...but didn’t dare to ask!

All you wanted to know about 

Electron Microscopy...
Introduction
This booklet is written for those who know little or nothing about electron microscopy and would like to know how an electron microscope works, why it is used and what useful results it can produce.

"With a microscope you see the surface of things. It magnifies them but does not show you reality. It makes things seem higher and wider. But do not suppose you are seeing things in themselves."

Feng-shen Yin-Te (1771 – 1810)
In The Microscope 1798

A publication of
FEI Electron Optics
FEI Company, one of the world’s leading suppliers of transmission and scanning electron microscopes.

Our commitment to electron microscopy dates back to the mid-1930s, when we collaborated in EM research programmes with universities in the UK and the Netherlands. In 1949, the company introduced its first EM production unit, the EM100 transmission electron microscope.

Innovations in the technology and the integration of electron optics, fine mechanics, microelectronics, computer sciences and vacuum engineering have kept FEI at the forefront of electron microscopy ever since.

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Nobody knows for certain who invented the microscope. The light microscope probably developed from the Galilean telescope during the 17th century. One of the earliest instruments for seeing very small objects was made by the Dutchman Antony van Leeuwenhoek (1632-1723) and consisted of a powerful convex lens and an adjustable holder for the object being studied (specimen). With this remarkably simple microscope (Fig. 1), Van Leeuwenhoek may well have been able to magnify objects up to 400x and with it he discovered protozoa, spermatozoa and bacteria and was able to classify red blood cells by shape.

The limiting factor in Van Leeuwenhoek’s microscope was the quality of the convex lens. The problem can be solved by the addition of another lens to magnify the image produced by the first lens. This compound microscope – consisting of an objective lens and an eyepiece together with a means of focusing, a mirror or a source of light and a specimen table for holding and positioning the specimen – is the basis of light microscopes today.

*Why use electrons instead of light?*

A modern light microscope (often abbreviated to LM) has a magnification of about 1000x and enables the eye to resolve objects separated by 0.0002 mm (see box A). In the continuous struggle for better resolution, it was found that the resolving power of the microscope was not only limited by the number and quality of the lenses but also by the wavelength of the light used for illumination. It was impossible to resolve points in the object which were closer together few hundred nanometres – see box B). Using light with a short wavelength (blue or ultraviolet) gave a small improvement; immersing the specimen and the objective lens in a medium with a high refractive index (oil) gave another small improvement but these measures together only brought the resolving power of the microscope to just under 100 nm.

The word is derived from the Greek mikros (small) and skopeo (look at). Ever since the dawn of science there has been an interest in being able to look at smaller and smaller details. Biologists have wanted to examine the structure of cells, bacteria, viruses and colloidal particles. Materials scientists have wanted to see inhomogeneities and imperfections in metals, crystals and ceramics. In the diverse branches of geology, the detailed study of rocks, minerals and fossils could give valuable insight into the origins of our planet and its valuable mineral resources.

**Resolution and Magnification (1)**

Given sufficient light, the unaided human eye can distinguish two points 0.2 mm apart. If the points are closer together, only one point will be seen. This distance is called the resolving power or resolution of the eye. A lens or an assembly of lenses (a microscope) can be used to magnify this distance and enable the eye to see points even closer together than 0.2 mm. Try looking at a newspaper photograph or one in a magazine through a magnifying glass for example.

**What is Electron Microscopy?**
In the 1920s it was discovered that accelerated electrons (parts of the atom – see box C) behave in vacuum just like light. They travel in straight lines and have a wavelength which is about 100,000 times smaller than that of light. Furthermore, it was found that electric and magnetic fields have the same effect on electrons as glass lenses and mirrors have on visible light. Dr. Ernst Ruska at the University of Berlin combined these characteristics and built the first transmission electron microscope (often abbreviated to TEM) in 1931. For this and subsequent work on the subject, he was awarded the Nobel Prize for Physics in 1986. The first electron microscope used two magnetic lenses and three years later he added a third lens and demonstrated a resolution of 100 nm (see box D), twice as good as that of the light microscope. Today, using five magnetic lenses in the imaging system, a resolving power of 0.1 nm at magnifications of over 1 million times can be achieved.

**The Nanometre**

As distances become shorter, the number of zeros after the decimal point becomes larger, so microscopists use the nanometre (abbreviated to nm) as a unit of length. One nanometre is a millionth of a millimetre (10⁻⁹ metre). An intermediate unit is the micrometre (abbreviated to µm) which is a thousandth of a millimetre or 1000 nm.

Some literature refers to the Ångström unit (abbreviated to Å) which is 0.1 nm and the micron for micrometre.

**The Electron**

One way of looking at an atom is to visualise it as a minute "solar system" in which electrons orbit like planets round a central nucleus. The nucleus consists of neutral and positively charged particles and the electrons have a compensating negative charge so that the atom is neutral. The electrons which are about 1800x lighter than the nuclear particles occupy distinct orbits each of which can accommodate a fixed maximum number of electrons.

When electrons are liberated from the atom, however, they behave in a manner analogous to light. It is this behaviour which is made use of in the electron microscope, although we should not lose sight of the electron’s role in the atom, which will be made use of later.

**Resolution and Magnification (2)**

The resolving power of a microscope determines its maximum magnification. It is only necessary to magnify the resolving power to 0.2 mm, the resolving power of the unaided human eye, for all the fine detail of an object to be revealed. Higher magnification will not reveal any more information and is unnecessary.

The resolving power of a microscope is one of its most important parameters. The reason that magnifications are often quoted is that it gives an idea of how much an image has been enlarged.
Transmission Electron Microscope (TEM)
The transmission electron microscope can be compared with a slide projector (Fig. 2). In the latter, light from a light source is made into a parallel beam by the condenser lens; this passes through the slide (object) and is then focused as an enlarged image onto the screen by the objective lens. In the electron microscope, the light source is replaced by an electron source (a tungsten filament heated in vacuum), the glass lenses are replaced by magnetic lenses and the projection screen is replaced by a fluorescent screen which emits light when struck by electrons. The whole trajectory from source to screen is under vacuum and the specimen (object) has to be very thin to allow the electrons to penetrate it.

