MyScope

training for advanced research

Scanning Electron Microscope

Training module

Nanyang Technological University has played an important contributing role to the development of this site via the provision of the Online Micro and Nano Characterisation Instruction website.

Support for this website has been provided by the Office for Teaching and Learning, an initiative of the Australian Government Department of Education, Employment and Workplace Relations.

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Calcium carbonate (CaCO$_3$) is commonly found in chalk, egg shells, snail shells, and the shells of many small marine organisms.

- Practice scanning electron microscopy using a virtual SEM
- Are you an SEM Champion?

Welcome to the online learning module for scanning electron microscopy. Through self-instruction, this unit will introduce you to:

- the basic principles of SEM operation;
- the origin of images and their formation;
- procedures for collecting and interpreting images;
- routines for preparing samples for investigation; and
- ways to work safely in an electron microscope laboratory.

You will also get the opportunity to “play” with a virtual Scanning Electron Microscope by following the guided instructions. This module will not make you an overnight expert in SEM, but it will provide the essential skills needed to begin experimentation and provide the scaffolding necessary to become a full-fledged electron microscopist through practice and perseverance.

At the end of the module menu is an online multiple choice test. You can do this at any time to determine how much you know about SEM, and repeat it as many times as you like. The result can be saved and sent to your teacher or supervisor if necessary.

If you do not wish to start at the beginning and go all the way through the site, the pages following this one give you the opportunity to tailor the site to your specific learning needs.

We wish you an enjoyable learning experience.
Frequently asked questions

Select an item to show relevant pages.

Image basics

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- Magnification range: what is it for an SEM? See answer in Background information - What is scanning electron microscopy? Magnification
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  - Perfecting an image - signal processing
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  - High vacuum mode and pump system
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What are the parts of the machine? See answer in
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**General**

What different kinds of SEMs exist and how do I choose between them? See answer in
  ● Types of SEM

When are cryo-preparation methods advantageous? See answer in
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What risks are involved in doing electron microscopy? See answer in
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SEM challenge

This is a secondary electron image taken at 25kV. The specimen is randomly distributed fine aluminium particles embedded in polymer resin and polished to a flat cross-section.

The specimen is mounted flat and in focus.

Why do some particles have a sharp edge and other particles have a “fuzzy” edge. What type of electrons are causing this effect? Note: SE1 are generated by the primary beam whereas SE2 are generated by backscattered electrons. The term SE includes all types.

Secondary electrons (SE) Secondary electrons type 1 (SE1) Secondary electrons type 2 (SE2) Backscattered electrons
A Scanning Electron Microscope (SEM) is a tool for seeing otherwise invisible worlds of microspace (1 micron = 10⁻⁶ m) and nanospace (1 nanometer = 10⁻⁹ m). By using a focussed beam of electrons, the SEM reveals levels of detail and complexity inaccessible by light microscopy.

SEMs can magnify an object from about 10 times up to 300,000 times. A scale bar is often provided on an SEM image. From this the actual size of structures in the image can be calculated.

Essentially, the way the scanning electron microscope "looks" at the surface 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. Unlike light photons, electrons cannot be focussed by glass lenses, but electromagnets are capable of guiding and concentrating electrons just as glass bends light.

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

1. **Resolution at high magnification.** Resolution can be defined as the least distance between two closely opposed points, at which they may be recognized 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).

2. **Depth of field** i.e. the height of a specimen that appears in focus in an image - 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. The use of "stereo pair" SEM images can give even greater information about the sample.

3. **Microanalysis** i.e. the analysis of sample composition including information about chemical composition, as well as crystallographic, magnetic and electrical characteristics.
One drawback to the SEM

One drawback to the use of the SEM is that it operates under vacuum and in many SEMs the samples must be rendered conductive to be viewed. This is often achieved by coating with a very thin layer of metal or carbon. However, there are a number of different types of SEMs which all have specific purposes, often associated with additional pieces of equipment like specialised stages or collectors. Some of these do not require dry or conductive samples. They include the following:

- Low vacuum scanning electron microscopy (LVSEM)
- Using cryo on a scanning electron microscopy (Cryo-SEM)
- Environmental scanning electron microscope (ESEM)
- Focused ion beam (FIB) technology
- E-beam lithography (EBL)

Fundamentally and functionally, electron microscopes are in many ways analogous to their optical counterparts (light microscopes: LM). This is somewhat surprising at first glance, given the contrast between the simple technology of the LM and the complex electronics, vacuum equipment, voltage supplies and electron optics system of electron microscopes. A comparison of these features is used frequently in textbooks as a starting point for any discussion of SEM.
What is an SEM (Parts of the machine)?

The typical scanning electron microscope laboratory contains a machine with three components:

1. the microscope column, including the electron gun at the top, the column, down which the electron beam travels, and the sample chamber at the base
2. the computer that drives the microscope, with the additional bench controls
3. ancillary equipment that, for example, analyses composition. This will be explored in the module on microanalysis rather than here under SEM.

These three components and some of the other elements they contain are described and illustrated below and in the figures.
Electron guns can be classified into two types

1. **Thermionic gun**
2. **Field emission gun**

<table>
<thead>
<tr>
<th>Emission</th>
<th>Thermionic</th>
<th>LaB6</th>
<th>Field Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (nm)</td>
<td>$1 \times 10^5$</td>
<td>$2 \times 10^4$</td>
<td>0.2</td>
</tr>
<tr>
<td>Brightness (A/cm².steradian)</td>
<td>$10^4 - 10^5$</td>
<td>$10^5 - 10^6$</td>
<td>$10^7 - 10^9$</td>
</tr>
<tr>
<td>Energy Spread (eV)</td>
<td>1 - 5</td>
<td>0.5 – 3.0</td>
<td>0.2 – 0.3</td>
</tr>
<tr>
<td>Operating Lifetime (hrs)</td>
<td>&gt;20</td>
<td>&gt;100</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Vacuum (torr)</td>
<td>$10^{-4} - 10^{-5}$</td>
<td>$10^{-6} - 10^{-7}$</td>
<td>$10^{-9} - 10^{-10}$</td>
</tr>
</tbody>
</table>

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.
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 types, their specifications and principles of operation are discussed in more detail in Principles of operation - The electron gun.
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. By amplifying and modulating the brightness of the detected electron signals an image is produced.
Magnetic lens system

The magnetic lens system consists of a:

1. Condenser lens
2. Objective lens
3. Scanning coils

Condenser lens

The condenser lens controls the intensity of the electron beam reaching the specimen. The objective lens brings the electron beam into focus (de-magnifies) on the specimen. The scanning coils deflect the electron beam horizontally and vertically over the specimen surface. This is also called rastering.

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.
Detectors

Secondary electron detector (SED) – Everhart-Thornley Detector

Due to the low energies of secondary electrons (SE) (~2 to 50 eV) they are ejected only from near-surface layers. Therefore, secondary electron imaging (SEI) is ideal for recording topographical information. To attract (collect) these low-energy electrons, a small bias (often +/- ve selectable but usually around +200 to 300V) is applied to the cage at the front end of the detector to attract the negative electrons towards the detector. [If the cage is negatively biased it functions as a BS detector]. A higher kV (e.g. 7 to 12kV) is applied inside the cage i.e. to the scintillator, to accelerate the electrons into the scintillator screen.

![Diagram of SED](ammrf.org.au/myscope/sem/background/whatissem/detectors.php)

Click Play to begin.

Backscattered electron detector (BSD) – solid state diode detector

The BSD is mounted below the objective lens pole piece and centered around the optic axis. As the specimen surface is scanned by the incident electron beam, backscattered electrons (BSE) are generated, the yield of which is controlled by the topographical, physical and chemical characteristics of the sample. Both compositional or topographical backscattered electron images (BEI) can be recorded depending on the window of electron energies selected for image formation.
See Principles of operation – Electromagnetic lenses, apertures and beam size for more on SEM theory.
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.
Specimen chamber

The specimen chamber is maintained at high vacuum that minimises scattering of the electron beam before reaching the specimen. This is important as scattering or attenuation of the electron beam will increase the probe size and reduce the resolution, especially in the SE mode. A high vacuum condition also optimises collection efficiency, especially of the secondary electrons.

Specimen stage

The specimen holder is fixed to the specimen stage by the dovetail locating slide. 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. The specimen stage can also rotate continuously.

See Principles of operation - High vacuum mode and pump system for more on specimen chamber theory.

Vacuum system

This will vary depending on the age and type of machine but as an example, it consists of an oil rotary (backing) pump and an oil diffusion pump. The backing pump is used for rough evacuation while the diffusion pump achieves higher vacuums. A fail safe circuit controls the vacuum sequence and maintains the appropriate vacuum (~10^-6 torr) in the optical column and specimen chamber. (Note: The diffusion pump will not work until the backing pump vacuum is adequate. Attempting to operate the 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. In these circumstances it is advisable to dry the sample before introduction into the microscope.
Main control panel

Main power
On some SEMs there is a key switch used to set the main power supply on or off.

Vacuum mode switch
Used to indicate High Vacuum (HV) or Low Vacuum (LV) mode. In LV mode the lamp is lit (on), when in HV mode the lamp is dim (off).

Specimen chamber vent
Used to indicate that the progress of bringing the specimen chamber and column to atmospheric pressure (lamp is blinking) and when venting is complete (lamp lights up).

Specimen chamber evac
Used to indicate that evacuation of the specimen chamber and column is in progress (lamp is blinking) and when evacuation is complete (lamp lights up).
Operation unit

Monitor

The Graphical User Interface (GUI) is displayed here.

