sample. To attract (collect) these low-energy electrons, a small bias (usually around +200 to 300V) is applied at the front end of the detector to attract the negative electrons towards the detector. A common type of SE detector is the Everhart-Thornley detector.
Backscattered electron detector (BSD)
The backscattered electron detector (BSD) is mounted below the objective lens pole piece and centred around the optic axis. Backscattered electrons (BSE) are generated as the specimen surface is scanned by the incident electron beam. The yield is controlled by the topographical, physical and chemical characteristics of the sample. BSE have higher energies than secondary electrons and can give us information from below the surface of the sample.
X-ray detectors
When the electron beam interacts with the sample, X-rays are emitted. The energy of these X-rays is dependent on the elements present in the sample. The most common system for detecting the X-rays emitted from the sample is the energy dispersive X-ray spectrometry (EDS). The EDS detector is based on a semiconductor crystal. The two most common types are the lithium-drifted silicon (SiLi) and the silicon drift detector (SDD).

CL (cathodoluminescence) detectors
Cathodoluminescence (CL) is the emission of light when a material is stimulated by an electron beam. The light is emitted in wavelength ranges from ultraviolet to infrared depending on the composition and structure of the sample. The light is collected by an optical system, then sent to a monochromator where it is separated into the component wavelengths.
**Beam/specimen interactions**

**Electron-matter interactions**
When the electron beam hits a sample it interacts with the atoms in that sample. There are a number of outcomes. Some electrons bounce back out of the sample (backscattered electrons), others knock into atoms and displace electrons which in turn, come out of the sample (secondary electrons); alternatively X-rays, and light or heat (in the sample) can be the result of these interactions. Generally most of the energy is dispersed as heat. We collect the electrons coming out of the material in order to produce the traditional SEM images (called micrographs).

Secondary electrons emerge from the surface layers of the sample and generate images that reveal the surface features. Edges, peaks and fine structures like crests tend to be particularly bright as the secondary electrons can escape easily from these features on a sample. However, there are also a variety of other factors that can influence the brightness of features in the SE image.

The volumes involved in the production of secondary electron (SE), backscattered electron (BSE) and X-rays, form into a shape that ranges from a tear-drop to a semi-circle within the specimen. This shape is called an interaction volume and its depth and diameter depends on the kV as well as the density of the specimen. Approximately the top 15nm of the volume comprises the zone from which SE can be collected, the top 40% is the region from which BSE can be collected and X rays can be collected from the entire region.
Electron-matter interactions can be divided into two classes: elastic scattering and inelastic scattering.

**Elastic scattering**
The electron trajectory within the specimen changes but its kinetic energy and velocity remains essentially constant. The result is generation of backscattered electrons (BSE).

**Inelastic scattering**
This occurs when the incident electron trajectory is only slightly perturbed, but energy is lost through the transfer of energy to the specimen. The result is the generation of:

1. phonon excitation (heating);
2. cathodoluminescence (visible light fluorescence);
3. continuum radiation (bremsstrahlung);
4. characteristic X-ray radiation;
5. plasmon production (secondary electrons);
6. Auger electrons (ejection of outer shell electrons).

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Modelling interactions

There are computer programs available that allow the interaction volumes to be modelled so that the best parameters for a given experiment can be chosen. One of the best known is Casino (Monte Carlo Simulation of electroN trajectory in sOlids). This program can be used to simulate many of the recorded signals in an SEM (secondary electrons, backscattered electrons and X-rays).
The SEM image is effectively made up of lines of image points, each point being the size of the beam spot at the sample surface. The ability of the SEM to resolve fine structures is limited by the diameter of this spot size (probe size). It is also limited by the number of electrons contained within the probe. If the probe is too small in relation to the area being imaged, it spends too little time on each image point to provide sufficient signal to form a good quality image. There is a finite relationship between magnification and the optimum probe size and it does vary from specimen to specimen.

The size of the spot affects resolution
The beam scans across the sample but is actually made up of moments when it dwells on the sample. Each moment, or spot as we see here, generates signal that makes up the final image.