Not all specimens can be made thin enough for the TEM. Additionally, there is considerable interest in observing surfaces in more detail. Early attempts at producing images from the surface of a specimen involved mounting the specimen nearly parallel to the electron beam which then strikes the surface at a very small angle. Only a very narrow region of the specimen appears in focus in the image and there is considerable distortion. The technique has not found wide application in the study of surfaces.

Penetration
Electrons are easily stopped or deflected by matter (an electron is nearly 2000x smaller and lighter than the smallest atom). That is why the microscope has to be evacuated and why specimens – for the transmission microscope – have to be very thin in order to be imaged with electrons. Typically, the specimen must be no thicker than a few hundred nanometres.

Scanning Microscopy
Imagine yourself alone in an unknown darkened room with only a fine beam torch. You might start exploring the room by scanning the torch beam systematically from side to side gradually moving down so that you could build up a picture of the objects in the room in your memory.

A scanning microscope uses an electron beam instead of a torch, an electron detector instead of eyes and a fluorescent screen and camera as memory.

**Fig. 2** The transmission electron microscope compared with a slide projector

**Fig. 3** A modern transmission electron microscope the Tecnai 12.
Scanning Electron Microscope (SEM)

It is not completely clear who first proposed the principle of scanning the surface of a specimen with a finely focused electron beam to produce an image of the surface. The first published description appeared in 1935 in a paper by the German physicist Dr. Max Knoll. Although another German physicist Dr. Manfred von Ardenne performed some experiments with what could be called a scanning electron microscope (usually abbreviated to SEM) in 1937, it was not until 1942 that three Americans, D. Zworykin, Dr. Hillier and Dr. Snijder first described a true SEM with a resolving power of 50 nm and a magnification of 8000x. Nowadays SEMs can have a resolving power of 1 nm and can magnify over 400 000x.

Figure 4 compares light microscopy (using transmitted or reflected light) with TEM and SEM. A combination of the principles used in both TEM and SEM, usually referred to as scanning transmission electron microscopy (STEM), was first described in 1938 by Dr. Manfred von Ardenne. It is not known what the resolving power of this instrument was. The first commercial instrument in which the techniques were combined was a Philips EM200 equipped with a STEM unit developed by Dr. Ong of Philips Electronic Instruments in the U.S.A. (1969). At that time, the resolving power was 25 nm and the magnification 100 000x. Modern TEMs equipped with a STEM facility can resolve 1 nm at magnifications of up to 1 million times.
There are four main components to a transmission electron microscope: an electron optical column, a vacuum system, the necessary electronics (lens supplies for focusing and deflecting the beam and the high voltage generator for the electron source), and software. A TEM from the Tecnai series comprises an operating console surmounted by a vertical column about 25 cm in diameter and containing the vacuum system, and control panels conveniently placed for the operator (Fig. 3).

The Transmission Electron Microscope

The column is the crucial item. It comprises the same elements as the light microscope as can be seen from the ray paths of light and electrons (Fig. 6). The light source of the light microscope is replaced by an electron gun which is built into the column. The glass lenses are replaced by electromagnetic lenses and the eyepiece or ocular is replaced by a fluorescent screen. The entire electron path from gun to screen has to be under vacuum (otherwise the electrons would collide with air molecules and be absorbed) so the final image has to be viewed through a window in the projection chamber. Another important difference is that, unlike glass lenses, electromagnetic lenses are variable: by varying the current through the lens coil, the focal length (which determines the magnification) can be varied. (In the light microscope variation in magnification is obtained by changing the lens or by mechanically moving the lens).

The electron gun
The electron gun comprises a filament, a so-called Wehnelt cylinder and an anode. These three together form a triode gun which is a very stable source of electrons. The tungsten filament is hairpin-shaped and heated to about 2700 °C. By applying a very high positive potential difference between the filament and the anode, electrons are extracted from the electron cloud round the filament and accelerated towards the anode. The anode has a hole in it so that an electron beam in which the electrons are travelling at several hundred thousand kilometres per second (see box G) emerges at the other side. The Wehnelt cylinder which is at a different potential, bunches the electrons into a finely focused point (Fig. 7).

If the specimen were not thin, the electrons would simply be stopped and no image would be formed (see box E "Penetration" on page 6). Specimens for the TEM are usually 0.5 micrometres or less thick. The higher the speed of the electrons, in other words, the higher the accelerating voltage in the gun, the thicker the specimen that can be studied.

The beam emerging from the gun is condensed into a nearly parallel beam at the specimen by the condenser lenses and, after passing through the specimen, projected as a magnified image of the specimen onto the fluorescent screen at the bottom of the column.

Other electron sources exist and these are discussed briefly under "Additional Techniques" on page 21.
**Electron Velocity**

The higher the accelerating voltage, the faster the electrons. 80 kV electrons have a velocity of 150 000 km/second (1.5 x 10^8 m/s) which is half the speed of light. This rises to 230 000 km/second for 300 kV electrons (2.3 x 10^8 m/s – more than three-quarters the speed of light).

Fig. 6 Ray paths of light in a light microscope (LM) compared with those of electrons in a transmission electron microscope (TEM).