Text icons

This set of icons is used for switching between scan modes, starting the auto function or opening an operation window.

Image display area

The image is displayed here so that specimen shift and stage movement can be monitored. Data such as accelerating voltage and magnification will be visible when the image in freeze mode.

Active data display

The current setting of the SEM is displayed here. By clicking each item, a dialogue box will appear so that adjustments can be made.

Operational keyboard

The operational keyboard is used in conjunction with the normal keyboard and mouse. The operation keyboard controls critical operations such as focussing in high magnification and astigmatism correction.

See the Operation example section for a step by step guide to using the operation unit.
Applications and practical uses - what the SEM can do

Scanning electron microscopy is a remarkably versatile technique. There are many different types of SEMs available, tailored to specific needs. **With SEM one can:**

- Image morphology of samples (e.g. view bulk material, coatings, sectioned material, foils, even grids prepared for transmission electron microscopy).
- Image compositional and some bonding differences (through contrast and using backscattered electrons).
- Image molecular probes: metals and fluorescent probes.
- 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 (while possible in many instruments it is generally done only in ESEM or during Cryo-scanning electron microscopy).
- Wet and dry samples while viewing them (only in an ESEM)
- View frozen material (in an SEM with a cryostage)
- Generate X-rays from samples for microanalysis (EDS; WDS)
- Study optoelectronic behaviour of semiconductors using cathodoluminescence
- View/map grain orientation/crystallographic orientation and study related information like heterogeneity and microstrain in flat samples (Electron backscattered diffraction).
- Electron diffraction using electron backscattered diffraction. The geometry may be different to a transmission electron microscope but the physics of Bragg Diffraction is the same.
What the SEM can't do

There are some things SEM can't do:

- **SEM cannot take colour images.** The colour is often added artificially in coloured SEM images. Note: Some SEMs can collect true colour images via a wavelength selective cathodolumenence (CL) detector.

- **SEM cannot image through water.** Note: An ESEM using a wet Scanning Transmission Electron Microscope (STEM) detector can be used to image through thin water films.

- **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.

- **SEM is not good for quantifying surface roughness at small scale.** Atomic Force Microscope (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.

- **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.**

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

- **Elemental analysis below micron scale.** Note: Analysis in the < 7kV range can provide elemental information on the sub-micron scale but is often problematical.

Where the SEM is not suitable, other techniques should be used. If in doubt consult the Technique Finder on the AMMRF web site.
Images from electrons

Electron images from the SEM can be used to achieve different information, for example for topographical, morphological, compositional, or crystallographical studies. There are a range of imaging techniques available.
Practical uses for the SEM

Scanning electron microscopy is used in all scientific fields, in engineering, archaeology and even in art. The following images in this section are examples from forensic science, materials science, biological science, medical science and digital art.

- **Forensic science** Examine and compare evidence.
- **Materials science** Metals, alloys, ceramics, polymers and biological materials.
- **Biological science** Large objects such as insects and animal tissues and small objects such as bacteria and viruses.
- **Soils and rock samples** Soils and geological samples.
- **Medical science** Determine the cause of illness and develop new treatments.
- **Digital art** Images taken from the SEM that have been modified as digital art.
Types of SEM

The different types of scanning electron microscopes in detail:

- Conventional (high vacuum) SEM
- Variable Pressure or Low Vacuum SEM
- Cryo on an SEM (Cryo-SEM)
- Environmental SEM
- Focused ion beam (FIB)
- Electron-beam (E-beam) lithography
Conventional (high vacuum) scanning electron microscopy (SEM)

This is the most common type of machine. It requires a dry, conductive sample (often achieved by applying a thin layer of metal to the surface with a technique called sputtering). The sample must be able to withstand a high vacuum. This type of machine is used for routine imaging, using either secondary electrons (SE) or backscattered electrons (BSE).
Variable Pressure or Low Vacuum scanning electron microscopy (LVSEM)

This type of machine is basically like a conventional SEM but has the advantage in low vacuum (LV) mode that the pressure can be adjusted in the sample chamber until the artefact of "electron charging" is removed from images. This charging artefact is the result of electrons from the electron beam building up in a nonconductive sample. The extra electrons then jump from the sample unpredictably, causing lines and streaks on the image. Alternatively the unpredictable electron discharge repels the beam, causing jumps in the image or the appearance of black patches.

This means LVSEM can be used to image the surface of non-conductive samples (no metal needs to be added to the surface of such samples). It is particularly useful for viewing polymers, biological samples, and museum samples that cannot be changed in any way, particulate samples, and geological materials. Imaging uses backscattered electrons (BSE).

Backscattered electron imaging (BSE) of nonconductive, uncoated samples can provide information about composition via the contrast of the image: whiter regions have a higher average atomic number than darker regions.

The LV mode can also be used to freeze-dry samples for viewing. The sample is placed on a conventional SEM mount, plunged into liquid nitrogen and then placed on the SEM machine stage. The chamber is pumped free from air (evacuated) and the sample left for about 10 minutes by which time it is dry. The technique works best on hydrated samples that have some basic structural integrity, such as plant tissue.

It should be noted that while LV mode allows adjustment of the pressure within the sample chamber this is not to the high degree achieved in an Environmental SEM.
Using cryo on a scanning electron microscope (Cryo-SEM)

Cryo stands for 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.
Environmental scanning electron microscope (ESEM)

This machine is designed to view a sample in its natural state, without the need for desiccation. Sample temperature and specimen chamber vapour pressure can both be controlled, allowing samples to be heated, cooled, wetted or dried.

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 also be condensed on the samples by going above 100% RH. Dynamic experiments can also be carried out on wet samples in real time, involving heating on a specialised hot-stage, anywhere up to 1500° C, cooling, wetting and 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.
Focused ion beam (FIB) technology

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, and this imaging information used to construct a 3D image. FIB can also be used to shape 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.
Electron-beam lithography (E-beam lithography or EBL)

EBL is a maskless lithography technique used for patterning of computer generated layout structures on photoresists on Si 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.

There are also the microanalysis techniques associated with SEM: Spectroscopy for analysing the elemental make up of samples, Electron Backscatter Diffraction (EBSD) for viewing crystallographic information, and Cathodoluminescence (CL) for imaging otherwise invisible microstructural defects and impurities in semiconductors and insulating materials.
The most important concept in scanning electron microscopy is the use of electrons. Much logically flows from this, such as that a vacuum is needed to generate the electron beam, that electrons are used for imaging, and that we need to understand the interactions of electron beam with the sample in order to interpret our images. The use of electrons also impacts on image resolution and image colour, and explains the 3D nature of the micrographs (photos). The second most important concept is that we are looking only at the surface of a sample, and penetrating only a small way into the sample with the electron beam. All these notions will be explored in more detail in the list of sections available under this component (Concepts in SEM). Specific terms can be explored at any time by using the Glossary.

Electron images are monochromatic (gray scale), not in colour, because electron wavelengths are much smaller than the visible light we see with our eyes. Any coloured images you see from a scanning electron microscope have been artificially coloured.

**Why is there no colour?**

Although electrons may have different wavelengths or energies, they do not have colour as we see it. Colours we see with our eyes correspond to different wavelengths of visible light, with violet being the shortest and red the longest. Secondary electron SEM images — the most common form of images produced by these machines — are effectively intensity maps of electrons collected by a detector. The intensity of brightness on the screen is in proportion to the number of electrons originally produced. SEM images are displayed as monochrome grayscale (or greyscale) digital images in which each pixel carries only intensity information in a shade of gray varying from black at the weakest intensity to white at the strongest. Sometimes these grayscale images can be post-processed to display false colour i.e. colourised grayscale.
Resolution

Definitions

- **Resolution** the ability to distinguish closely spaced points as separate points.
- **Resolution Limit** smallest separation of points which can be recognized as distinct.
- **Resolving Power** resolution achieved by a particular instrument under optimum viewing conditions.

The concepts of resolution and magnification are often confused. Any image can be magnified without limit, but most will become blurry if we do this. A crisp image, sometimes referred to as a sharp image, is what is wanted. This means

- the image must be properly focused and
- the image must have adequate resolution.

In this image below, bacteria from a yoghurt culture have been placed on the head of a pin (a). When this image is magnified, even a small amount (see b) blurry detail is apparent. But when the machine is adjusted to achieve high resolution, individual bacteria are easily seen even at higher magnification (image c has been magnified 83 times more than image a).
What is resolution?

It is the ability to distinguish closely spaced points as separate. Resolution can also be understood as the least distance between two closely opposed points, at which they may be recognized as two separate entities. The smallest distance we can see between points in a light microscope (LM) is about 200 nm [There are 1000 nm (= nanometers) in 1 mm] whereas a typical scanning electron microscope (SEM) can distinguish gaps smaller than 10 nm.

Resolution is dependent on wavelength of the beam we use to see the material, and this explains why the electron beam, with its much shorter (smaller) wavelengths is able to provide better detail than the light microscope. Visible light has a longer wavelength than an electron beam. In this image we see the waves in blue. As tip-to-tip wavelength gets smaller, the ability to resolve the spots gets better.
In more detail

The first breakthrough in the development of the electron microscope came when Louis de Broglie advanced his theory that the electron had a dual nature, with characteristics of a particle or a wave. De Broglie combined some of the principles of classical physics with quantum theory and developed an equation to calculate the very small wavelengths of these particles (electrons).

