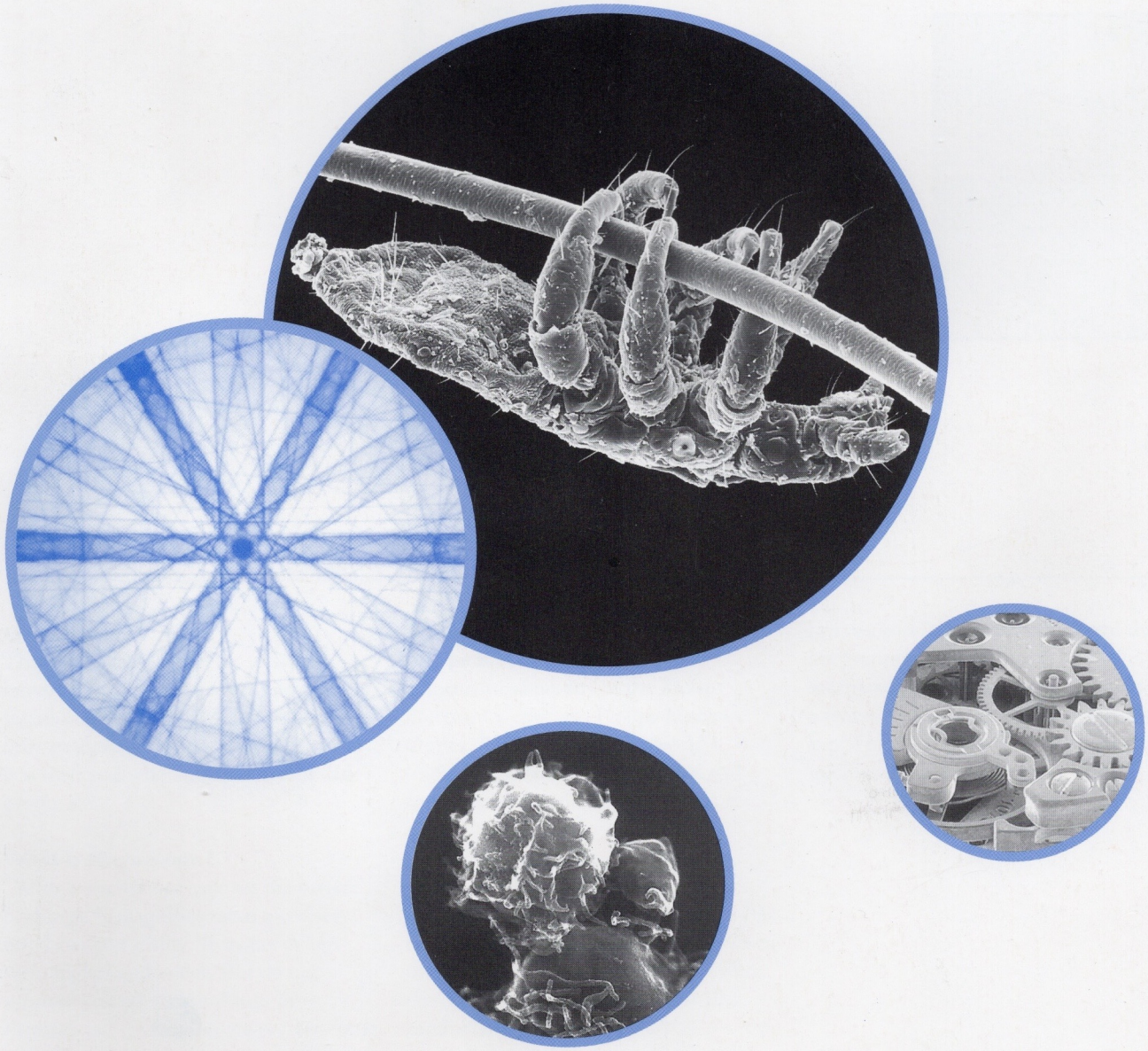
All you wanted to know about Electron Microscopy,

but didn't dare to ask.