**Electron Density**

How many electrons impinge on the specimen? A typical electron beam has a current of about 1 picoampere (10^-12 A). One ampere is 1 coulomb/sec. The electron has a charge of 1.6 x 10^-19 coulomb. Therefore approximately 6 million electrons per second impinge on the specimen.
What happens in the specimen during the electron bombardment?
Contrary to what might be expected, most specimens are not affected by the electron bombardment as long as it is kept under control. When electrons impinge on the specimen, a number of things can happen:

a. Some of the electrons are absorbed as a function of the thickness and composition of the specimen; these cause what is called amplitude contrast in the image.
b. Other electrons are scattered over small angles, depending on the composition of the specimen; these cause what is called phase contrast in the image.
c. In crystalline specimens, the electrons are scattered in very distinct directions which are a function of the crystal structure; these cause what is called diffraction contrast in the image.
d. Some of the impinging electrons are reflected (these are called backscattered electrons).
e. The impinging electrons can cause the specimen itself to emit electrons (these are called secondary electrons).
f. The impinging electrons cause the specimen to emit X-rays whose energy and wavelength are related to the specimen’s elemental composition.
g. The impinging electrons cause the specimen to emit photons (or light); this is called cathodoluminescence.
h. Finally, electrons which have lost an amount of energy because of interaction with the specimen can be detected by an Energy Loss Spectrometer which is the equivalent of a prism in light optics.

In a standard TEM the first two phenomena contribute to the formation of the normal TEM image for non-crystalline (biological) specimens, while for crystalline specimens (most non-biological materials), phase contrast and diffraction contrast are the most important factors in image formation. It is necessary to add accessories or peripheral equipment to the basic microscope in order to exploit the additional information which can be obtained by studying the last five interactions listed above.

The electromagnetic lenses
Figure 10 shows a cross-section of an electromagnetic lens. When an electrical current is passed through the coils (C), an electromagnetic field is created between the pole pieces (P) which form a gap in the magnetic circuit. By varying the current through the coils, the magnification of the lens can be varied. This is the essential difference between the magnetic lens and the glass lens. Otherwise they behave in the same way and have the same types of aberration: spherical aberration (the magnification in the centre of the lens differs from that at the edges), chromatic aberration (the magnification of the lens varies with the wavelength of the electrons in the beam) and astigmatism (a circle in the specimen becomes an ellipse in the image).

Spherical aberration is a very important characteristic which is largely determined by the lens design and manufacture. Chromatic aberration is reduced by keeping the accelerating voltage as stable as possible and using very thin specimens. Astigmatism can be corrected by using variable electromagnetic compensation coils.

The condenser lens system focuses the electron beam onto the specimen under investigation as much as necessary to suit the purpose. The objective lens produces an image of the specimen which is then magnified by the remaining imaging lenses and projected onto the fluorescent screen.

If the specimen is crystalline there will be a diffraction pattern at a different point in the lens known as the back focal plane. By varying the strength of the lens immediately below the objective lens, it is possible to enlarge the diffraction pattern and project this onto the fluorescent screen. In the Tecnai series of TEMs, the objective lens is followed by four lenses: a diffraction lens, an intermediate lens and two projector lenses. To guarantee a high
lens and the diffraction lens can be selected from outside the column as dictated by circumstances.

**Observation and recording of the image**

The image on the fluorescent screen can be observed through a large window in the projection chamber (some models even have two windows). In order to examine fine detail or to assist correct focusing of the image, a special fine grain focusing screen can be inserted into the beam and observed through a high-quality 12x binocular viewer.

As in any other branch of science, a permanent record of what has been observed with the eye is desired. Antony van Leeuwenhoek painstakingly drew what he saw in his microscope. The invention of photography enabled microscopists to photograph what they saw. Electrons have the same influence on photographic material as light. Therefore it is only necessary to replace the fluorescent screen with a photographic film in order to record the image. In practice the fluorescent screen hinges up to allow the image to be projected on the film below. It is also possible to use a TV camera to record the image digitally, making it suitable for subsequent enhancement and analysis.

TV observation is useful for instructional purposes or other occasions when group viewing is desired. It can also be used for recording dynamic phenomena using a video tape recorder or as the input signal for an image analysis system.

**Stability and magnification**

On the way from the filament to the fluorescent screen, the electron beam passes through a series of apertures with different diameters. These apertures stop those electrons which are not required for image formation (e.g. scattered electrons). Using a special holder carrying four different apertures, the diameter of the apertures in the condenser lens, the objective lens and the diffraction lens can be selected from outside the column as dictated by circumstances.

**Box I**

When a wave passes through a periodic structure whose periodicity is of the same order of magnitude as the wavelength, the wave emerging is subject to interference which produces a pattern beyond the object. The phenomenon can be observed when ocean waves pass through a regular line of posts or when a street lamp is seen through an umbrella. The street lamp appears as a rectangular pattern of spots of light, bright in the centre and then getting fainter. This is caused by diffraction of light by the weave of the umbrella fabric and the size and form of the pattern provide information about the structure (closeness of weave and orientation). In exactly the same way electrons are diffracted by a crystal and the pattern of spots on the screen of the microscope gives information about the crystal lattice (shape, orientation and spacing of the lattice planes) – see Fig. 11.

**Box II**

**Diffraction**

When a wave passes through a periodic structure whose periodicity is of the same order of magnitude as the wavelength, the wave emerging is subject to interference which produces a pattern beyond the object. The phenomenon can be observed when ocean waves pass through a regular line of posts or when a street lamp is seen through an umbrella. The street lamp appears as a rectangular pattern of spots of light, bright in the centre and then getting fainter. This is caused by diffraction of light by the weave of the umbrella fabric and the size and form of the pattern provide information about the structure (closeness of weave and orientation). In exactly the same way electrons are diffracted by a crystal and the pattern of spots on the screen of the microscope gives information about the crystal lattice (shape, orientation and spacing of the lattice planes) – see Fig. 11.
Vacuum
Normal atmospheric air pressure is around 760 mm of mercury. This means that the pressure of the atmosphere is sufficient to support a column of mercury 760 mm high. Modern physicists use the Pascal (Pa). Normal air pressure = 100 000 Pa; residual pressure in the microscope = 2.5 x 10^{-5} Pa. At this pressure, the number of gas molecules per litre is about 7x10^{12} and the chance of an electron striking a gas molecule while traversing the column is almost zero.