Table 1 shows the resolving power for the light microscope. Note that it improves as the wavelength of the illuminating light decreases. Abbe (1893) showed, using the Abbe equation (see below), that the smallest resolvable distance is about half the wavelength of the illumination used. This distance is the *ultimate* resolving power of any instrument. This is called the *Abbe Criteria of Resolution*.

Table 1. Resolution achieved with visible light

<table>
<thead>
<tr>
<th>Light Source</th>
<th>Red</th>
<th>Green</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>700</td>
<td>546</td>
<td>436</td>
</tr>
<tr>
<td>Resolution (nm)</td>
<td>250</td>
<td>190</td>
<td>160</td>
</tr>
</tbody>
</table>

To explain this more fully, the resolving power of the optical (light) system can be expressed as Abbe’s equation:

\[ R = 0.61 \frac{\lambda}{NA} \]

where:

- **R** is the (minimal resolvable) distance between distinguishable points (in nm),
- **\( \lambda \)** is the wavelength of the illumination source (in nm),
- **NA** is the numerical aperture of the objective lens.

The optimal resolving power for a light microscope is obtained with ultraviolet illumination (\( \lambda = 365 \)) if a system with the optimal NA is used (1.4). In this example:

\[ R = 0.61 \times 365 / 1.4 \]

\[ R = 159 \text{ nm} \]

The wavelengths of electrons in an SEM are much smaller than the wavelength of ultraviolet light (see Table 2). For example UV light has a wavelength of 365nm whereas an electron beam has a wavelength of around 0.005nm: an impressive difference.

Table 2. Wavelengths of electron beams generated at different accelerating voltages (compare with Table 1)

<table>
<thead>
<tr>
<th>Accelerating Voltage (kV)</th>
<th>Wavelength (nm) (symbol = ( \lambda ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.0055</td>
</tr>
<tr>
<td>100</td>
<td>0.0039</td>
</tr>
<tr>
<td>200</td>
<td>0.0025</td>
</tr>
<tr>
<td>1,000</td>
<td>0.0012</td>
</tr>
</tbody>
</table>
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 are bounced back out of the sample (backscattered electrons), others knock into atoms and displace electrons that, 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 heat is how most of the energy is dispersed. We collect the electrons coming out of the material in order to produce the traditional SEM images (called micrographs).

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.

Show the interaction volumes of: Secondary electrons (SE) Backscattered electrons (BSE) X-ray all
More detail

Electron-matter interactions can be divided into two classes:

1. **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).

2. **Inelastic scattering** – 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:
   - phonon excitation (heating);
   - cathodoluminescence (visible light fluorescence);
   - continuum radiation (bremsstrahlung);
   - characteristic x-ray radiation;
   - plasmon production (secondary electrons);
   - auger electrons (ejection of outer shell electrons).

Click **Play** to begin.
History

The first commercial SEM was produced by the Cambridge Instrument Company and became available in 1965.

In 1923, Busch demonstrated that a beam of electrons could be focused by magnetic or electric fields. This opened the way for the development and construction of the first TEM in 1932, by Knoll and Ruska, and the first SEM in 1938 by M. von Ardenne. Zworykin et al. (1942) produced an SEM with a potential resolution of 50 nm. These early SEMs had little better than LM performance. Great improvements in the design, particularly in the area of detection and the use of SE were made by O.W. Oatley and his group at Cambridge University. McMullan (1953) constructed a prototype SEM in the Cambridge Laboratories and by 1955 development had achieved a resolution of 25 nm. The first commercial SEM was produced by the Cambridge Instrument Company and became available in 1965 with a resolution of about 20-25 nm.

Since then, the SEM has evolved to become one of the most versatile tools of the physical and biological sciences. Today, many SEMs are commercially available and they all contain the same essential features: an electron optical column in which an electron beam is generated under high vacuum, and focussed to a tiny spot on the specimen surface. The specimen response is detected and then displayed in visual mode.
Scanning electron microscopy in practice

- Principles of SEM operation
- Sample preparation
- Virtual SEM
- Health and safety
Principles of SEM operation

A scanning electron microscope is a machine comprised of an electron generating component called the gun, a column through which the electron beam travels, a series of lenses to shape the electron beam, the sample chamber at the base, and a series of pumps to keep the system under vacuum. Below are some topics that help explore inside the machine and how the electron beam interacts with the sample.

- Layout
- Magnification
- The electron gun
- Filament saturation
- High vacuum mode and pump system
- Electromagnetic lenses, apertures and beam size
- Acceleration voltage vs. specimen type
- Generating an image
- Perfecting an image
- Troubleshooting
- Basic guide
Layout

Basic principles of the SEM

- **Scanning** moving back and forth
- **Electron** uses a focussed electron beam
- **Microscopy** designed to enlarge an image

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 (LM), the electrons in a scanning electron microscope (SEM) never form a real image of the sample. The SEM image is in the form of a serial data stream i.e. it is an electronic image. It is a result of the beam probe 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 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 i.e. a point-by-point translation. Increased magnification is produced by decreasing the size of the area scanned.

In more detail

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. 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.
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 a picture of the tiny spheres that are produced from a lit party sparkler.
More detail

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.

Large spot size

Small spot size

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 amount 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 adjoining 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).
The electron gun

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 gun is made up of a number of components.

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. An actual assembly, minus Wehnelt cap, is shown in the photograph. 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 10Åµm in diameter whereas the tungsten crystal is sharpened to a much narrower tip.

Below the cap sits an anode, which, being positive, attracts the electrons away from the filament. If the filament is broken, the beam current will not increase on the SEM because no electrons can be produced (see SEM Operation).
The electron gun is used to provide a large, stable current in a small electron beam.

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 emitting of thermal electrons. The emitted electrons are those that have overcome the work function energy of the material.

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.

Two important parameters for any electron gun are the amount of current produced and the current stability. At the saturation point the beam is most stable.

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 on intensity values, projected as a function of the position upon the specimen. Since the micrograph is acquired over a period of time of minutes (at the slow scan rate used for photographs of high quality), any changes in the filament emission 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.
Saturating the filament

An important factor in using a thermionic gun is understanding **saturation** of the filament. The more current that is put through the filament, the greater the emission of electrons from the tip region. However a point is reached where the emission is at 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).

![Graph showing filament current against electron emission](image)

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

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: read about how to saturate a filament below.

**How to saturate a filament**

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 **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 (100x), 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.

Table 3 A comparison of SEM filament types

<table>
<thead>
<tr>
<th>Emission</th>
<th>Thermionic</th>
<th>LaB6</th>
<th>FE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (angstroms)</td>
<td>1 x 10^6</td>
<td>2 x 10^5</td>
<td>&lt;1 x 10^2</td>
</tr>
<tr>
<td>Brightness (A/cm^2.steradian)</td>
<td>104 – 10^5</td>
<td>105 – 10^6</td>
<td>107 – 10^9</td>
</tr>
<tr>
<td>Energy Spread (eV)</td>
<td>1 – 5</td>
<td>0.5 – 3.0</td>
<td>0.2 – 0.3</td>
</tr>
<tr>
<td>Operating Lifetime (hrs)</td>
<td>&gt;20</td>
<td>&gt;100</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Vacuum (torr)</td>
<td>10^-4 – 10^-5</td>
<td>10^-6 – 10^-7</td>
<td>10^-9 – 10^-10</td>
</tr>
</tbody>
</table>

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.
High vacuum mode and pump system

This is the normal mode of operation for the SEM. A high vacuum minimises scattering of the electron beam before reaching 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.

Because 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 the detectors can be used (but only semi-qualitavely) in the so-called '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 materials.

Note: In low vacuum operational mode it is not the low energies of SEs that limits their use; the main reason is due to arcing in the Everhart-Thornley detector as a result of the kV scale bias on the scintillator.

Choose an option to change the pressure mode of the sample chamber. Ready mode Evacuation mode Vent mode
Electromagnetic lenses, apertures and beam size

A series of electromagnetic lenses and apertures are used to reduce the diameter of the source of electrons and to place a small, focused beam of electrons (or spot) onto the specimen.

An electromagnetic lens is a coil of wire through which current flows. Because the current flow produces a magnetic field at right angles, the field pushes inwards into the hole in the centre. This acts to shape a beam of electrons travelling in their natural spiral path down the central hole.

Focus and lenses

The purpose of a lens is to change the path of the rays in a desired direction. Glass or transparent plastic may bend light and so are used in optical lenses. However, glass or plastic lens will stop electrons. Therefore, it is not appropriate to use glass or plastic as lenses in an electron microscope. Since electrons are charged particles and they can be bent in a magnetic field. Lenses for electrons are constructed with ferromagnetic materials and windings of copper wire. These produce a focal length which 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 is at the top and the objective lens at the bottom. Each does a different job. The condenser lens convergences the cone of the electron beam to a spot below it, before the cone flares out again and is converged back again by the objective lens and down onto the sample. This initial convergence can be 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 objective lens also has some influence over the diameter of the spot size of the electron beam on the specimen surface. But its main role is in focusing the beam onto the sample. Note: a focused beam produces a smaller spot on the surface than an under or over-focused beam.

### Aperture

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.
An aperture holder: this 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.

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.

For more detail on the topic of focus see 3.1.8: Generating an image and 3.1.10: Role of sample height.
A large aperture is chosen for low magnification imaging to increase 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 of fewer electrons and therefore a less bright image.

Table 4: 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.

<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 that 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.
Acceleration voltage vs. specimen type

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 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.