Each dwell time (seen as a spot in the image) generates electrons that are used to make up the image on the screen. We see edges and dips and bumps on a sample because of changes in the number of electrons coming off the sample at that point. As we go up in magnification, and drop our beam probe size down to a smaller and smaller spot, we see more detail (see line 2 in the diagram). But there is a limit. The limit of magnification is the point where no variation in signal (electrons generated from the sample) is obtained from adjacent points on the sample. This performance limit is dependent on the composition and structure of the specimen being examined. For example, specimens such as metals with a high atomic number (Z) produce a high yield of electrons and achieve a higher useful magnification than low Z samples (e.g. carbon and plastic).
Secondary electron (SE) images

For routine scanning electron microscope images, secondary electrons (SE) are usually used to image the surface. Secondary electrons are low energy electrons formed by inelastic scattering and have energy of less than 50eV. The low energy of these electrons allows them to be collected easily. This is achieved by placing a positively biased grill on the front of the SE detector, which is positioned opposite one side of the specimen. The positive grill attracts the negative electrons and they go through it into the detector. This is the case for the Everhart-Thornley detector, which is most commonly used but some machines have another kind of in-lens SE detector.

The major influence on SE signal generation is the shape (topography) of the specimen surface. Secondary electrons provide particularly good edge detail. Edges (and often pointy parts) look brighter than the rest of the image because they produce more electrons.

The image below shows protuberances (bumps) on the wing of an insect. Notice the whiter edge to each bump.
The contribution of BSE to images collected with the SE detector

The primary function of the SE detector is to attract low energy secondary electrons. These SEs are generated from approximately the top 15nm of the surface. Unless the SEM is specially set up to minimise the BSE contribution, the image produced by the detector will always contain an amount of sub-surface information derived from high energy BSE. As a general rule, the higher the kV the more sub-surface information is picked up by the detector due to various backscattered effects (elastic scattering effects).

At 2kV you will see a lot more surface detail than at 20kV, but this surface detail may be due to contamination. One important skill in operating an SEM is to choose the correct kV for your specimen such that you gather information from the depth of the specimen that interests you, with the least contribution from surface contamination above or unimportant structures below.
So far only the secondary electrons produced by interaction of the primary electron beam with the sample have been discussed. These are termed SE1 electrons. There are a number of different types of secondary electrons. Backscattered electrons can generate secondary electrons. These are termed “SE2”. Interaction of the beam with the sample chamber, pole piece etc. can also produce secondary electrons. These are termed “SE3”.

The following image is from the secondary electron detector and shows a silicon wafer surface at the same magnification but at different beam energies (kV): a = 5kV, b = 10kV, c = 15kV, d = 20kV. Note that the features in image d are much more rounded than in image a because there is more information coming from below the surface of the sample.
How do I get a good image - A basic guide to using an SEM

Not all SEMs use the same sequence of steps. The exact steps you use in operating an SEM will depend on the type of machine you are facing. Here are some general notes on what to expect.

Specimen preparation

This is vital to getting good quality information from your sample. A poorly mounted or incorrectly processed sample can lead to viewing artefacts. In simple terms, an SEM sample should be dry and electrically conductive to get the best results. This section is designed to help you choose what processes may be needed to prepare your sample. Although the information we present is fundamental, it is recommended that you consult recent published research papers in your area of research to check on current techniques being used. Note: for samples that need coating, consider whether compositional information is required (e.g. X-ray spectroscopy techniques) and use carbon coating if this is the case.
Specimen preparation

Biological samples
Biological samples usually have a high water content. If the sample is placed into the SEM vacuum without special preparation, it can be significantly damaged as the vacuum draws water out of the sample. This can also lead to contamination of the SEM chamber. For most samples, simple air-drying is not an appropriate method because of the changes to the sample that can be caused during the drying (think about the difference between a grape and a sultana). The steps involved in drying a biological sample usually include fixation, dehydration and drying. Once the sample is fully dried it can be mounted on an SEM stub and then coated with a thin layer of a conductive material (eg. gold).

Polymer samples
Polymer samples can be either wet or dry. If they are wet (or have significant water or liquid content) they need to be dried before they can be examined in the SEM. Polymer materials do not usually need to undergo fixation. However, any liquid within them must be removed. Once the sample is fully dried it can be mounted on an SEM stub and then coated with a thin layer of a conductive material (eg. gold).

Non-biological samples
Non-biological samples (eg. metals) are often inherently dry. However, there can be liquid within the sample and many samples require at least some drying. If the sample is inherently conductive it will not require coating.