Vacuum
Electrons behave like light only when they are manipulated in vacuum. As has already been mentioned, the whole column from gun to fluorescent screen and including the camera is evacuated. Various levels of vacuum are necessary: the highest vacuum is around the specimen and in the gun; a lower vacuum is found in the projection chamber and camera chamber. Different vacuum pumps are used to obtain and maintain these levels. The highest vacuum attained is of the order of a ten millionth of a millimetre of mercury (see box J).

To avoid having to evacuate the whole column every time a specimen or photographic material or a filament is exchanged, a number of airlocks and separation valves are built in. In modern TEMs the vacuum system is completely automated and the vacuum level is continuously monitored and fully protected against faulty operation.

The electronics
To obtain the very high resolution of which modern TEMs are capable, the accelerating voltage and the current through the lenses must be extremely stable. The power supply cabinet contains a number of power supplies whose output voltage or current does not deviate by more than one millionth of the value selected for a particular purpose. Such stabilities require very sophisticated electronic circuits.

Improved electron optical design has made possible a number of increasingly more complicated electron-optical techniques. This in turn has created the need for more ease of operation. Digital electronic techniques in general and microprocessor-based techniques in particular play an important role in this respect. Modern electron microscopes employs a fast, powerful PC (personal computer) to control, monitor and record the operating conditions of the microscope. This results in a dramatic reduction in the number of control knobs compared with earlier models and a microscope which is very easy to use. Furthermore it allows special techniques to be embedded in the instrument so that the operator can carry them out using the same controls. The PC can be attached to a network to allow automatic back-ups to be made and results to be downloaded to other workstations. The microscope can always be brought up to date by installing new software or by replacing the computer with the latest model.

Fig. 14 Bend contours in a semiconductor.

Fig. 15 Arrangement of atoms in ceramic.

Fig. 16 Chromosome from a human cancer cell.

Fig. 17 Woven fabric (SEM image).
Specimen orientation and manipulation
With most specimens it is not sufficient to move them only in the horizontal plane. Although the specimen is thin, there is nevertheless information in the image coming from various depths within the specimen. This can be seen by tilting the specimen and taking stereo photographs. In order to define the axis of tilt, it is necessary to be able to rotate the specimen. Crystalline specimens need to have a second tilt axis perpendicular to the first tilt axis in order to be able to orient a part of the specimen so as to obtain the required diffraction pattern. These requirements can be fulfilled in a device called a goniometer.

The goniometer is a specimen stage designed to provide, in addition to X and Y translation of the specimen, tilt about one or two axes and rotation as well as Z movement (specimen height) parallel to the beam axis. It is usual also to provide for heating, cooling and straining of the specimen for specialised experiments in the microscope. The goniometer is mounted very close to the objective lens; the specimen is actually located in the objective lens field between the pole pieces because it is there that the lens aberrations are smallest and the resolution is highest.

The goniometer itself provides motorised X, Y and Z movement and tilt about one axis. The specimen is mounted near the tip of a rod-shaped holder which in turn is introduced into the goniometer through an air lock. It is the specimen holder rod which provides the extra tilt axis or the rotation or heating, cooling or straining, a special holder being needed for each purpose.

Specimen preparation
A TEM can be used in any branch of science and technology where it is desired to study the internal structure of specimens down to the atomic level. It must be possible to make the specimen stable and small enough (some 3 millimetres in diameter) to permit its introduction into the evacuated microscope column and thin enough (less than about 0.5 micrometres) to permit the passage of electrons.

Every branch of research has its own specific methods of preparing the specimen for electron microscopy. In biology for example, tissues are sometimes treated as follows: first there is a chemical treatment to remove water and preserve the tissue as much as possible in its original state; it is then embedded in a hardening resin; after the resin has hardened, slices (sections) with an average thickness of 0.5 micrometres are cut with an instrument called an ultramicrotome equipped with a glass or diamond knife. The tiny sections thus obtained are placed on a specimen carrier – usually a 3 mm diameter copper specimen grid which has been coated with a structureless carbon film 0.1 micrometre thick.

In metallurgy the following method of preparation is sometimes applied: a 3 mm diameter disc of material (thickness say 0.3 mm) is chemically treated in such a way that in the centre of the disc the material is fully etched away. Around this hole there will usually be areas that are sufficiently thin (approximately 0.1 micrometre) to permit electrons to pass through. In a semiconductor it is sometimes desired to cut out a section of material perpendicular to the surface in order to investigate a defect. This is done by ion-beam etching. We shall return to this in a later section.

A technique which is gaining importance in the field of structural biology is that of freezing the specimen and observing it in the frozen state.

It is beyond the scope of this booklet to describe the many specimen preparation techniques that are used nowadays. What can be said is that the preparation of a tiny specimen for TEM is often relatively complicated and certainly more complicated than the operation of present-day TEMs.

Scanning Transmission Electron Microscopy (STEM)
The technique can be applied in a SEM but there is much more interest in applying it in TEM because of the lenses beneath the specimen which greatly expand the number of possibilities for gathering information. This technique was first demonstrated by FEI in 1969. Today most TEMs can be equipped with this facility and what was once an attachment is now an integral part of the instrument.

The latest TEM from FEI offers a resolution of 1 nm at a magnification of more than 1 million times.

Once a scanning facility has been introduced into the TEM column, it is possible to take advantage of the backscattered electrons as well as of the secondary electrons which are emitted during specimen bombardment.

Fig. 13 A site-specific specimen from a 200 mm semiconductor wafer is thinned to about 300 nm. Cuts are made to partially detach the section. The wafer is tilted to 60° and FIB cuts made at the base and sides. From here, milling to final thickness will be done at low beam currents and final "release" cuts will be made at 0° tilt.
A scanning electron microscope, like the TEM, consists of an electron optical column, a vacuum system and electronics. The column is considerably shorter because there are only three lenses to focus the electrons into a fine spot onto the specimen; in addition there are no lenses below the specimen. The specimen chamber, on the other hand, is larger because the SEM technique does not impose any restriction on specimen size other than that set by the size of the specimen chamber.

The electronics unit is more compact: although it now contains scanning and display electronics which the basic TEM did not, the lens supplies and the high voltage supplies are considerably more compact.