Show the interaction volumes of: Low voltage High voltage Both voltages
More detail

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. The disadvantage associated with the use of higher kV are addressed in detail under troubleshooting.

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.

Table: 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>
Generating an image

For routine scanning electron microscope images, secondary electrons (SE) form the usual image of 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 off to 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 there is another kind of In-lens SE detector in some machines.

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 shows protuberances (bumps) on the wing of an insect. Notice the whiter edge to each bump.

Backscattered (BS) electrons are high-energy electrons (>50 eV) from the primary incident beam that are ejected back out from the samples. 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, a grain of sand that is made up of a titanium mineral looks whiter than a grain made of a silicon material (Ti versus Si). In the image, the left picture is taken using backscattered electrons. Here there is a difference in contrast between the grains labelled Si and Ti where as in the right image, taken using secondary electrons, there is no difference in contrast between these grains. The sample is a mixture of mineral sand.

To increase the yield of SE emitted from the specimen, heavy metals such as gold or platinum are routinely used to coat specimens. An extremely thin layer is applied (~10 nm). This coating is applied for two main reasons: (1) Non conductive
specimens are often coated to reduce surface charging that can block the path of SE and cause distortion of signal level and image form; and (2) Low atomic number (Z) specimens (e.g. biological samples) are coated to provide a surface layer that produces a higher SE yield than the specimen material.

Because secondary electrons have very low energies, only those produced at the surface of the sample are able to escape and be collected by the SE detector. Electrons emitted from a surface that faces away from the detector or which is blocked by the topography of the specimen, will appear darker than surfaces that face towards the detector. This topographical contrast due to the position of the SE detector is a major factor in the "life-like" appearances of SE images. In the image of the beetle, the electron detector is in the top left corner, hence that region looks brightest. It is, however, not the only factor that contributes to the contrast and brightness in an SEM.

Backscattered electron images

Backscattered (BS) electrons are incident (primary) beam electrons that have been re-emitted from the sample through elastic scattering. They are high-energy electrons (>50 eV) that have undergone simple or multiple elastic scattering events within the specimen. 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.
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.

**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.

**BSE detectors:** Typically, BS detectors are solid state and comprised of a piece of silicon wafer. The incident beam passes through a hole in the detector before striking the sample. The Silicon diode is divided into sectors (quadrants) that can be summed or subtracted depending on the nature of image required. The normal BSE signal is referred to as COMP (compositional) and provides information about the average atomic number of the sample. A TOPO mode (topographic) is also available which provides surface landscape information and includes no compositional contrast.

The differences between SE, BS COMP and BS TOPO modes can clearly be seen using a Cu grid on a carbon background. In the image below, the top third is a typical SE image, the middle third is BS COMP mode and the lower third is BS TOPO mode.

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 Z 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.

As a general rule, 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.

**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, however, 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) (see Electron-Matter Interactions).
For example 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. The following image is from the secondary electron detector and shows a metal surface at the same magnification but with different beam energies (different kVs): 5kV; 10kV; 15kV; 30kV (across 1-4). Note that subsurface and compositional information is apparent in frames 3 and 4 because the SE detector is also gathering backscattered electrons.

More detail

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”
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 a 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.
Role of sample height

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 (grainier 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.

1. 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.
2. 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.
3. 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 (\(\alpha\)) 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>
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<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.
What is 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. At 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 be distorted to form an ellipse, due to a range of factors such as level of machining accuracy and the material of the pole-piece, imperfections in the casting of the iron magnets and 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 best 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.
Scan rate and signal to noise ratios

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.

More detail

The image quality of the 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 the displaying of this signal (S/N). Noise pulses are derived from such sources as beam brightness, condenser lens settings (spot size), and SE detector ‘gain’, 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. Thus, at condenser lens settings for high resolution (small spot sizes), the quantity of electrons reaching the specimens to interact with 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.
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 (therefore the graininess of the image). At low magnifications we use a larger spot size than at higher magnifications.

Images taken at the same magnification, kV, and working distance but using different spot sizes show these affects. The difference in blurriness (resolution) is easily seen across the series

The spot size on the far left side is 60 and the spot size on the far right is 17 (the series is 60, 50, 40, 30 and 17). The way spot size is reported depends on the make of the machine used. The image is of a cross section through carbon fibres (note the round profiles) embedded in a polymer. Some extraneous material is present on the surface.

More detail

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.
Troubleshooting: edge effect, charging, sample damage

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

1. 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.

2. 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.

![Diagram showing edge effects](image)

Changes in the interaction volume with topography

3. Charging

**Charging** is produced by build-up of electrons in the sample and their uncontrolled discharge, and 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. 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 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.

If available, an SEM with low vacuum capability or an Environment Scanning Electron Microscope (ESEM) can be used to control charging.

4. 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 volatilise 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.

5. 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. One way to work around this artefact is to take micrographs at low magnification, before moving to higher magnification.

Brass surface with a rectangle of carbon contamination from scanning the beam at a higher magnification.
A basic guide to using an SEM

Not all SEMs are used in the same sequence of steps but a general guide is provided here of what might be expected.

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:

1. Sample insertion
2. Beam adjustment
3. Image capture

See Operation example for a visual and more detailed tutorial on this topic.
Sample insertion and beam activation

1. To use the SEM you must first place a sample in the sample chamber. Some machines have a setting that says 'exchange position'. If so, check that the receiving stage inside the chamber is set at the exchange position.

2. 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.

3. Once the sample is placed in the sample chamber, you must remove the air by pumping it out again. This is called evacuation. It is worth bearing in mind that your sample should be able to survive exposure to a vacuum. Some specialised sample preparation may be needed (see main menu: Sample preparation). However, there are a range of SEMs and some of them work at lower vacuums, allowing wet samples to be viewed. Others allow viewing of frozen samples.

4. While you are waiting for the machine to reach its correct vacuum you can select the electron beam voltage that you will need to use (but don’t turn it on yet). Delicate samples such as many polymers and biological materials are better viewed under a beam of lesser intensity than most geological materials or metals.

5. Once the chamber has reached a suitable vacuum (this will be different on different machines), you can turn on the electron beam.
Beam Adjustment

1. Choose a low magnification to start with and ensure your contrast and brightness are adjusted so you can see an image. Adjust the scan speed to TV rate for easy viewing.

2. It may be necessary to adjust the probe size (spot size) to match the magnification. Choose a large spot size for low magnification and a small one for high magnification.

3. Focus the image. There may be a course and a fine focus control.

4. Correct the stigmators (x and y) if the image is excessively streaky or blurry and focus again.

5. You may be required to align the machine at this stage. If so there will be detailed instructions supplied to you. This might include changing an aperture to a larger or smaller size, adjusting the gun controls to provide the brightest image, and adjusting the "wobbler" to align the beam axis.

6. Once the machine is ready to use, move your sample about with the X and Y translators until you are looking at a region of the sample you want to see in more detail. You may want to rotate or tilt the sample to get the best angle of view. Check that you have enough room around your sample before tilting so as not to bump into anything in the chamber. Sometimes you will need to change the height (z-axis) of your sample (lower for greater depth of focus or to allow tilting; higher for greater resolution)

7. Increase your magnification.

8. Adjust the brightness and contrast again for a comfortable view.

9. Now adjust your focus and correct the stigmators until the image is crisp and in focus.
Image Capture

1. Different brands of SEM have slightly different processes for photography. To take a photograph you may need to adjust the contrast and brightness against a scale inbuilt in the machine, otherwise you can rely on your eyes. You will need to set the information up that you want at the bottom of your photograph, such as magnification or a scale bar. The basic principle involved in taking the photo is that you slow the scan rate of the beam down. Normal viewing occurs at “TV” rate, but the slower the scan rate, the more signal you collect as the beam scans the sample. This leads to a better, less “grainy” or “noisy” image.

2. Once the **photo** is taken you need to **save** it to a file for later access.

3. Now continue using the machine or remove the sample and replace it with a new one. To change samples you will need to return your sample to the settings necessary for sample exchange. In some machines this may involve returning to an initial setting, or exchange position. Turn the beam off and let air into the chamber.

4. Once you have finished your viewing session, take your sample **out** of the chamber. There may be a specific way the laboratory manager wants the machine left. You will be instructed in this. Otherwise, just remember to leave the **beam off** and the sample chamber **evacuated** (empty of air/ under vacuum).
Sample preparation

This is vital to getting good quality information from your sample. A poorly mounted or incorrectly processed sample can lead to viewing artefacts. This section is designed to help you choose what processes may be needed to prepare your sample. Text and video help guide you through processes.

Click the linked items below to read information or watch videos about each process.

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.
Virtual SEM - choose a sample

Please select a sample you would like to view under the SEM.

- Sparkler
- Bone
- Slag
- Rock
Virtual SEM - sparkler

Interactive simulation of a scanning electron microscope (SEM) imaging a children's sparkler. Shows the affect of such settings as accelerating voltage, spot size, Z-depth, filament current, magnification, and astigmatism.

Adobe Flash player is required to use the simulator.

Would you like to try this simulation in test mode?
Virtual SEM - bone

Interactive simulation of a scanning electron microscope (SEM) imaging a sample of bone. Shows the affect of such settings as accelerating voltage, spot size, Z-depth, filament current, magnification, and astigmatism.

Adobe Flash player is required to use the simulator.

Would you like to try this simulation in test mode?
Virtual SEM - slag

Interactive simulation of a scanning electron microscope (SEM) imaging welding slag. Shows the affect of such settings as accelerating voltage, spot size, Z-depth, filament current, magnification, and astigmatism.