Mounting the sample
SEM samples are attached to a support called a stub. This process is a very important part of preparing the sample. Because SEM is a surface imaging technique, the part of the sample that is of interest must be uppermost on the stub. There must be a continuous electrical connection between the stub and the sample so that the charge does not build up. This connection can be achieved using conductive tapes or glues in combination with a conductive coating.

Sample insertion
To use the SEM you must first place a sample in the sample chamber. Since the sample chamber is kept under vacuum, you must introduce air into the chamber in order to open it and place the sample inside on the stage. This is called venting the chamber. Some machines have a small specimen exchange chamber attached to the main chamber and this is the region vented. Note: the specimen chamber is kept under vacuum in order to keep it clean and maintain the quality of the vacuum. If the chamber is not kept under vacuum the pumping time increases and contamination can build up inside the chamber.

Evacuation
Once the sample is placed in the sample chamber, you must remove the air by pumping it out. This is called evacuation. When the chamber has reached a suitable vacuum (this will be different on different machines), you can turn on the electron beam.
# Accelerating Voltage

In theory, an increase in accelerating voltage will result in a higher signal and lower noise in the final image (micrograph). But the situation is not so simple. There are some disadvantages:

* Reduction in the resolution of structural details of the specimen surface in SE mode
* Increased electron build up in insulating samples, causing charging artefacts
* Increased heating and the possibility of specimen damage

With a higher accelerating voltage the electron beam penetration is greater and the interaction volume is larger. Therefore, the spatial resolution of micrographs created from those signals will be reduced. The number of backscattered electrons (BSEs) will increase. For secondary electron (SE) imaging at typical voltages (say 15 keV), BSEs can enter the secondary electron detector and degrade resolution because they come from deeper in the sample.

Accelerating voltage (kV or keV) is the voltage difference between the filament and the anode which accelerates the electron beam towards the anode. The accelerating voltage (kV or High Tension) of a typical SEM ranges from 0 to 30kV. In particular, the greater the kV, the greater the power of penetration by the beam into the sample.

A working guide to the selection of an appropriate accelerating voltage is provided in the table. Experimentation is always necessary to determine the optimum settings for any sample.

### A working guide to the selection of an appropriate accelerating voltage.

<table>
<thead>
<tr>
<th>keV range</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5kV</td>
<td>delicate or uncoated specimens</td>
</tr>
<tr>
<td>5-10kV</td>
<td>coated biological samples</td>
</tr>
<tr>
<td>10-30kV</td>
<td>physical science samples</td>
</tr>
</tbody>
</table>
An aperture is a minute hole in a strip of metal that is placed in the path of the electron beam in order to restrict or limit electron progress down the machine column. The aperture stops electrons that are off-axis or off-energy from progressing down the column. It can also narrow the beam below the aperture, depending on the size of the hole selected.

Objective lens (OL) aperture: This aperture is used to reduce or exclude extraneous (scattered) electrons. An optimal aperture diameter should be selected for obtaining high-resolution secondary electron images.

The objective aperture arm fits above the objective lens in the SEM. It is a metal rod that holds a thin plate of metal containing four holes. Over this fits a much thinner rectangle of metal with holes (apertures) of different sizes. By moving the arm in and out different sized holes can be put into the beam path.

In the image below the arm holds a thin metal strip with different sized holes that line up with the larger holes. The metal strip is called an Aperture strip.

A large aperture is chosen for low-magnification imaging to increase the signal, and for BSE and microanalysis work. A smaller aperture is chosen for high-resolution work and better depth of focus but has the disadvantage that fewer electrons reach the sample and therefore results in a less bright image.
The following table shows some examples of aperture size and purposes. Note: A numerical scale may be provided for different apertures. For example 1, 2, 3 and 4 may be used. This can run in either direction with the largest number for the largest aperture diameter or the largest number for the smallest aperture, depending on the instrument.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Aperture diameter (microns)</th>
<th>Probe current</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>30</td>
<td>Smallest</td>
<td>Ultrahigh resolution; Low probe current; Large depth of field</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td></td>
<td>Usual observation</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td></td>
<td>High resolution at high probe current; Reduced depth of field</td>
</tr>
<tr>
<td>1</td>
<td>110</td>
<td>Largest</td>
<td>Observation at high probe currents; Shallow depth of field</td>
</tr>
<tr>
<td>0</td>
<td>1,000</td>
<td>--------------</td>
<td>Axis alignment</td>
</tr>
</tbody>
</table>

During an alignment procedure to produce a good image, the aperture needs to be checked to ensure it is centred around the beam axis. This is done by using the Wobbler control. If the image is seen to shift from side to side then the aperture needs adjusting in the X or Y direction (in and out or side to side) and is adjusted with tiny turns of the appropriate knobs until the image stops shifting.