The Scanning Electron Microscope

All the components of a SEM are usually housed in one unit (Fig. 5 page 7). On the right is the electron-optical column mounted on top of the specimen chamber. In the cabinet below this is the vacuum system. On the left is the display monitor, the keyboard and a "mouse" for controlling the microscope and the camera. All the rest is below the desk top which gives the whole instrument its clean appearance.

The electron gun at the top of the column produces and electron beam which is focused into a fine spot less than 4 nm in diameter on the specimen. This beam is scanned in a rectangular raster over the specimen. Apart from other interactions at the specimen, secondary electrons are produced and these are detected by a suitable detector. The amplitude of the secondary electron signal varies with time according to the topography of the specimen surface. The signal is amplified and used to cause the brightness of the electron beam in a cathode ray tube (CRT) to vary in sympathy. Both the beam in the microscope and the one in the CRT are scanned at the same rate and there is a one to one relationship between each point on the CRT screen and a corresponding point on the specimen. Thus a picture is built up (see Fig. 18). The ratio of the size of the screen of the viewing monitor (CRT) to the size of the area scanned on the specimen is the magnification (see box K). Increasing the magnification is achieved by reducing the size of the area scanned on the specimen. Recording is done by photographing the monitor screen (or, more usually, a separate high resolution screen), making videoprints or storing a digital image.

The electron gun

The electron gun consists of a filament and Wehnelt cylinder and is the same as that in a TEM. Also the illumination system, consisting of electron gun, anode and condenser lenses is not much different. The final lens focuses the beam onto the surface of the specimen to be studied.
Scanning Electron Microscope

In the introduction, the functioning of a SEM was compared, with some restrictions, with a reflected light microscope (Fig. 3). Another valid comparison is illustrated in Fig. 18. The electron beam trajectories in a SEM are very similar to those in a TV monitor. Both devices have an electron gun, both are evacuated, both have deflection coils.

The electrons produced when the primary beam strikes the specimen in a SEM are detected and turned into an electrical signal; in the monitor, the primary electrons are turned into light to produce an image on the fluorescent screen.
What happens in the specimen during electron bombardment?
When discussing the TEM, it was seen that when electrons strike the specimen several phenomena occur. In general, five of these phenomena are used in an ordinary SEM (see box M).

a. The specimen itself emits secondary electrons.
b. Some of the primary electrons are reflected (backscattered electrons).
c. Electrons are absorbed by the specimen.
d. The specimen emits X-rays.
e. The specimen sometimes emits photons (= light).

All these phenomena are interrelated and all of them depend to some extent on the topography, the atomic number (see box M) and the chemical state of the specimen. The number of backscattered electrons, secondary electrons and absorbed electrons at each point of the specimen depends on the specimen’s topography to a much greater extent than the other properties mentioned. It is for this reason that these three phenomena are exploited primarily to image the specimen’s surface.

Electron detection
Detectors for backscattered electrons and secondary electrons are usually either a scintillation detector or a solid state detector. In the former case, electrons strike a fluorescent screen which thereupon emits light which is amplified and converted into an electrical signal by a photomultiplier tube. The latter detector works by amplifying the minute signal produced by the incoming electrons in a semiconductor device. When the specimen is not directly connected to earth but via a resistor, the electrons that are not reflected generate a potential difference across the resistor. This changing potential difference can be amplified and the resulting signal used to produce a third sort of image on the monitor. This facility also permits the study of (dynamic) electrical phenomena in electronic devices such as integrated circuits.

Magnification and resolution
In the SEM, the magnification is entirely determined by the electronic circuitry that scans the beam over the specimen (and simultaneously over the fluorescent screen of the monitor where the image appears).

Magnification can be as high as 300 000x which is usually more than sufficient. In principle the resolution of a SEM is determined by the beam diameter on the surface of the specimen. The practical resolution however depends on the properties of the specimen and the specimen preparation technique and on many instrumental parameters such as beam intensity, accelerating voltage, scanning speed, distance from the last lens to the specimen (usually referred to as the working distance) and the angle of the specimen surface with respect to the detector. Under optimum conditions a resolution of 1 nm can be attained (see box N).

Observation and recording of the image
A SEM is usually equipped with two image monitors, one for observation by the operator and the other, a high resolution monitor, is equipped with an ordinary photo camera (which can be 35 mm or Polaroid).

To facilitate the observation and correct choice of the parameters mentioned above, FEI SEMs have an image store in which the image is built up scan by scan and displayed at TV speed so that there is a steady, flicker-free image on the viewing monitor. Images are digital and can be stored electronically for subsequent enhancement and analysis.

Image treatment
Because the image in a SEM is completely electronically produced, it can be subject to all kinds of treatment using modern electronics. This includes contrast enhancement,
inversion (black becomes white etc.), mixing of images from various detectors, subtraction of the image from one detector from that produced by a different detector, colour coding and image analysis. All these techniques may be applied if it suits the primary aim of extracting the best possible information from the specimen.

Vacuum
In general a sufficiently low vacuum for a SEM is produced by either an oil diffusion pump or a turbomolecular pump in each case backed by a rotary pre-vacuum pump. These combinations also provide reasonable exchange times for specimen, filament and aperture (less than 2 minutes) without the need to use vacuum airlocks. The SEM vacuum system is fully automatically controlled and protected against operating failures.

Electronics
It goes without saying that in a SEM the voltages and currents required for the electron gun and condenser lenses should be sufficiently stable to attain the best resolution. Similarly the stability of the electronic circuitry associated with the detectors should be extremely well controlled. Stabilities of 1 part per million are no exception.

All the electronic units are housed in the microscope console and are controlled by a personal computer (PC) using a keyboard and mouse.

Application and specimen preparation
A SEM can be used whenever information is required about the surface of a specimen. This applies in many branches of science and technology as well as life sciences. The only requirement is that the specimen can withstand the vacuum of the chamber and the electron bombardment.