Adobe Flash player is required to use the simulator.

Would you like to try this simulation in test mode?
Virtual SEM - rock

Interactive simulation of a scanning electron microscope (SEM) imaging a sample of rock. Shows the affect of such settings as accelerating voltage, spot size, Z-depth, filament current, magnification, and astigmatism.

Adobe Flash player is required to use the simulator.

Would you like to try this simulation in test mode?
Occupational health and safety in an EM lab

Occupational Health and Safety (OH&S) is an important aspect of electron microscopy laboratory and any workplace. Your health and safety (and that of others) is, and should be, the prime concern of any employer. Injuries can come at a great cost to you and the university/organisation, in both lost-time and money, as well as your general wellbeing. Major accidents/incidents may result in loss of productivity, large fines, and compensation payouts, loss of funding, increased insurance costs and even jail/fines for management/supervisors found guilty of negligence. It is in their interests to look after you.

Any workplace can be a dangerous place and unfamiliar surroundings may have hazards you may not be aware of. Knowledge of hazards is key defence. Trial and error is not a valid method of work. Experienced workers have developed safe ways of working in EM labs and every lab aims to adapt (and achieve) these current "best practices" to their unique situation and update their procedures regularly. Many accidents in workplaces happen within the first month of starting work.
EM Laboratory Hazards

Lab hazards may appear as an obvious and immediate hazard – e.g. cold burns from liquid nitrogen spills, while other dangers may be less visible. These “hidden dangers” can be just as, or even more, dangerous - e.g. suffocation (due to the depletion of oxygen) by the evaporation of liquid nitrogen.

Other hazards you may encounter in an EM lab include:

- **Chemical**: These are the major hazards present in a lab and include toxic, corrosive and suspected carcinogenic chemicals. e.g. biological fixatives (glutaraldehyde, osmium tetroxide) are designed to kill cells quickly and they will do the same to your cells. Some samples may also be a chemical hazard.

- **Electrical**: High voltage is present in electron microscopes and other electrical equipment. Covers afford protection and should not be removed. Liquids of any volume should be used with great caution around electrical equipment.

- **Thermal**: High or low temperatures e.g. hot plates, dry ice, cryogens (liquid Nitrogen).

- **Muscular**: This includes strains and ergonomic issues e.g. sitting for long periods at a microscope without adjusting the monitors or chair, may cause muscle strains.

- **Mechanical hazards**: (moving equipment parts, sharp edges) e.g. razorblades, polishers, saws. Electrical cables or water lines may also present a tripping hazard.

Less common hazards that may be present include:

- **Microbiological**: Live samples may cause infections, irritations or allergic reactions– these should only be present in labs designed to handle them (PC2 or PC3 laboratories).

- **Acoustic**: Noisy pumps, equipment and sudden loud noises (e.g. opening of improperly connected or unconnected gas cylinders).

- **Radiation**: (Uranium stains – used in TEM; and X-rays from EM’s) EM's generate high energy X-rays. However, exposure to these X-rays is practically impossible, due to microscope design. Improper modification of microscopes may cause leaks.

**Hazard awareness – the induction process**

As with any workplace, individuals should be inducted into the area to familiarize individuals with the local OH&S systems and be made aware of the general hazards in the workplace. This lab induction should contain basic information such as location and use of first aid and spill kits, fire-extinguishers, emergency contacts and procedures. Emergency exits and assembly points should also be explained. Location or sources of MSDS’s (Material Safety Data Sheets) are often included. Detailed information on hazards relating to individual processes or equipment may not always be presented in an initial induction. This information is best presented by the expert(s) in that process, usually at the start of training in that process. Often trainers will refer to Standard Operating Procedures or risk assessments to explain these hazards. Workplaces are required to document your understanding of these procedures or the risk assessments, and you are quite often asked to "sign-off" on a document or acknowledge this in some other way such as by passing a quiz.
Risk assessments

In EM labs you will often find risk assessments for the use of equipment including:

- Individual microscopes
- Coaters,
- Ovens,
- Critical Point Dryers and other sample preparation equipment.

You will also find risk assessments for sample preparation chemical techniques including:

- Chemical fixation
- Dehydration
- Methods for drying samples (e.g. HMDS)

A risk assessment is a formal analysis of hazards involved in a process, to determine how dangerous a process is – i.e. the risk. It incorporates measures to make it safe to do the process - the controls. Risk assessments are required to be conducted in every workplace by law. (When do you do a risk assessment? Assessments should be conducted by experienced persons, before any new process is undertaken, when changing the process or if an incident has occurred. Assessments should also be routinely re-assessed.)

Hazard are the potential dangers present in workplace task. The risk is the overall likelihood that an injury (or other detrimental event) will occur because of the hazard.

To determine the risk, you have to look at 3 factors.

1. **Consequence** – The most likely outcome if control measures failed and an incident occurred. (It is not the worst case scenario.) Consequences can be personal injury, environmental or economic damage. They range from Minor (e.g. first aid, small bruises) to Catastrophe (e.g. many fatalities, extensive environmental/financial cost) with several categories in-between.

2. **Exposure** – How often the process takes place. This is not how often, for example, “you spill a chemical on yourself”, but how often you “undertake the process using that chemical”. Your exposure to a process may range from Very Rare (has never happened yet) to Continuous (occurs many times per day).

3. **Probability** – The likelihood that the consequence you have chosen will occur while undertaking the process. Probability ranges from Practically Impossible to Almost Certain.

Once individual ratings have been determined for these 3 factors, a risk rating can be calculated for the process. This risk can be calculated using a variety of systems. These include line-bar systems, number systems or risk matrices. The higher the consequence, exposure or probability - the greater the risk. Risk categories (and actions recommended) are listed below. Your resulting risk may be categorized as follows:

<table>
<thead>
<tr>
<th>Risk level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Risk is acceptable, it is ok to continue process, and no immediate improvements are needed.</td>
</tr>
<tr>
<td>Moderate to Substantial</td>
<td>Risk is a concern. Situation is not an emergency but procedures should be improved as soon as practicable.</td>
</tr>
<tr>
<td>High to Very High</td>
<td>Immediate corrective action is required. Process should be stopped until risk is reduced.</td>
</tr>
</tbody>
</table>

With any risk assessment, additional controls should be implemented until the resulting risk is considered low (at the very least). The effect of controls (no matter what the risk rating) should be monitored and reviewed to provide continual...
improvements. Your aim is to produce a risk assessment document that comes out with a low risk for the process (e.g. handling your sample and generating your data). You rework the document with new ideas on how to control the risks until it comes out in this “safe” category.

Here is a list of types of control measures, from most to least effective.

1. **Eliminate** the hazard is the first choice
2. **Substituting** a less hazardous material, process or equipment
3. **Redesigning** the equipment or work process,
4. **Isolating** the hazard through engineering – separating the worker from the hazard
5. **Administrative** controls involve minimizing exposure to a risk through the use of procedures or instruction.
6. **Personal Protective Equipment (PPE)** is used as a last resort when exposure to risk is not or cannot be minimized by other means.

When any chemicals are involved in a process, it is good practice to complete a Chemical Risk Assessment as well as the Standard Risk Assessment. A Chemical Risk Assessment incorporates the chemical properties of all chemicals involved in the process into the assessment of the task. It includes the crucial information from the Material Safety Data Sheets (MSDS) such as hazardous properties, routes of exposure, required control measures, first aid, waste disposal and spill procedures.

Workplaces are required by law to have on hand MSDS’s for all hazardous substances – including your sample (in some cases). MSDS’s can be obtained from the manufacturer, supplier or from on-line databases. MSDS’s for unique chemicals (e.g. of your creation) or your unique samples may not exist. This is where a chemical risk assessment (produced by experts in the material – you) is crucial in keeping all those who may contact your material safe.

As many hazards in an EM lab are of a chemical nature, the Chemical Risk Assessment may be a more practical guide to a chemical process than the Standard Risk Assessment alone. Your sample is a form of chemical and you may be required to submit a chemical risk assessment. This should address these issues. You may not need to write a risk assessment from scratch. Other colleagues may have worked on similar material and their risk assessment may suit your situation. Research groups often develop sample risk assessments for submission to other labs. EM labs often have prepared assessments for common samples and you may be able to adapt these to match your sample.