Focus

The best focus is achieved when the diameter of the electron beam is at a minimum at the surface of the sample. The image should be sharp and well defined.
Spot size

The size (cross sectional diameter) that the cone of the beam makes on the surface of the sample affects 1) the resolution of the image and 2) the number of electrons generated and therefore the graininess of the image. At low magnifications we use a larger spot size than at higher magnifications.

**The size of the spot affects resolution**

The beam scans across the sample but is actually made up of moments when it dwells on the sample. Each moment, or spot as we see here, generates signal that makes up the final image.

When images are taken at the same magnification, kV, and working distance but using different spot sizes, the difference in blurriness (resolution) is easily seen across the series. The way spot size is reported depends on the make of the machine used.
The number of dwell points (spots in the row in the diagram) is constant for any one magnification so too small a small spot size will result in gaps where no signal is generated and too large a spot size will result in overlap and averaging of the signal.

Spot size changes as a number of machine parameters are modified. For example the spot size is larger at long WD (working distance) than at short WD. A smaller objective lens aperture produces a smaller spot size. Also, a higher current though the condenser lens (labelled spot size on the control panel or software) creates a smaller spot on the sample no matter what WD is used. Therefore when the WD is small, the condenser lens setting high, and the aperture small, we see the smallest possible spot size. These three parameters interact and need to be considered carefully to achieve the best image because they also affect other parameters such as field of focus and strength of the electron signal.

The image below is of a diatom taken at three different spot sizes. At the largest spot size (spot 5) the image shows less detail than the smallest spot size (spot 1). However, the brightness of the image is reduced in the smallest spot size.
Working distance

Sample height, or working distance (WD), refers to the distance between the bottom of the SEM column and the top of the sample. Within the sample chamber the sample stage can be wound up closer to the end of the column (a short working distance) or dropped down lower (a long working distance).

The shorter the working distance, the smaller the diameter of the beam is at the sample surface. So, when possible, the WD is kept at 10mm or smaller for high-resolution imaging. The disadvantage is that focal depth is drastically reduced at small WD. This can be offset by using a smaller objective aperture and putting up with the reduction in electrons that comes with this choice (grainer image).

Depth of field

In many SEMs an external working distance (Z) control is used to either raise or lower the specimen. This value is often mistaken for an accurate WD. However, the true working distance (WD) is measured electronically as the point of focus on the sample surface to the SEM column above. There are three reasons why the value of the external Z control (mechanical control) and the WD provided on the image screen are different.

The ‘on screen’ value of the WD is only an accurate measurement if the electron beam is focused accurately onto the specimen surface. An under-focused or over-focused image will provide a false WD value as well as a blurry image. The value of the external Z and even a true WD from an accurately focused specimen will be different because both measurements may be taken from different points on the specimen holder.

Specimens that are not uniformly flat will have a different true WD for different topographical features. The WD impacts on the depth of field and resolution of the SEM image. As the WD is increased the beam divergence angle is decreased which provides a greater depth of field. The “trade-off” for an increased WD is that the electron beam must travel a greater distance from the gun and therefore has a larger spot size on the specimen.

<table>
<thead>
<tr>
<th>WD</th>
<th>5 to 30mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of field</td>
<td>Shallow ------------ Deep</td>
</tr>
<tr>
<td>Resolution</td>
<td>High ------------ Low</td>
</tr>
</tbody>
</table>
Depth of field refers to the zone in which the specimen appears acceptably in focus to the eye. This “range” over which the image appears to be in focus is typically several thousand times greater in an SEM than in the light microscope and results in the almost 3 dimensional appearances of many SEM micrographs.
Contrast and brightness

Perfecting an image - Signal processing

The SEM image is a constructed (virtual) intensity map (either digital or analogue) of numbers of electrons ejected from the sample material. The electron signal from each dwell point in the SEM is displayed in a sequence, as pixels on a line on a screen, line by line to build the image. The strength of the signal at each point is a reflection of the electrons generated from the topography or composition. Through signal processing each quantum of signal information (gained from each dwell point of the beam) can be changed to some new value that bears a rigorous relationship to the original one, before it is displayed. In this way we can adjust the signal to change contrast and brightness of our final image.

