6. Electron microscopy

There is a limit below which it is not possible to resolve an image in light microscopy. However, structural information on a specimen can still be obtained, by using electrons instead of light. The principles involved are similar, although the operational practicalities are somewhat different. An electron microscope can be used to obtain magnification in the range $10^6 \times$. Gas molecules scatter (diffract) electron beams, so the vast majority of studies involving electron microscopy have to be at very low pressures, typically $1.33 \times 10^{-3} - 1.33 \times 10^{-5} \text{ Nm}^{-2}$. This limits the range of materials that can be studied using this technique to dry, solid specimens that are stable at these very low pressures.

The theory

The interaction of materials with electrons is shown in Fig. 1.

![Figure 1: Interaction of electrons with materials](image)

One major similarity between light and electron microscopy is that images can be formed from the radiation that is transmitted through the specimen or from radiation that comes back towards the radiation source, be it a lamp or an electron gun. In the case of electron microscopy, different conditions are necessary for generating and detecting the radiation. Scanning electron microscopes are useful for displaying images of surface structures, which are generated by secondary electrons. The transmission electron microscope relies on the primary electrons passing through the specimen to give high resolution images of internal structures of samples (which must be less than $1 \times 10^{-7} \text{ m}/0.1 \mu \text{m}$ thick).

X-rays are formed when a primary electron strikes an inner shell electron of an
atom in the specimen and gives it sufficient energy to ionise. Once the inner shell electron has been removed an electron from a higher energy orbital will drop down to the lower level and emit its excess energy as an X-ray photon. The energies of the photons produced are characteristic of the elements from which they have been formed. (The gap left at the higher level can then be filled by an electron from a level higher still, so that a range of characteristic X-ray energies is observed.)

The scanning electron microscope (SEM)

**Electron beam formation and focusing**

The most important signals to consider in the SEM are:

1. secondary electrons;
2. backscattered electrons; and
3. X-rays.

Secondary electrons are usually used to provide the image because the electron beam is not spread out and resolution is often very high, usually in the range $5 \times 10^{-9} – 2 \times 10^{-8}$ m ($5–20$ nm). This type of electron is generated as a result of inelastic scattering of the incident electrons (Fig. 2). The secondary electrons have low energies, typically $3–8 \times 10^{-19}$ J ($2–5$ eV) although they can be as high as $8 \times 10^{-18}$ J ($50$ eV). The inelastically scattered incident electrons can continue and cause other events.

![Figure 2](image-url)

**Figure 2** Scattering of electrons and X-ray formation

Backscattered electrons are the primary beam electrons that have been scattered elastically by the nuclei in the sample (Fig. 2). These electrons are useful for imaging the atoms in a specimen by atomic number contrast. This is because low atomic number samples give low emissions of backscattered electrons while high atomic...
number samples give high emissions of these electrons. The backscattered electrons have higher energies than secondary electrons – usually from approximately $8 \times 10^{-18}$ J (50 eV) up to the energy of the primary beam electrons.

The electrons can be scattered from relatively deep positions within the sample – typically up to $1 \times 10^{-7}$ m (100 nm), but because the spread of electrons is relatively large, the resolution of any image from these electrons is low (perhaps $2 \times 10^{-8}$ m) compared with secondary electron images.

The incident electrons are usually generated by passing an electric current through a tungsten filament at the top of a column (other methods exist such as applying a potential to a lanthanum hexaboride single crystal). A voltage, usually in the range 300 V to 40 kV, is applied between the electron source (the cathode) and the rest of the column (the anode). This voltage accelerates the electrons down the column, towards the specimen. Whereas light rays are focused in a light microscope by glass lenses, electrons are focused in an electron microscope by electromagnetic lenses (Fig. 3).

The condenser lens is used to collimate the electron beam which the objective lens focuses onto the specimen, producing a ‘probe’ of diameter ca $1 \times 10^{-8}$ m (10 nm). Scanning coils are then used to direct the beam across the specimen in a series of parallel lines so that when the parallel scans are put together a two dimensional image is obtained (similar to a domestic television set). Scan rates can be as fast as 25 frames per second for immediate study, or as slow as several minutes per scan if more clearly defined images are required for a photographic record.