Many specimens can be brought into the chamber without preparation of any kind. If the specimen contains any volatile components such as water, this will need to be removed by using a drying process (or in some circumstances it can be frozen solid). Non-conducting specimens will charge up under electron bombardment and need to be coated with a conducting layer. Because a heavy element like gold also gives a good yield of secondary electrons and thereby a good quality image, this is the favourite element for coating. In addition it gives a fine grain coating and is easily applied in a sputter coater. The layer required to ensure a conducting layer is quite thin (about 10 nm). All in all, the preparation of specimens to be investigated by SEM is not as complicated as the preparation of specimens for TEM.

Sometimes it is impossible or undesirable to prepare a specimen for SEM: many specimens in forensic science, for example, silicon wafers used in IC manufacture, and integrated circuits themselves which need to be studied whilst in operation. In such cases special techniques need to be employed in order to obtain satisfactory images. Low voltage SEM is one of these techniques. The modern SEM can be adapted to all these special techniques, sometimes with the addition of a suitable accessory.
**Specimen orientation and manipulation**

As has been mentioned, the quality of the image in a SEM depends on the orientation and the distance of the specimen from the detectors and the final lens. The specimen stage allows the specimen to be moved in a horizontal plane (X and Y direction) and up and down (Z direction) and rotated and tilted as required. These movements are often motorised and controlled by the PC.

The various SEM models in a range differ in the size of their specimen chambers allowing various sizes of specimens to be introduced and manipulated. The maximum specimen size also determines the price because the larger the specimen chamber, the larger the goniometer movement needed and the larger the pumping system needed to obtain and maintain a good vacuum.

The simplest model accepts specimens of a few centimetres in diameter and can move them 50 mm in the X and Y directions. The largest chamber accepts samples up to 200 mm in diameter and can move them 150 mm in each direction. All models allow samples to be tilted up to high angles and rotated through 360 degrees.

There are special stages for heating, cooling and straining specimens but because of the wide variety of possible sample sizes, these stages are produced by specialist firms.

**ESEM**

As mentioned above, samples for conventional SEM generally have to be clean, dry, vacuum compatible and electrically conductive. In recent years the Environmental Scanning Electron Microscope (ESEM) has been developed to provide a unique solution for problematic samples. Examples of specimens which pose problems are wool or cotton tissue, cosmetics, fats and emulsions (e.g. margarine).

Attempts to view a specimen containing volatile components by placing it in an environmental chamber (see box O) isolated from the main column by one or more differential pumping apertures used to be hampered by the lack of a suitable electron detector which can work in the atmosphere of the chamber. The Gaseous Secondary Electron Detector makes use of cascade amplification (see Fig. 20) not only to enhance the secondary electron signal but also to produce positive ions (see page 22) which are attracted by negative charge on the insulated specimen surface and effectively suppress charging artefacts.

![Fig. 21 Ice cream in the Cryo-SEM: the amount of emulsion in relation to air and water (ice) is an important parameter to study.](image_url)

### Box O

**Environmental Chamber**

The pressure-temperature phase diagram for H$_2$O indicates that true "wet" conditions only exist at pressures of at least 600 Pa at 0°C (environmental microscopists usually refer to 4.6 Torr = 4.6 mm of mercury – see box J). In the range 650 to 1300 Pa ($5 \times 10^{-1}$ Torr) therefore the specimen may be observed whilst at equilibrium with water.
Signal Amplification in the ESEM

Fig. 20 Gas ionisation amplifies the secondary electron signal and, in a non-conductive specimen, positive ions are attracted to the specimen surface as charge accumulates there.

Fig. 22 House mites have been filmed live and moving in the ESEM.

Fig. 23 Biomineralisation by a number of organisms illustrates an astounding range of skeletal ultrastructure. Photos courtesy of Natural History Museum, London.

Fig. 24 The cochlea is the hearing organ inside the ear. It is the vibration of the fibres which transmits sounds to the brain.
More information from the specimen
As already mentioned, the electrons bombarding the specimen cause it to emit X-rays whose energy is determined by the elemental composition. When a suitable detector is placed near the specimen (the goniometer allows for this), it is possible to detect very tiny quantities of elements. Quantities down to one thousandth of a picogram ($10^{-12}$ g) or less can thus be detected (see box P). The detector in question is called an Energy Dispersive X-ray Detector (EDX). With this detector, spectra are acquired showing distinctive peaks for the elements present with the peak height indicating the element concentration. This applies to both SEM and TEM.

It has also been mentioned that some of the electrons lose energy when they travel through the specimen. This loss of energy can be measured with an Electron Energy Loss Spectrometer (EELS). This detector is mounted under the projection chamber of the TEM.

Additional Techniques

Scanning Transmission Electron Microscopy
If the specimen in a SEM is sufficiently transparent for electrons to be transmitted through the specimen, these can be collected with a suitably placed detector. This STEM technique, sometimes applied in a SEM is more often encountered in a TEM because use can also be made of the lenses below the specimen. It is discussed on page 13.

X-ray Analysis
To return for a moment to the analogy of a person in an unknown darkened room with a fine beam torch (box F). While scanning the room to determine its topography, the viewer would also be aware of the colour of objects. Likewise by looking at the wavelengths of X-rays emitted (their "colour") we can determine which chemical elements are present at each spot and the intensity of the X-rays is a measure of the element concentration.

The impinging electron in the primary beam may cause an electron in an atom of the specimen to be removed from its orbit (if the atom is near the surface, it can become a secondary electron). The atom is left in an excited state (it has too much energy) and it returns to its stable state by transferring an electron from an outer orbit to replace the one removed (see box C). Finally the outermost missing electron is replaced by one from the free electrons always present in a material. Each of these transfers results in the release of surplus energy as an X-ray quantum of characteristic wavelength (in the case of outer to inner orbital transitions) or a light quantum (in the case of free electron to outer orbit transfers). We can see that different materials produce different colours by examining a colour television screen with a magnifying glass. Different phosphors produce different colours of light under the impact of the scanning electron beam.
The other phenomena that occur when the electron beam goes through the specimen (backscattering of the incident electrons and the emission of secondary electrons) are exploited by using the STEM technique which was discussed on page 13.