In most cases the unprocessed image contains enough “natural contrast” for the operator to extract useful information from the image. Natural contrast can be considered as the contrast contained in the signal that comes immediately from the specimen + detector system. If the natural contrast is too low or too high, then signal changes corresponding to important detail may be lost. In this case we see the image as having a lot of black or white regions. A good quality image has a gradation of greys with very little of the image fully black or white. Signal processing techniques manipulate the natural contrast so that the eye can perceive information through contrast in the image. Although signal processing allows the user to manipulate the natural contrast, there is no addition of information, only enhancement of that already present. This image of part of a small hive beetle shows too little contrast on the left and too much contrast on the right. The central image is correct. The image on the left can be adjusted after collection, by modifying the spread of greyscale “Levels” in software like Photoshop, but the image on the right is not able to be corrected since the pure black and white areas are absolute (no further data can be retrieved from these regions).

It should be noted that signal processing can greatly change the appearance of an image relative to that which might usually be expected, and therefore the SEM operator is under an obligation to state whether processing has taken place. Normally however, it is considered routine to adjust the image quality using the contrast and brightness knobs (“contrast control” and “black level control”). However, if some other differentiation had been applied to give a crisp appearance to an SE image, a written report should describe the exact nature of the processing.

Older models of SEMs generally have a graphical display of contrast and brightness that can be used to adjust the image. More modern machines rely on automatic adjustment (ACB buttons), supplemented by machine operator preferences corrected by eye, using the contrast and brightness controls.

Tilting to increase SE contrast

Another mechanism to increase SE contrast in an image is to tilt the sample so that it is at an angle to the probe (typically 30° to 60°). As a result of tilting, more SE are generated per unit of projected specimen area and this enhances contrast by making the distribution of light and dark areas, more pronounced.
Magnification and calibration

Magnification

Magnification is the enlargement of an image, or portion of an image. In a scanning electron microscope this is achieved by scanning a smaller area. In the images, the beam is indicated by arrows on a sample.

As a smaller region is scanned, what we see is the object getting bigger. In the micrographs the image is magnified from 900x to 10,000x across the three frames. This is an image of the tiny spheres that are produced from a lit party sparkler.
The magnification of the image is usually given as a value on the screen (e.g., 2000x). There will also be a scale bar on the image which represents an accurate unit of distance. The magnification number is only accurate when the image is displayed on the microscope monitor. As soon as the image is opened on another screen, or printed in a paper, the magnification number is likely to be incorrect. The scale bar, however, is embedded in the image and will scale as the image is displayed in different formats.

**Calibration**

Basic maintenance of an SEM includes regular checks of the calibration of the magnification. A standard sample (such as a calibration grid) is imaged under standard conditions. The features on the image are measured and compared to the given magnification or scale bar to ensure that the correct dimensions are achieved. If not, there are calibration procedures that can be followed. The magnification displayed on the screen may include an error of 2 - 5% under standard conditions. In many cases, this level of uncertainty is acceptable. However, if the work being done requires a high level of accuracy it is important to calibrate the system using conditions that are exactly the same as the experimental conditions and a calibration standard that has features that closely match the size of the features you wish to measure experimentally. For example, if you need to measure the size of particles which are expected to be 500nm in diameter, your calibration sample should contain features of the same size.
Scan rate and signal to noise

It is conventional to reduce the scan rate when collecting an image for later use or publication. The slower scan rate allows more electrons to be collected at each point along the line of the beam scan. This produces a better quality image. The image quality from an SEM is limited by the spot size and the ratio of the signal (S) produced by the electron beam to the noise (N) imparted by the electronics of the instrument in displaying this signal (S/N). Noise pulses are derived from such sources as beam brightness, condenser lens settings (spot size), and SE detector sensitivity, and may impart a salt-and-pepper, grainy appearance to the image. When the SEM is set up for high-resolution imaging it will often have a low S/N ratio and appear grainy. This may be unavoidable. The image quality in an SEM and hence its S/N ratio is improved as the total number of electrons recorded per picture point is increased.