![Figure 3](image_url)  
Figure 3  The scanning electron microscope (SEM)
The sample is held on a movable stage in a chamber at the base of the column. The stage enables the specimen to be moved in the x, y and z directions, and also allows for tilt and rotational adjustments to be made. (Some electron microscopes have air locks so that the sample can be changed while keeping the remainder of the column under vacuum.)

One limitation is that samples that are non-conductors of electricity have to be treated before they can be studied with the electron microscope. A plasma of gold ions is sputtered onto the sample at very low pressure and a thin film of gold forms on its surface. This coating inhibits image distortion by sample charging, and does not normally affect surface detail because the gold coating can only be detected at relatively high magnifications. Gold is often used because it is an excellent electrical conductor and being a heavy metal, has a high secondary and back scattering electron yield.

**Image formation**

Secondary electrons are useful for high resolution imaging. They are attracted by a grid, typically set at +200 to +600 V potential, in front of a scintillation detector. They are further accelerated by a potential of about 10 kV onto the scintillation detector surface, where their energy is converted to visible light. The light emitted passes down a perspex light guide to a photomultiplier tube where it is converted to an electrical current. This signal can be amplified to produce an image on a cathode ray tube (a television screen). A large number of secondary electrons results in a bright image on the screen.

Photographic images are produced by placing a camera in front of a suitable screen and moving images can also be recorded by using videotape. Images can be clarified by removing unwanted background ‘noise’ with the aid of a computer.

The magnification can be changed by changing the area of the sample scanned while keeping the screen size constant. A large magnification is achieved by scanning a very small area of the sample. The images obtained have an advantage over light microscopy images because they have a ‘three dimensional’ quality and have an appreciably greater depth of field ca 300 x better (see diagram of polymer bead, page 166).

**Chemical analysis**

The X-rays produced when primary electrons interact with the sample have energies characteristic of the elements contained in the specimen. A solid state detector can be used to measure the energy of the X-rays formed and, when used in conjunction with a computer, can be used to identify the atoms present. The systems are capable of identifying elements with atomic numbers 5–92 (boron to uranium) simultaneously (Fig. 4). The sample used was copper mounted on an aluminium base using a silver based adhesive.

Once the elements in a sample have been identified by the energies of the X-rays emitted from it, it is possible to programme a computer to display the location of the different elements in different colours on a screen. This can be done so that one element is shown per image, or many elements shown in the same image. Individual atoms cannot be ‘seen’, but their distribution in a sample can.

**The transmission electron microscope (TEM)**

**Electron beam formation and focusing**

Electron beam formation is similar to that in the SEM, but does not require the scanning coils (Fig. 5). The accelerating potential between the cathode (the electron source) and the anode (the rest of the column) tends to be higher than for the SEM –
usually 80–200 kV, although instruments with very high resolution might require a potential of 1 MV. Higher potentials are necessary to give the electrons sufficient energy to penetrate the specimen. The pressure inside the instrument also has to be lower to achieve high resolution images, typically $1.33 \times 10^{-4} - 1.33 \times 10^{-5}$ Nm$^{-2}$.

Electromagnetic condenser coils collimate the beam, which then strikes the specimen. The specimen must be dry, solid, stable, and capable of withstanding the heating effect of the electron beam. It must also be extremely thin (of the order $1 \times 10^{-7}$m) and transparent or semi-transparent to the beam. Micromted sections of samples can be used (a microtome is an instrument used for cutting thin slices from a sample), and techniques such as etching and chemical staining also can be used to visualise the detail in the final image.

**Image formation**

Once the electron beam has been passed through the specimen it is magnified and focused by an image forming electromagnetic lens (the ‘objective’). It then strikes a fluorescent screen where the energy of the electrons is converted to visible light, forming an image. The image can be viewed through a lead glass window. Alternatively, the screen can be replaced by a camera so that a photographic image is recorded.

If scanning coils are used, high resolution images can be obtained from the secondary electrons detected. Alternatively, the X-rays emitted can be analysed. If the TEM is put into scanning mode (STEM) it is possible to deduce where in a bulk sample small amounts of material are. Under favourable conditions, as little as $10^{-16}$ g of a substance can be detected in STEM mode.