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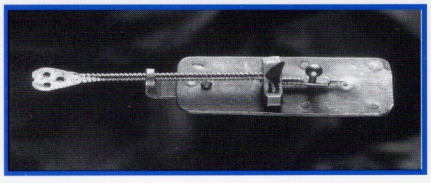

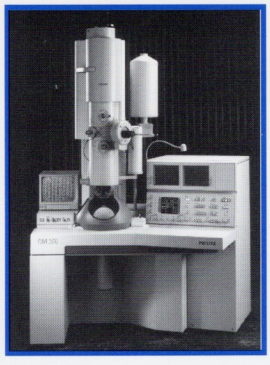

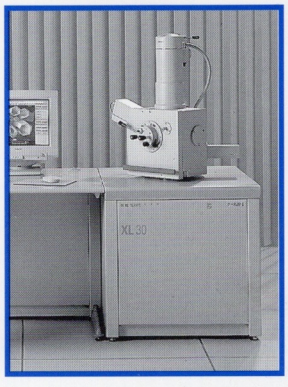

Introduction
This booklet is written for those who know little or nothing about electron microscopy and would like to know how an electron microscope works, why it is used and what useful results it can produce.

What is Microscopy ?
"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 Philips Electron Optics
A specialist company of the Philips Electronics concern, Philips Electron Optics, PEO, is one of the world's leading suppliers of transmission and scanning electron microscopes.

Philips' commitment to electron microscopy dates back to the mid- 1930's, when it 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 Philips at the forefront of electron microscopy ever since.

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What is electron microscopy ?

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 a valuable insight into the origins of our planet and its valuable mineral resources.

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 (figure 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.

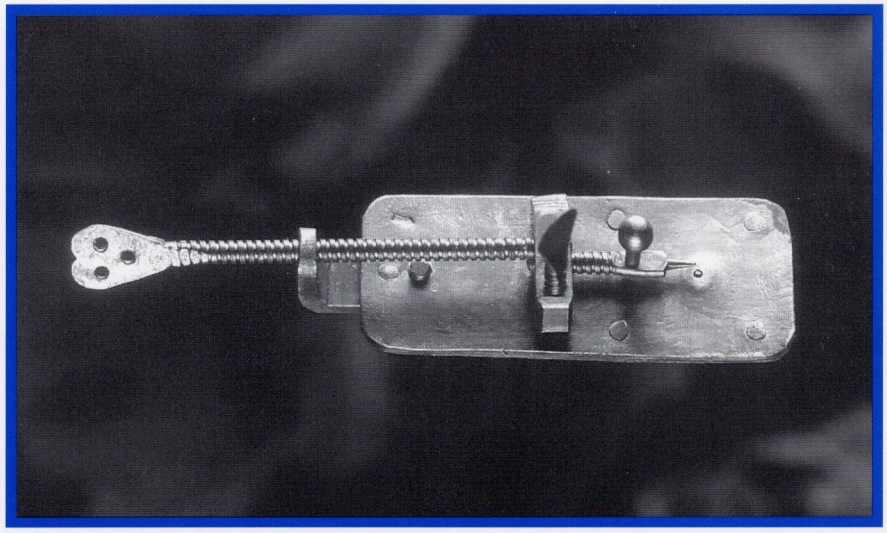


Fig. 1 One of the 550 light microscopes made by Antony van Leeuwenhoek.

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 right). 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 than the wavelength of the light (a few hundred nanometres – see box next page). Using light with a short wavelength (blue or ultraviolet) gave a small improvement; immersing the specimen and the front of the objective lens in a medium with a high

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.

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.