Tungsten (W) filaments characteristically have low yields of electrons resulting in a low brightness image. Therefore, at condenser lens settings for high resolution imaging, (small spot sizes) the quantity of electrons reaching the specimen is low. Therefore SE production is low and a high current must be used in the SEM electronics (e.g. the photomultiplier tube) in order to produce an image. This results in an unfavourable S/N ratio. To overcome the limitation of W filaments, and to improve the S/N ratio, bright sources such as the field emission gun (FEG) have been developed.
Image artefacts and trouble-shooting

Getting the perfect image takes knowledge and practice and is a trade off between many factors. There are a number of problems that can be encountered.

Astigmatism

Astigmatism can be one of the hardest adjustments to correct accurately in images, and requires practice. The image in the centre shows a correctly focused image that has also been corrected for astigmatism. On the left and right are examples of poorly corrected astigmatism, seen as streaking of the image. To allow accurate imaging, the electron beam (probe) should be circular in cross section when it reaches the specimen. The probe cross section can become distorted to form an ellipse. This is due to a range of factors such as the level of machining accuracy and the material of the pole-piece, imperfections in the casting of the iron magnets and or the copper winding. This distortion is called astigmatism and causes focus difficulties. Bad or “gross” astigmatism can be seen as “streaking” in the image in an X direction that changes to the Y direction as the image passes through focus from under focus to over focus. At exact focus the streaking vanishes and focus can be correctly achieved if the spot size is suitable.

To make the probe circular, a stigmator is used. This comprises electromagnetic coils placed around in the microscope column in quadruple, sextuple or octagonal orientations. These allow adjustment of the shape of the beam and can be applied to correct for any major lens distortions. An image is generally regarded as free of astigmatism when it does not streak in one direction or the other when the objective lens is adjusted to under or over focus at around 10,000x magnification. Astigmatism is usually negligible in an image at less than 1000x.

The best procedure to correct for astigmatism is to set X and Y stigmators to zero offset (i.e. no astigmatism correction) then fine focus the sample as well as possible. Then adjust either the X or Y stigmator controls (not both) for the best image and refocus the image. When the best image has been obtained with one stigmator, use the other stigmator to get the sharpest possible image. Refocus the image: if the astigmatism has been corrected there will be no streaking of the image as it is focused.

Lack of detail of surface structures

At high kV the beam penetration and diffusion become larger and result in signal (electrons coming out of the sample) being generated from deeper within the specimen. This can obscure fine surface structures. It will also increase BSE and so the image will start to show changes in contrast based on composition. The solution for obtaining fine surface structure is generally to use lower kVs such as 5-10kV.
**Edge effects**

Edge effects are due to the enhanced emission of electrons from edges and peaks within the specimen. They are caused by the effects of topography on the generation of secondary electrons and are what gives form and outline to the images produced by the secondary electron detector. Electrons preferentially flow to, and are emitted from, edges and peaks. Poor signal intensity occurs in those regions shielded from the detector, such as depressions. Topographic contrast is also enhanced by back scattered electrons emitted from regions of the sample facing towards the detector. Lowering the beam kV can reduce edge effect.

Changes in the interaction volume with topography

**Notes:**
- Points and edges produce more electrons.
- e-beam
Charging
Charging is produced by build-up of electrons in the sample and their uncontrolled discharge. This can produce unwanted artefacts, particularly in secondary electron images. When the number of incident electrons is greater than the number of electrons escaping from the specimen then a negative charge builds up at the point where the beam hits the sample. This phenomenon is called charging and it causes a range of unusual effects such as abnormal contrast and image deformation and shift. Sometimes a sudden discharge of electrons from a charged area may cause a bright flash on the screen. These make it impossible to capture a uniform image of the specimen and may even be violent enough to cause small specimens to be dislodged from the mounting stub. The level of charge will relate to (1) the energy of the electrons and (2) the number of electrons. The energy of the electrons is related to the kV (i.e. high kV = high energy) so reducing kV can reduce charging. The number of electrons relates to a number of parameters including, beam current, the emission level of the gun, the spot size, and the apertures between the gun and the specimen. So reducing the number of electrons by adjusting these parameters can also reduce charging.