**Examples**

1. Transport and analysis of a SEM sample
2. Using a SEM

### Major hazards in SEM labs

<table>
<thead>
<tr>
<th>Major Hazards in SEM Labs</th>
<th>Actions/ Effects</th>
<th>Possible Controls</th>
</tr>
</thead>
</table>
| **Liquid Nitrogen**       | Cryo-burns, Asphyxiation, Brittlement of materials. May liquify and concentrate oxygen to levels that can allow combustion on warming. | ● PPE: Cryo-gloves, safety glasses, face shield, cryo-aprons, and enclosed shoes.  
| **Glutaraldehyde / Formaldehyde** | Toxic, corrosive, sensitiser. Vapours may cause serious damage to eyes. Environmental toxin. | ● PPE: Gloves, protective eyewear, lab coat, enclosed shoes.  
  ● Spill Kit. |
<table>
<thead>
<tr>
<th>Substance</th>
<th>Description</th>
<th>PPE Protection Measures</th>
</tr>
</thead>
</table>
| Osmium tetroxide                  | Toxic, corrosive. Vapours may cause serious damage to eyes/lungs. At high concentrations or in crystalline form, it has a stronger toxic vapour effect than glutaraldehyde. | - Gloves, protective eyewear, lab coat, enclosed shoes.  
- Spill Kit containing milk powder (neutralizer). |
| Acids                             | Corrosive                                                                   | - Gloves, protective eyewear, lab coat, enclosed shoes.  
- Spill Kit. |
| Sodium Cacodylate buffers         | Toxic, possible skin sensitiser. Environmental toxin (contains arsenic).     | - Gloves, protective eyewear, lab coat, enclosed shoes. |
| Samples                           | May be toxic, corrosive, carcinogenic, irritants, oxidizers.......          | - Sample dependent.  
- Appropriate labelling required. |
| Hexamethydisilazane - HMDS        | Highly toxic; flammable; corrosive; irritant; liberates H₂ gas.              | - Gloves, protective eyewear, lab coat, enclosed shoes.  
- Engineering: Fumehood, correct containers, bunding.  
- Spill Kit. Safety shower/eyewash. |
| Ethanol, Methanol, Acetone – dehydration or cleaning liquids. | Flammable; irritant to eyes; toxic (methanol); may cause dizziness.          | - Gloves, protective eyewear, lab coat, enclosed shoes.  
- Engineering: Fumehood, correct containers, bunding.  
- Spill Kit. Safety shower/eyewash. |
| Electrical hazards – High voltage equipment | Electrocution                                                               | - High voltage components are enclosed. Areas or equipment undergoing servicing are roped off from access by unqualified persons. |
| Ergonomics - Microscope workstations | Muscular stains may result from extended use of equipment. Workstations employing ancillary equipment (e.g. multiple monitors, computers) may have poor ergonomics if not modified to suit individual users. | - Chair, monitor height, keyboard distance should be modified to suit individual user. Regular breaks should be taken (5 minutes every hour). |
Risk assessment example: transport and analysis of a SEM sample

The Risk Analysis process involves:

- Determining the context and hazards. Risk assessments are for individual processes (what might be perfect for one situation/sample may not suit another). Individual types of samples may have their own risk assessment, but there is nothing wrong in considering a range of sample types in one assessment, if the hazards and controls are similar. A generic assessment for say, a non-hazardous solid sample, may be used as a basis but individual sample types may have their own unique hazards and the assessment should be modified accordingly.

- Considering what control measures are/can be used to minimize the hazards. Using the hierarchy of control measures - Elimination, Substitution, Redesign, Administrative & Personal Protective Equipment (PPE). MSDS's, industry guidelines or state regulations may give you information on what controls are best.

- Analysing the risk, Determining the Consequences. Exposure and Probability and applying these to a risk calculator.

- Determining the overall risk (and adding additional controls). The overall risk should be low. Additional controls can be applied to any risk assessment (whether low risk or not) and effectiveness of controls should be checked on a regular basis.

<table>
<thead>
<tr>
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<th>Current Controls</th>
<th>Risk Analysis</th>
<th>Resulting Risk</th>
</tr>
</thead>
</table>
| **Risk Situation 1: Exposure to Sample** | **ENGINEERING:** Sample transported in sealed container **ADMINISTRATIVE & TRAINING:** Correct labelling of sample required and monitored. Should include health and risk statements, owner and contact number. Risk assessment available on site. This containing spill and first aid procedures. All users of SEM trained in appropriate care of samples during transportation and use. | **Risk Analysis Details**
**Consequence:** Minor - No injury is likely from short term contact with sample. **Exposure:** Occasional - Individuals transport sample to EM labs approximately once per week on average. **Probability:** Conceivable - No exposure to samples has resulted in injuries but it is conceivable. | **Risk Level:** Low  
**Suggested Action:** Risk is normally acceptable |

**Notes:** Samples may be processed in lab of origin and mounted on standard SEM stubs before transport to EM laboratories for further analysis. 

**Hazard Event:** Person contacts sample.

<table>
<thead>
<tr>
<th>Risk Situation 2: Sample &amp; Microscope interactions</th>
<th>Current Controls</th>
<th>Risk Analysis</th>
<th>Resulting Risk</th>
</tr>
</thead>
</table>
| **Notes:** Sample properties must be considered before use in the microscope. Interaction with the beam or vacuum may liberate volatile components/loose particles which may affect/damage microscope parts. Sample size must also be considered (care must be taken with sample insertion and height adjustment to avoid detector/microscope damage). All new sample types should be discussed with trained staff before use. Hazard Event: Specimen is inserted into microscope and releases components or physically contacts delicate internal microscope parts which subsequently require cleaning/replacement. | **ADMINISTRATIVE:** Written standard working procedure. Restricted access. Documented consideration of sample risk to microscope – this risk assessment. Instruction manual provided. Access restricted to trained individuals. Samples are required to be “dried” (de-volatised) under vacuum for at least 1 day before microscope use. **TRAINING:** Job specific & general OHS induction. All staff and clients are trained and tested in safe operating procedures. | **Risk Analysis Details**
**Consequence:** Substantial - Most likely consequence from hazard event would result in a slight disruption in over all centre activities and/or a substantial financial loss ($5,000 to $50,000 - parts, staff/engineer time) **Exposure:** Occasional - Individuals, on average, use a microscope approximately once a week. **Probability:** Remotely possible - It would be a remote possibility that control measures may fail and a hazard event occurs. Incidents have occurred in the past. | **Risk Level:** Low  
**Suggested Action:** Risk is normally acceptable |

**Chemical Risk Assessment**

The Chemical risk assessment should complement the Standard Risk Assessment. It is more of a “hands on” assessment
relating to the chemical components of the sample (including the chemicals used to mount it) and contains MSDS information. It can be useful to refer to an original MSDS, which can be sourced online (e.g. Chemwatch) or from the supplier. Often an MSDS for your sample does not exist, and you may need to refer to its individual components. MSDS’s are often written for industrial situations – with large volumes of that chemical present. The MSDS may need to be interpreted and applied to this specific situation. (e.g. A substances MSDS spill procedure of “shovel into suitable container or drum, wear P3 mask” may not be relevant for a 1 gram, non-powdered form of the material you may have). This summary chemical risk assessment is for a sample that does not have any special health effects or special clean up procedures.

<table>
<thead>
<tr>
<th>Substances and Properties</th>
<th>Summary of existing controls and effectiveness</th>
<th>Risk Control</th>
<th>Risk Analysis Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAMPLE COMPONENTS:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - Super glue cw# 90335</td>
<td>Engineering: Sample transported in sealed container</td>
<td>Elimination or substitution of hazardous chemical/s: Not possible. <strong>Required engineering controls</strong>: Isolation/containment: Transportation in sealed plastic containers recommended for maintaining samples. Administrative controls (including training requirements): Training (job specific / general OHS induction): Training on first use.</td>
<td><strong>Consequence</strong>: Minor - Sample composed of small volumes (&lt;1g) and in a solid form. Health effects of the original chemical components are unlikely. No injury is likely from short term contact with sample. <strong>Exposure</strong>: Occasional - Individuals transport sample to EM labs approximately once per week on average. <strong>Probability</strong>: Conceivable - No exposure to samples has resulted in injuries but it is conceivable. <strong>Risk is</strong>: Significant but controlled. As a general guide, if you are working with a hazardous substance, it will always be “a significant risk” – but it can be a controlled risk.</td>
</tr>
<tr>
<td></td>
<td>Administration: Correct labelling of sample required and monitored.</td>
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<td></td>
<td>Training: All users of SEM trained in appropriate care of samples during transportation.</td>
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<td></td>
<td>PPE: Gloves (Nitrile/latex) recommended when handling samples.</td>
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<td></td>
<td>Current Controls are: Effective and maintained well.</td>
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<tr>
<td>2 - Dry conductive carbon paint: Graphite cw# 10065</td>
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<td></td>
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<tr>
<td>Form: solid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conc: 100%</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Haz Substance: No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG Class: None</td>
<td></td>
<td></td>
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<tr>
<td>Health Effects: None</td>
<td></td>
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<tr>
<td>Hazardous Reactions: None</td>
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<tr>
<td>3 - SAMPLE: Fixed (sterile) biological specimen – Rat skin.</td>
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<td>Form: solid</td>
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<tr>
<td>Conc: 100%</td>
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<tr>
<td>Haz Substance: No</td>
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<tr>
<td>DG Class: None</td>
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<tr>
<td>Health Effects: None</td>
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<tr>
<td>Hazardous Reactions: None</td>
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<tr>
<td>4 – Mounting stub:</td>
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<tr>
<td>Aluminium cw# 10039</td>
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<tr>
<td>Form: solid</td>
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</tr>
<tr>
<td>Conc: 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haz Substance: No</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>DG Class: None</td>
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<td></td>
<td></td>
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<tr>
<td>Health Effects: None</td>
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<td></td>
</tr>
<tr>
<td>Hazardous Reactions: None</td>
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<td></td>
</tr>
<tr>
<td>FOR ENTIRE SAMPLE:</td>
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</tr>
<tr>
<td>Route of Exposure: None</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>likely</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Evidence of Exposure: None</td>
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<tr>
<td>Storage Location: Returned to lab of origin.</td>
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</tbody>
</table>

Example 2: Using a SEM.
Risk assessment example: using a SEM

The Risk Analysis process involves:

- Determining the context and hazards. Risk assessments are for individual processes. What might be perfect for one situation/machine may not suit another. Individual pieces of hazardous equipment should have their own risk assessment. A generic assessment for say, a SEM, may be used as a basis but individual instruments may have their own unique hazards and the assessment should be modified accordingly.