**Figure 4** Identification of elements from their characteristic X-rays in a scanning electron microscope
Applications

Electron microscopy does have some limitations. Images of living systems cannot be obtained, and the preparation of a specimen can be complicated and expensive. Without programming a computer to assign different colours to different grey tones the images produced are always in monochrome (ie they are not in colour). However, a wide range of materials can be studied by electron microscopy. These include: powder particles; soaps; hair; teeth; bacteria; timber; plastics; metals and foils; plant and animal tissues; lotions, creams and emulsions (eg ice creams) [see Advanced Techniques, page 171]; foodstuffs and oils; and packaging.
Scanning electron microscope images

Electron microscopy has been used in industry to study the pore size in a Polyhipe (poly high internal phase emulsion). To the naked eye and under low magnification the polymer appears similar to expanded polyphenylethene (polystyrene). However, under the scanning electron microscope it is possible to see the fully interconnected open pore structure of the material – it is about 90 per cent air (Fig. 6). By measuring the pore sizes (about $5 \times 10^{-6}$ m) and its structure, its properties can be determined.

![Internal structure of 'polyhipe'™](image)

**Figure 6** Internal structure of ‘polyhipe’™
It is also possible to study the effect of shampoos on human hair. Under the electron microscope the structure of a hair can be seen clearly, and the changes in the structure of the hair before and after treatment with a shampoo can be followed. Other fibres, such as wool, dog hairs, nylon etc, can be identified from their structures and any scales (cuticle) covering them.

![Image of human hair broken under tension and showing the outer cuticle and the inner cortex](image)

**Figure 7** Human hair broken under tension and showing the outer cuticle and the inner cortex

Soiled fabrics can also be studied by using the electron microscope, because dirt particles which are bound to different fibres can be seen. The efficiency of different types of soap and detergent in removing the particles can then be determined.
Figure 8  Cotton fibres with soil particle attached

Figure 9  Dental plaque
**Figure 10**  Knuckle joint on a bee’s head where the antenna is attached
Transmission electron microscope images
Using the TEM images of carbon dioxide gas hydrate crystals can be obtained (Fig. 11). The image was obtained by forming a carbon replica of the surface and shadowing the replica with tungsten, and it is possible to see images of the crystals embedded with an ice (1h) matrix. (The magnification is x $10^4$.)

![Figure 11](image)

**Figure 11** Carbon dioxide gas hydrate crystals

TEM can also be used to look at thin sections. Figure 12 is of Crambe abyssinica, a commercial oil seed. The thin section was obtained by fixing with potassium mangate (VII) and embedding in epoxy resin before sectioning with a microtome. Under magnification of $4 \times 10^3$ the dark areas mainly represent protein and cell walls, nuclei and starch bodies can be seen clearly.
Advanced techniques

Some materials do not lend themselves to electron microscopy because of their physical state or stability. However, useful images can still be formed. For example, replicas of liquids and soft solids are formed under high vacuum by evaporating platinum/carbon at an oblique angle onto a previously frozen sample. This produces ‘shadows’ which highlight changes in topography, and improve image contrast. Carbon is then evaporated from a gun mounted directly above the sample forming a continuous thin film layer. Later, at room temperature and pressure, the original sample material is dissolved away and the remaining carbon–platinum/carbon film (literally a ‘carbon copy’) is placed in the TEM for examination. Alternatively, it is sometimes possible to use the frozen sample as the specimen itself. In such cases they are typically frozen to –180 °C so that their vapour pressures are insignificant and the heating effect of the incident electrons is unlikely to cause them to melt.

It is sometimes inappropriate to put certain samples into an electron microscope – eg skin in the study of the effect of a moisturising cream. In this case a sample of skin would have to be removed from the subject before and after application of the cream. This is clearly unacceptable, so a negative of the skin is taken by painting a liquid polymer over the skin and peeling it off once it has cured to a rubber-like consistency. A positive is then made by pouring an epoxy resin (such as ‘Araldite’) into the polymer ‘mould’ and removing the polymer once the resin has set. The positive is then coated with gold. The result is a specimen that is easy to handle and is stable to the vacuum and the electron beam.

Scanning electron microscopes are available that are capable of extremely high resolution – down to approximately 8 x 10^-10 m (0.8 nm). These require a very high vacuum – of the order of 1.33 x 10^-8 Nm^-2. Such instruments are not cheap, however, and currently (1992) cost about £500 000. Transmission electron microscopes are capable of even greater resolution – to about 1.4 x 10^-10 m (0.14 nm).