In this image of a small hive beetle, the horizontal bright and dark bands are a result of charging. A sample preparation solution to such a problem can be to recoat the sample with a thicker layer of platinum, this is done to increase the conductivity of the surface so that sufficient electrons can escape to avoid charging and damage at the surface. Alternatively such samples can be pre-treated with osmium tetroxide vapour to enhance conductivity in the joint regions that are difficult to coat effectively with metal.

The streaking and enhanced contrast in this image of organic secretion from an adult jewel beetle are also due to charging. Loose materials such as particles often suffer from charging. A sample preparation solution is to reduce the amount of sample on the mount so that all the material is in contact with the base adhesive, and coat it with a metal such as platinum or gold.

An SEM with low vacuum capability or an environmental scanning electron microscope (ESEM) can be used to control charging.
Specimen damage

Irradiating a specimen with an electron beam results in a loss of the beam energy to the sample in the form of heat. A higher kV results in a higher temperature at the irradiated point and this can damage (e.g. melt) fragile specimens, such as polymers or proteins, and evaporate waxes or other sample components. This can ruin a sample (as well as contaminate the SEM chamber). The solution is to lower the beam energy, sometimes down to a few kV. Increasing the working distance can also help since it produces a larger spot size on the sample for the same beam energy but this has the disadvantage of reducing resolution.

Beam-related contamination

Beam-related contamination refers to the deposition of material (e.g. carbon) in a region on the sample where the beam has been scanning. This is a result of the interaction of the electron beam with gaseous molecules (such as hydrocarbons) in the vacuum chamber.

One way to work around this artefact is to take micrographs at low magnification, before moving to higher magnification. This artefact can also be reduced by ensuring that the sample is as clean as possible before it is placed into the SEM chamber. For example, it is good practice to wear gloves when handling samples to prevent contamination by finger grease etc.
Specialised SEM Techniques - CL

When an electron beam interacts with a luminescent material, visible light may be emitted as a result of electronic transitions in the band gap of the material. This can give the researcher information about the properties of the material and any defects or impurities which may be present. A CL system consists of a light collector, a transmitter and a detector.
In conventional SEM, the sample must be both completely dry and conductive before it can be effectively imaged. The processes used to dry samples can introduce changes in the sample. The changes introduced by preparation are well-understood, but it can sometimes be useful to view a sample in its natural state. The ESEM is designed to do this. Sample temperature and specimen chamber vapour pressure can both be controlled, allowing samples to be heated, cooled, wetted or dried. This allows dynamic experiments to be performed.

Relative humidity (RH) can be controlled within the chamber by adjusting the temperature of the conventional stage (±20°C) along with the pressure.

For example a relative humidity of 100% can be achieved by combination of low temperature (e.g. 4°C) and high water vapour pressure (e.g. 6.1 Torr).

The advantage of using 100% RH is that the sample is not being dehydrated as it is being imaged. Water can be condensed on the samples by going above 100% RH. Dynamic experiments can be performed on wet samples in real time, involving heating (on a specialised hot-stage), cooling, wetting or drying. The samples can be imaged while these dynamic processes are occurring.

Some examples of experiments that can be undertaken in the ESEM include the determination and imaging of melting dynamics for physical science materials; determination of crystallisation dynamics; and imaging of biological processes, for example pollen tube growth in real time through wetting of pollen.
Cryo-SEM - Cold stage

Cryo indicates frozen. A cryo-scanning electron microscope is a conventional SEM that has been fitted with specific equipment that allows samples to be viewed in the frozen state. This is particularly useful for directly viewing hydrated (wet) samples, delicate biological samples, hydrogels, food, biofilms, foams, fats and waxes, suspensions, pharmaceuticals and nanoparticles. The sample can be snap frozen outside the machine and then inserted in its frozen state, or placed into the machine in an unfrozen state and frozen more slowly in the machine. It is imaged using either secondary electrons (SE) or backscattered electrons (BSE). Frozen samples can also be fractured or cut during preparation to reveal internal structures.
FIB

This technology involves using an ion beam (typically gallium ions) directed onto a hard sample. The beam is focussed to an extremely fine probe size (<10 nm) onto the surface of a specimen. The sample can be sectioned or shaped with the ion beam while it is being monitored by scanning electron microscopy (SEM). FIB can cut 10-nm-thick sections from very hard materials. These sections can be taken off as sequential sections, each viewed in turn with the SEM mode. This imaging information is used to construct a 3D image. FIB can also be used to shape thin slices or needles that can then be viewed by other techniques such as transmission electron microscopy or atom probe tomography. It can also be used for deposition of materials in a small area (approx 100nm) from chemical vapour from specific gasses.