**Other electron sources**
The same type of hairpin-shaped tungsten filaments are used in both TEM and SEM. The higher the temperature of the filament, the more electrons are generated by the electron gun and the brighter the image. The lifetime of the filament however is reduced and a practical compromise sets a limit on the brightness of the tungsten gun. Two brighter sources of electrons have become popular in recent years: the lanthanum hexaboride (LaB₆) gun and the field emission gun (FEG). In the former, a lanthanum hexaboride crystal when heated gives off up to 10x more electrons than tungsten heated to the same temperature. In the latter, electrons are pulled out of a very fine pointed tungsten tip by a very high electric field. Electron densities up to 1000 times those from tungsten emitters can be obtained with the FEG.

The larger number of electrons or the greater electron density provided by these sources permits reductions in beam diameter which results in better resolution in both SEM and STEM. It also permits more accurate X-ray analysis in both instruments to be obtained. The gains are not however proportional to the increase in brightness: other factors such as lens aberrations also play a role.

Field emission TEMs and SEMs cost more than regular instruments but provide much higher performance.
**Ion Beam technology**

So far this booklet has been about electron microscopy and what useful information can be obtained using a primary electron beam. However, electrons are not the only charged particles that can be accelerated and focused using electrical and magnetic fields. It was explained earlier (see box C) that the atom consists of a positively charged nucleus surrounded by electrons in orbits. Normally the atom is neutral because there are equal numbers of protons and electrons. An atom that has lost one or more of its outermost electrons has a positive charge and can be accelerated, deflected and focussed in a similar way to its negatively charged cousin the electron.

The difference lies in the mass (analogous to weight – see box Q). The electron is almost 2000x lighter than the lightest ion and ions can be up to 250x heavier still. The relatively low-mass subatomic electrons interact with a sample and release secondary electrons which, when collected, provide high quality images at near atomic-level resolution. The greater-mass ions dislodge surface particles, also resulting in displacement of secondary ions and electrons. The ion beam directly modifies or mills the surface with submicron precision. Secondary electrons and ions are used for imaging and compositional analysis.

By carefully controlling the energy and intensity of the ion beam, it is possible to carry out very precise micro-machining to produce minute components or to remove unwanted material. Examples of applications are the production of thin-film heads for reading computer storage media and the removal of material round a defect in a semiconductor device to allow the defect to be studied in a transmission electron microscope (see Fig. 13, page 13).

How is such a fine beam controlled and how does the operator know when the micro-machining is finished? Quite simply by having a detector to observe the secondary electrons produced by the impact of the ions. By having a scanning electron microscope as an integral part of the same instrument, the point of impact of the ion beam can be observed non-destructively and the operator can control the ion beam process precisely. These instruments are called dual-beam instruments.

**FIBs in IC Manufacturing**

By precisely focusing a high current density ion beam, a FIB workstation can not only remove material and expose defects, but also deposit new conducting paths or insulating layers, analyse the chemical composition of a sample and view the area being modified, all within submicron tolerances. IC manufacturers find it a cost-effective tool with a versatile range of applications that can reduce time-to-market and improve yields. During IC design and testing, these products speed the fabrication and debugging of prototypes. Focused ion beam technology gives rapid access to design problems, then gas injection techniques allow fast cut-and-splice modifications to the circuit. Used in defect review, a FIB unit can improve manufacturing yields by analysing surface and subsurface defects on wafers from the manufacturing (fab) line. It can also cross-section critical structures to provide three-dimensional monitoring of process performance. As a failure analysis tool, it speeds the diagnosis of IC malfunction and the location of subsurface defects. As micro-electronic structures get smaller, contamination control becomes more important. Some of the new FIB models feature robotic wafer handling and automatic wafer alignment to comply with the cleanroom requirements of the fab line.

**FIBs in Materials Science**

For materials science applications, a FIB unit can cross-section complex alloys and layered materials and prepare extremely thin TEM specimens. An ion beam’s submicron milling has uses in the manufacture and test of microelectromechanical systems, such as acceleration detectors. Other hard surface applications include micromachining fresnel lenses and sharpening scanning tunnelling microscope tips.

**FIBs in Biological Science**

Used in the biological sciences, an ion beam’s precise cut can remove the surface of a cell or prepare a cross-section of a virus to allow for detailed examination under an electron microscope. It is opening up new possibilities in the field of structural biology.

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**Weight and Mass**

Everything has mass. Even a weightless astronaut has the same mass he had before he took off. Weight is the force of gravity acting on a mass. If the gravity is lower, as on the moon, we weigh less, but our mass is still the same. That’s why scientists refer to mass rather than weight. Mass is independent of the frame of measurement.

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www.fei.com
The emission of light photons by a material.

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

Cleanroom
Room containing extremely low numbers of dust particles used for the manufacture of semiconductors.

Column
The evacuated electron beam path, the electromagnetic lenses and the aperture mechanisms are accommodated in a vertical column (see page 8).

Condenser lens
Part of the Illumination system between the gun and the specimen designed to concentrate the electron beam into a parallel or a finely focused spot on the specimen (or, in the case of the scanning electron microscope, into the objective lens).

CRT
Cathode ray tube. The screen of a conventional TV set or a PC monitor is the front end of a cathode ray tube. Behind is an electron gun producing an electron beam, lenses to focus it and a scanning system to make the beam scan a raster (q.v.) on the screen to produce an image.

Crystal
A material in which the atoms are ordered into rows and columns (a lattice) and because of this periodicity, electrons, whose wavelength (q.v.) is about the same size as the spacing between atoms, undergoes diffraction (q.v.).

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

Diffraction
Deviation of the direction of light or other waves when the wavefront passes the edge of an obstacle.

Diffraction contrast
Image contrast caused by the removal of electrons (or light) from the beam by scattering by a periodic (e.g. crystaline) structure in the specimen.