- Considering what control measures are/can be used to minimize the hazards. Using the hierarchy of control measures - Elimination, Substitution, Redesign, Administrative & Personal Protective Equipment (PPE). Industry guidelines or state regulations may give you information on what controls are best.

- Analysing the risk. Determining the Consequences. Exposure and Probability and applying these to a risk calculator.

- Determining the overall risk (and adding additional controls). The overall risk should be low. Additional controls can be applied to any risk assessment (whether low risk or not) and effectiveness of controls should be checked on a regular basis.

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<tr>
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<th>Current Controls</th>
<th>Risk Analysis</th>
<th>Resulting Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Situation 1: Tripping over hoses or leads behind microscopes</td>
<td>Current Controls: ENGINEERING: Ramp/covers over leads. Barricading. ADMINISTRATIVE &amp; TRAINING: Restricted area signage. Inform all clients that they are not to walk around the back of microscopes (access restricted to specific personnel).</td>
<td>Risk Analysis Details Consequence: Substantial - Possible disabling injury requiring medical treatment. Exposure: Rare - Access to area limited to trained Engineers &amp; CMM staff and rarely visited. Probability: Conceivable - This event may happen but has not occurred so far.</td>
<td>Risk Level: Low Suggested Action: Risk is normally acceptable</td>
</tr>
</tbody>
</table>

Notes: There may be leads and vacuum hoses on the floor behind the scanning electron microscope that are required for its operation and which are unable to be relocated. Hazard Event: Trip and fall, resulting in hitting the floor or equipment.

<table>
<thead>
<tr>
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<th>Resulting Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Situation 2: Slipping due to flooding</td>
<td>Current Controls: ADMINISTRATIVE: Scheduled instrument maintenance / Lab monitoring. Detailed spill procedures - Use of signage to warn of wet floor. Mop/ Vacuum floors using wet/dry vacuum cleaner. Seal off area for mopping. Lab appropriate shoes required - monitored by staff.</td>
<td>Risk Analysis Details Consequence: Substantial - Possible disabling injury requiring medical treatment. Exposure: Rare - Flooding and water spills occur rarely in areas where slippage may result. Probability: Unusual but possible - Slippage due to water spills is an unusual event in the laboratory</td>
<td>Risk Level: Low Suggested Action: Risk is normally acceptable</td>
</tr>
</tbody>
</table>

Notes: Water spills occur occasionally in the laboratory (e.g. leaking water chiller lines) creating slippery surfaces. Hazard Event: Person slips and contacts equipment or floor.
<table>
<thead>
<tr>
<th>Hazard</th>
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<th>Resulting Risk</th>
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</thead>
</table>
| **Risk Situation 3: Electrocution** | **Current Controls:** ISOLATION: All high voltage components are enclosed and raised above floor level (to avoid water leaks/spills) and meet required Australian Standards. ADMINISTRATION: Access restrictions. Room access restricted to users trained in correct operation of microscope. Restricted access (service personnel only) and signage at rear of microscope (where most high voltage cables/components are sited). Room access restricted during service work and panels replaced as soon as practical. TRAINING: Instrument specific. Includes specific reference to high voltage hazard. Only instrument manager nominated and qualified service personnel are allowed to access restricted zones and remove panels or modify microscope. | **Risk Analysis Details** Consequence: Very serious - Most likely outcome from exposure to high voltage components is death. Exposure: Occasional - Individuals use microscope on average once a week. Probability: Practically impossible - It would be practically impossible during standard operation of the microscope for an individual to receive a fatal electric shock. | **Risk Level:** Low  
**Suggested Action:** Risk is normally acceptable |

**Notes:** SEM contains high voltages capable of serious or fatal shocks to individual users. **Hazard Event:** Individual removes panel and contacts exposed high voltage cable or components.

| Risk Situation 4: Electrocution - through flooding | Current Controls: ISOLATION: All high voltage components are enclosed Components are raised above floor level (to avoid water leaks/spills) and meet required Australian Standards. This includes power boards, leads and plugs. ADMINISTRATION: Room access restricted to users trained in correct emergency procedures. Restricted access (service personnel only) and signage at rear of microscope (where most high voltage cables/components are sited). TRAINING: Instrument specific. Includes specific reference to high voltage hazard. Only instrument manager nominated and qualified service personnel are allowed to access restricted zones where potential leaks may occur. | **Risk Analysis Details** Consequence: Very serious - Most likely outcome from exposure to high voltage components is death. Exposure: Unusual - Water leaks do occur. Probability: Conceivable - It is conceivable a water leak may contact high voltage and cause electrocution but has not happened. | **Risk Level:** Low  
**Suggested Action:** Risk is normally acceptable |

**Notes:** Electron microscopes contain high voltages and are cooled by pressurised water. Water leaks may contact high voltage components. **Hazard Event:** Water cooling line breaks/ leaks causing flooding and contact with high voltage circuitry/ power board. Individual contacts electrified water.
<table>
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<th>Resulting Risk</th>
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</thead>
<tbody>
<tr>
<td><strong>Risk Situation 5: Liquid Nitrogen spills causing cryo burns</strong></td>
<td><strong>Current Controls:</strong> ADMINISTRATIVE: Prescribed training and recording of competency. The routine use of LN2 requires Lab specific training, risk assessments and recorded competency for high risk activity. TRAINING: “Hands on” – conducted by competent staff member. Coverage includes, for example, correct handling practice by a trained staff member i.e. pour slowly and be aware of potential for sudden “boiling” and spraying of LN2. PPE: Wearing of appropriate approved PPE, face shield AND safety glasses for eyes, cryo-gloves to protect hands, enclosed shoes with trousers covering openings into shoes. Training in correct use of PPE is always required.</td>
<td><strong>Risk Analysis Details</strong>&lt;br&gt;&lt;strong&gt;Consequence:** Minor - Most likely injury would only requires first aid treatment e.g. minor burn. <strong>Exposure:</strong> Frequent - Liquid nitrogen is used frequently with the electron microscope for a range of different purposes. <strong>Probability:</strong> Conceivable - Probability is conceivable but has never happened with the current control measures</td>
<td><strong>Risk Level: Low</strong>&lt;br&gt;&lt;strong&gt;Suggested Action:** Risk is normally acceptable**</td>
</tr>
</tbody>
</table>

**Notes:** Liquid nitrogen is required for a range of purposes with the scanning electron microscope e.g. cryo-SEM, anticontaminant traps, EDS detector dewars.<br><strong>Hazard Event:** Cryo burns to the body.

| **Risk Situation 6: Spill of liquid nitrogen causing oxygen depletion** | **Current Controls:** ADMINISTRATIVE: Prescribed training and recording of competency. The routine use of LN2 requires Lab specific training, risk assessments and recorded competency for high risk activity. TRAINING: Coverage includes, for example, correct handling practice by a trained staff member i.e. pour slowly and be aware of potential for sudden “boiling” and spraying of LN2. PPE: Wearing of appropriate approved PPE, face shield AND safety glasses for eyes, cryo-gloves to protect hands, enclosed shoes with trousers covering openings into shoes. Training in correct use of PPE is always required. | **Risk Analysis Details**<br><strong>Consequence:** Very serious - Asphyxiation may result from exposure. **Exposure:** Occasional - Liquid nitrogen is used in volumes capable of producing asphyxiation approximately once per week per individual. **Probability:** Conceivable - Has not happened but is conceivable. | **Risk Level: Low**<br><strong>Suggested Action:** Risk is normally acceptable** |

**Notes:** Liquid nitrogen is regularly used to maintain diffusion pumps. Small volumes (~2 L) are used per microscope.<br><strong>Hazard Event:** Large spill of liquid Nitrogen produces oxygen displacement and collapse of individual.

| **Risk Situation 7: Ionizing radiation exposure** | **Current Controls:** ENGINEERING: Microscope design and shielding. Design features contain ionizing radiation when produced (eg shielding) and prevent generation of an electron beam if chamber is open (electronic safety controls). Time delay in chamber venting and/or double-door systems, coupled with the rapid dissipation of radiation, prevents residual hazardous radiation being present on opening. ADMINISTRATIVE: Restricted access policy. Only certified engineers, or those directly supervised by them, are allowed to modify/repair microscope. TRAINING: Clients are trained to follow standard operating procedures. | **Risk Analysis Details**<br><strong>Consequence:** Serious - Most likely consequence of long term exposure to this radiation is a permanent disability. **Exposure:** Occasional - Individuals use microscopes once a week on average. **Probability:** Practically impossible - It would be practically impossible for an individual to be exposed to this radiation. | **Risk Level: Low**<br><strong>Suggested Action:** Risk is normally acceptable** |