When milling a region or cutting a piece out of a sample, it is important to firstly lay down a strip of metal that will stop the ion beam from eroding that region. This allows a well defined edge to be achieved on the area being excised.

Machines can have both ion and electron columns on a single instrument (called dual beam instruments). The advantage of dual beam machines is that they allow specimens to be imaged in detail using the electron beam, without damaging the surface of the specimen with the ion beam.
Energy dispersive X-ray spectroscopy (EDS or EDX) is an analytical technique used to investigate the elemental or chemical characteristics of a sample. When an electron beam interacts with a sample, X-rays are emitted. The energy of these X-rays is characteristic of the elements present in the sample. This technique is discussed in detail in the Microanalysis MyScope module.
EBSD

Electron Backscatter Diffraction (EBSD) is a technique which allows the investigation of the structure, phase and crystal orientation of crystalline materials. It can be used to reveal texture, grain size and morphology, defects and deformation. The technique requires an EBSD detector installed onto an SEM. The specimen needs to be as flat and smooth as possible so that topography effects do not interfere with the analysis.
Electron beam lithography (EBL) is a maskless lithography technique used for patterning of computer generated layout structures on photoresists on silicon wafers. Upon irradiation of focused electron beam, electron-sensitive resists undergo chain-scission or crosslinking, resulting in solubility switch of materials during the subsequent development process (remove/retain exposed material in development depending on the tone of the resist). To date, EBL remains the highest resolution patterning tool in lithography, it is widely used in photomask fabrication and low volume production of semiconductor components.
Backscatter

Backscattered (BS) electrons are high-energy electrons (>50 eV) from the primary incident beam that are ejected back out from the sample. These BSE are used to produce a different kind of image. Such an image uses contrast to tell us about the average atomic number of the sample.

For example, an area of a sample that is rich in iron will be brighter than an area that contains silicon. The image below shows a mixture of grains. The brightest one (top left) is high in iron. The grain on the right is high in calcium and the central grain contains magnesium, iron and silicon. The back scattered image shows that this grain is zoned. The slightly brighter area in the centre is higher in iron.

The higher the average atomic number, the more primary electrons are scattered (bounced) back out of the sample. This leads to a brighter image for such materials.

The backscattered electron has an energy up to the incident beam energy and is usually very near that energy. The greater energy of BSE, compared with SE, means that BSE produced from deeper within the interaction volume are able to escape from the sample and be collected by the BSE detector, so BSE images have lower spatial resolution than SE images. In other words, the BSE can travel further in the sample before coming out again and so the information they carry is less restricted to the surface detail. This results in reduced resolution.
Coating

It is important to leave the sample uncoated (in its natural state) if compositional information is required because the practice of coating samples with metals obscures this. If the sample is non-conductive then it can be coated with carbon (a low atomic number material) which will enhance conductivity without obscuring the compositional detail from below.

Topography and BSE

In order to get the best compositional information using BSE, it is preferable to use a flat sample. Otherwise the topography will interfere with the signal reaching the detector. For a smooth (e.g. polished) specimen the most dense material provides the highest (brightest) signal level and the least dense the lowest signal level. In this way, BSE images provide information on compositional heterogeneity through atomic number contrast. BSE greyscale differences indicate the average atomic number of the phases present and thus allow the recognition and classification of the phases, but they do not indicate either the elements present or the concentration levels.

Differences in elemental composition or concentrations that can be observed clearly by BSE imaging can successfully be assessed with microanalysis using energy dispersive analysis. However, different phases in material can appear the same with BSE and these too, can successfully be assessed with microanalysis.

ECCI

Another BSE imaging technique used in a scanning electron microscope for example in studying defects in metals is electron channelling contrast imaging (ECCI). This can detect and characterise dislocation structures in bulk specimens. The change in diffraction of the backscattered electrons as they interact with a dislocation in the material results in a higher backscattering coefficient than for the matrix; so individual dislocations appear as bright lines in a darker matrix.
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