EDX
Energy Dispersive X Ray Analysis or Spectroscopy. An EDX spectrometer makes a spectrum (q.v.) of X-rays emitted by the specimen on the basis of their energy. An alternative method of making the spectrum, Wavelength Dispersive Spectroscopy (WDS), exists but is not referred to in this book.

EELS
Electron Energy Loss Spectroscopy (or Spectrometry) is defined on page 20.

Electron
Fundamental atomic particle rotating in an orbit around the nucleus of the atom (see page 3). Free electrons can easily flow in a conductor. In a semiconductor, they can be extracted into a vacuum by heat and or an electrical field.

Electron microscope
A microscope (q.v.) in which a beam of electrons is used to form a magnified image of the specimen.

SEM
Environmental Scanning Electron Microscopy is defined on page 18.

Excitation
The input of energy to matter leading to the emission of radiation.

Excited atom
An atom which has lost one of its inner electrons (see also ion) has a higher energy (the extra energy is that needed to remove the electron). It seeks to return to its ground state by reemitting its electrons and emitting the excess energy as an X-ray quantum.

Fab line
The semiconductor production line which is located in a clean room (q.v.).

FEG
Field Emission Gun is defined on page 21.

FIB
Field ion beam instrument. A beam of ions replaces the beam of electrons. Dual beam instruments also have an electron column.

Flament
Metal wire, usually in the form of a hairpin, which, when heated in vacuum, releases free electrons and so provides the source of electrons in the electron microscope.

Fluorescent screen
Large plate coated with a material (phosphor) which emits light (fluoresces) when bombarded by electrons. In the TEM, the image is formed on the fluorescent screen (see page 11).

Focal length of a lens
The distance (measured from the centre of the lens) at which a parallel incident beam is brought to a focus.

Focus
The act of making the image as sharp as possible by adjusting the objective lens (q.v.).

Goniometer
Specimen stage allowing linear movement of the specimen in two or more directions and rotation of the specimen in its own plane and tilting about one or more axes (see page 13).

Ground state
The lowest energy state of an atom. All electrons in their proper places and no charge.

Ion
An atom which has lost one or more of its outer electrons. It has a positive charge.

Ion getter pump
Vacuum pump in which the movement of ions in strong magnetic field drag molecules of gas and emit them in the cathode of the pump.

Lattice
Regular array of atoms in a crystal (q.v.).

Lens
In a light microscope, a piece of transparent material with one or more curved surfaces, which is used to alter systematically the direction of rays of light in an electron microscope, a similar effect is achieved on a beam of electrons by using a magnetic (or electrostatic) field (see pages 5 & 10).

Microscope
Unit of length (distance). One micrometre (abbreviated to µm) is a thousandth of a millimetre or 1000 nm (see box D, page 5).

Microtome
Instrument designed to extend man’s visual capability, i.e. to make visible minute detail that is not seen with the naked eye.

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

Nonomter
Unit of length (distance). One nanometre (abbreviated to nm) is a millth of a millimetre (10⁻⁹ metre) (see box D, page 5).

Objective lens
In a TEM, this is the first lens after the specimen whose quality determines the performance of the microscope. In a SEM it is the last lens before the specimen and produces the extremely fine electron spot with which the specimen is scanned.

Oil diffusion pump
Vacuum pump where the pumping action is produced by dragging action of a stream of oil through an orifice.

Phase
Relative position in a cyclical or wave motion. It is expressed as an angle, one cycle or one wavelength (q.v.) corresponding to 180°.

Phase contrast
Image contrast caused by the conversion of phase differences in light leaving the object into amplitude differences in the image.

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

Photomultiplier
Electrometer tube in which light is amplified without interference (noise) to produce an electrical signal.

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

Primary electrons
Electrons in the beam as it leaves the gun and impinges on the specimen.

Quantum

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

Refraction
Change in speed (and sometimes direction) when light or electrons pass through a specimen.

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

Resolving power
The ability to make points or lines which are closely adjacent in an object distinguishable in an image. (see box B, page 5).

Resolution
The act or result of displaying fine detail in an image. (see box A, page 4)

Rheumming pump
Vacuum pump in which the pumping action is produced by moving packets of air from one side of a rotating cylinder to another by means of an eccentric drum.

Scanning
Process of investigating a specimen by moving a finely focused electron beam systematically in a raster over the surface. The same technique in the display monitor leads to an image in which every point corresponds to a point on the specimen.

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

Secondary electrons
Electrons originating in the specimen which are emitted under influence of the primary beam.

SEM
Scanning Electron Microscopy is defined on page 7.

Secondary electron detector
Electron detector used in SEM or STEM in which the electron to be detected causes a small change in the semiconductor material which is amplified by making the semiconductor part of an electrical circuit.

Spectrometer
Instrument for obtaining a spectrum (q.v.).

Spectrum
A display produced by the separation of a complex radiation into its component wavelengths or energies.

Spherical aberration
See aberration. The magnification in the centre of the lens differs from that at the edges.

Sputter coater
Instrument for coating a non-conducting specimen with a very thin uniform layer of a conducting element such as gold to make it suitable for examination in a scanning electron microscope without danger of it charging up.

STEM
Scanning Transmission Electron Microscopy is defined on page 7.

TEM
Transmission Electron Microscopy is defined on page 6.

Turbomolecular pump
Vacuum pump in which the centrifugal force of fast rotating disc rotates molecules from the axis to the periphery.

Vacuum
Space from which (most) gases and vapours have been removed (see box 1, page 12).

Wavelength
The distance on a periodic wave between two successive points at which the phase is the same.

Wehnelt cylinder
An electrode between the cathode (filament) and the anode (earth) in an electron gun which focuses and controls the electron current in the beam and keeps it constant (see Fig. 7, page 9).

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

X-rays
Extremely short wavelength radiation. In the electron microscope, an X-ray photon (q.v.) is emitted when an excited atom releases its excess energy and returns to its ground state.