**Notes:** Interactions between the electron beam and sample/stage generate ionizing radiation during normal operation. The relatively low kV of standard SEM beams results in low energy x-ray production. Engineering features isolate hazardous ionizing radiation from the operator to minimise exposure to this radiation.<br><strong>Hazard Event:** Individual removes shielding or replaces viewing port with non-lead glass which allows ionizing radiation to escape.
<table>
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</thead>
<tbody>
<tr>
<td><strong>Risk Situation 8: Falling from ladder</strong></td>
<td><strong>Current Controls:</strong> ENGINEERING: Appropriate ladders (of Aust. Std) are to be used to fill traps and dewars – no stools. ADMINISTRATIVE: Procedure done by trained staff. Dedicated ladders. Clients are not routinely required to fill EDS dewars as this is done by trained staff. Ladders (of Aust. Standard) dedicated to fill traps and dewars are provided. TRAINING: All staff/clients using ladders trained. Trained and tested by demonstration in ladder use in accordance with manufacturer’s instructions (i.e. locked &amp; fully extended, do not stand above second from top rung, do not reach beyond side of ladder, 3 points of contact at all times).</td>
<td><strong>Risk Analysis Details</strong>&lt;br&gt;&lt;strong&gt;Consequence:** Substantial - It is possible that this incident could result in a permanent or lost time injury. &lt;strong&gt;Exposure:** Frequent - Dewars are filled at least 3 times per week with LN2 and this requires the use of a ladder. <strong>Probability:</strong> Conceivable - The probability is determined to be conceivable but has not happened.</td>
<td><strong>Risk Level:</strong> Low</td>
</tr>
<tr>
<td><strong>Notes:</strong> With some microscopes, climbing short ladders is required to fill traps/dewars with liquid nitrogen from height.</td>
<td><strong>Hazard Event:</strong> Person falls from ladder.</td>
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</table>

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<thead>
<tr>
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<th>Resulting Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk Situation 9: Sample &amp; Microscope interactions</strong></td>
<td><strong>Current Controls:</strong> ADMINISTRATIVE: Written safe working procedure. Restricted access. Instruction manual provided. Access restricted to trained individuals. Samples are required to be “dried” (de-volatised) under vacuum for at least 1 day before microscope use. TRAINING: Job specific / &amp; general OHS induction. All staff and clients are trained and tested in safe operating procedures.</td>
<td><strong>Risk Analysis Details</strong>&lt;br&gt;&lt;strong&gt;Consequence:** Substantial - Most likely consequence from hazard event would result in a slight disruption in overall centre activities and/or a substantial financial loss ($5,000 to $50,000 - parts, staff/engineer time) &lt;strong&gt;Exposure:** Occasional - Individuals, on average, use a microscope approximately once a week. <strong>Probability:</strong> Remotely possible - It would be a remote possibility that control measures may fail and a hazard event occurs. Incidents have occurred in the past.</td>
<td><strong>Risk Level:</strong> Low</td>
</tr>
<tr>
<td><strong>Notes:</strong> Sample properties must be considered before use in the microscope. Interaction with the beam or vacuum may liberate volatile components/loose particles which may affect/damage microscope parts. Sample size must also be considered (care must be taken with sample insertion and height adjustment to avoid detector/microscope damage). All new sample types should be discussed with trained staff before use. <strong>Hazard Event:</strong> Specimen is inserted into microscope and releases components or physically contacts delicate internal microscope parts which subsequently require cleaning/replacement.</td>
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ammrf.org.au/myscope/sem/practice/safety/example2.php
<table>
<thead>
<tr>
<th>Hazard</th>
<th>Current Controls</th>
<th>Risk Analysis</th>
<th>Resulting Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk Situation 10:</strong> Ergonomic Issues Including: Visual fatigue</td>
<td><strong>Current Controls:</strong> ADMINISTRATIVE: Bookings are restricted to 2 hours (generally) during prime time. Trainers observe and document demonstrated correct ergonomic procedures. TRAINING: Individuals trained in correct ergonomic procedures. Clients are advised: • to stretch and to take regular breaks in accordance with organisations screen based equipment guidelines. • to change between sitting and standing postures to offset static loads on muscles and change compressive forces on intervertebral discs.</td>
<td><strong>Risk Analysis Details</strong> Consequence: Substantial - Disabling injury requiring medical treatment e.g. carpal tunnel problems. Exposure: Occasional - Individuals, on average, use a microscope approximately once a week. Use of the keyboard and mouse occurs frequently during operation of the electron microscope. Probability: Remotely possible - It would be a remote possibility that control measures may fail and a hazard event occurs. Incidents have occurred in the past.</td>
<td><strong>Risk Level:</strong> Low</td>
</tr>
<tr>
<td><strong>Notes:</strong> Persons using the scanning electron microscope spend most of the session time viewing a CRT or LCD screen.</td>
<td><strong>Suggested Action:</strong> Risk is normally acceptable</td>
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<tr>
<td><strong>Hazard Event:</strong> Eyestrain.</td>
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<tr>
<td><strong>Prolonged sitting</strong></td>
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<tr>
<td><strong>Notes:</strong> Computer operation is usually performed in a seated posture. The chair design and adjustment directly affect the persons posture when viewing the computer screen and operating the input devices (eg mouse or hand gesture touch pad, keyboard, voice activation software). Poor chair design or adjustment will result in awkward postures and will increase the risk of musculoskeletal injury particularly to the back and shoulders when sitting for most of the hours worked. <strong>Hazard Event:</strong> Prolonged periods of uninterrupted sitting results in lower back, neck and shoulder pain during and after task completion. A chair without seat height adjustment, backrest angle tilt and backrest height is used; awkward and unsupported posture results.</td>
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<tr>
<td>Keyboard, mouse and joystick use</td>
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<tr>
<td><strong>Notes:</strong> Use of a keyboard, mouse, joystick to enter and manipulate information on a computer screen is a major feature of EM work. Addition of ancillary equipment, not designed for specific work station, may increase the effects of keyboard overuse. <strong>Hazard Event:</strong> Continuous keyboard mouse or joystick use without rest breaks due to a high volume of work. Strain of the shoulder, forearm and wrist.</td>
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</table>

### Chemical Risk Assessment

The Chemical risk assessment should complement the Standard Risk Assessment. It is more of a “hands on” assessment relating to the chemical component of the task and contains MSDS information.
### Substances and Properties

#### Summary of existing controls and effectiveness

**Elimination or substitution of hazardous chemical/s:** not possible as liquid nitrogen is an essential requirement.

**Required engineering controls:** Oxygen sensors.

**Other engineering controls:** General ventilation
General air conditioning in large rooms or open areas is suitable.

**Administrative controls (including training requirements):** Training (job specific / general OHS induction). All staff and clients are trained in correct handling procedures. Written emergency procedures. Posted on site. Written safe working procedure. General liquid Nitrogen use risk assessment and SOP.

**Personal protective equipment:**
- **Gloves:** Cryo-gloves
- **Eye protection:** Safety glasses with side protection. Face shield for large volume work
- **Coat/apron:** Cryo-apron/ lab coat
- **Footwear:** Enclosed shoes
- **Other:** Long pants (cuffs over shoes) recommended attire for large volume work.

### Risk Control

**Current Controls are:** Effective and maintained well.

**Engineering:** general ventilation.

**Administrative:** Appropriately signage. Small volume dispensers provided.

**PPE:** cryo-gloves, eye protection with side protection, cryo apron/ lab coat, enclosed shoes.

**Training:** Correct handling procedures.

### Risk Analysis Details

**Consequence:** Very serious - Asphyxiation may result from exposure.

**Exposure:** Occasional - Liquid nitrogen is used in volumes capable of producing asphyxiation approximately once per week per individual.

**Probability:** Conceivable - Has not happened but is conceivable.

**Risk is:** Significant but controlled - As a general guide, if you are working with a hazardous substance, it will always be “a significant risk” – but it can be a controlled risk.

---

**1 - Liquid nitrogen**

**Form:** liquid  
**Conc:** concentrated  
**Haz Substance:** Yes  
**DG Class:** 2.2 - Non flammable, non toxic gas

**Health Effects:** Asphyxiant

**Hazardous Reactions:** Cryogenic

**Route of Exposure:** Inhalation, Eye & skin contact

**Evidence of Exposure:** none

**Storage Location:** 30 litre dewar in lab

**Waste disposal procedures:** Evaporated in well ventilated areas - Fumehood or external to building.  

**Storage incompatibilities:** Nil.

**Special safety instructions:** Skin: For cold burns, immerse in cold water. Inhaled: Fresh air, rest , keep warm, if breathing shallow, see medical help - give oxygen.
Additional material

- Recommended reading
- Useful links
- Operation example
Recommended reading


Useful links

General information

● earth.leeds.ac.uk/research/facilities/sem.htm
● www.mos.org/sln/SEM/index.html
● www.ingentaconnect.com/content/asp/jspm
● en.wikipedia.org/wiki/Environmental_scanning_electron_microscope

Micrographs

● cgc.rncan.gc.ca/geochem/model/eds_processing_e.php
● www.bath.ac.uk/ceos/psgallery.html
● www5.pbrc.hawaii.edu/microangelo/
● www.rit.edu/cias/photo/ipt/a-sem/

Micrographic art

● www.ldeo.columbia.edu/res/fac микро/

History

● www2.eng.cam.ac.uk/~bcb/semhist.htm
● www2.eng.cam.ac.uk/~bcb/cwo1.htm

Training manuals

● www.entomology.ksu.edu/DesktopDefault.aspx?tabindex=111&tabid=351
● micro.magnet.fsu.edu/primer/java/electronmicroscopy/magnify1/
● www.unl.edu/CMRAcfem/semoptic.htm
● en.wikipedia.org/wiki/Scanning_electron_microscope
● bama.ua.edu/~hsmithso/class/bsc_656/websites/sem.html

Just for fun

● www.mnh.si.edu/highlight/sem/
● www3.ntu.edu.sg/NanoCluster/events/nanoasart/
Example of machine operation using a JEOL instrument

Please select the type of image you would like to view under the SEM.

- Secondary electron (SE)
- Backscattered electron (BSE)