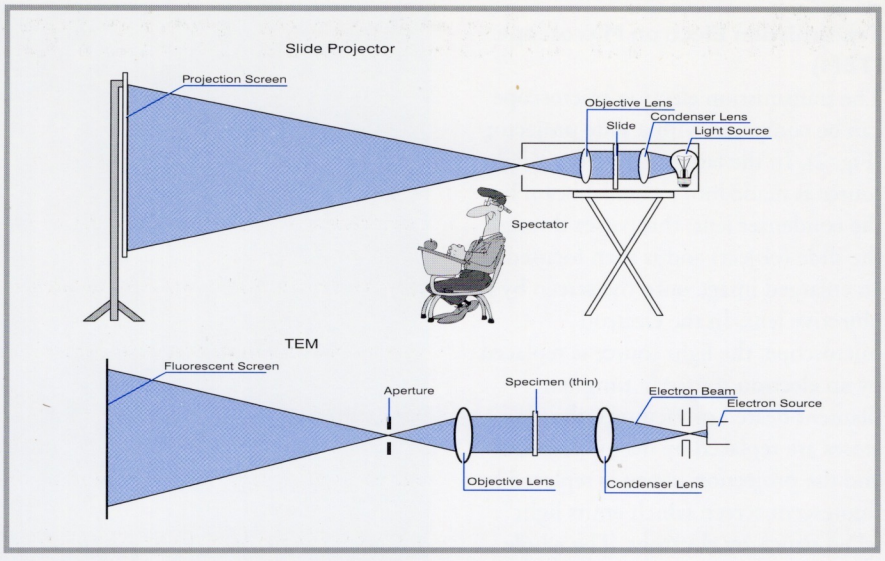


Fig. 2 The transmission electron microscope compared with a slide projector.

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 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.

In the 1920s it was discovered that accelerated electrons (parts of the atom) 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, 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.

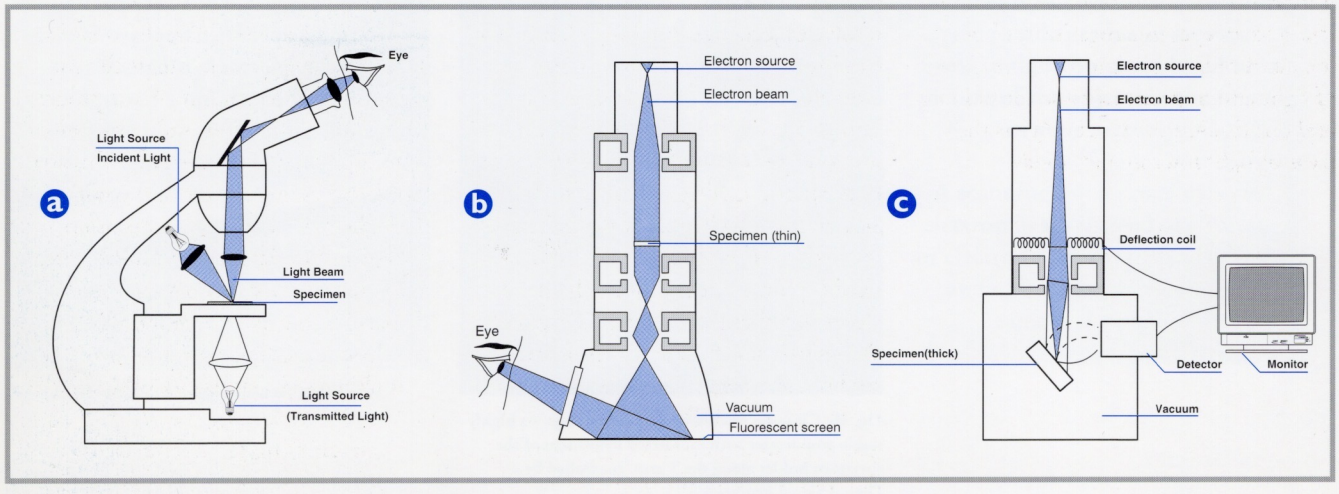


Fig. 3 Comparison of the light microscope (a) with transmission (b) and scanning (c) electron microscopes.

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.

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 electron microscope – have to be very thin in order to be imaged with electrons. Typically, the specimen must not be thicker than a few hundred nanometres.

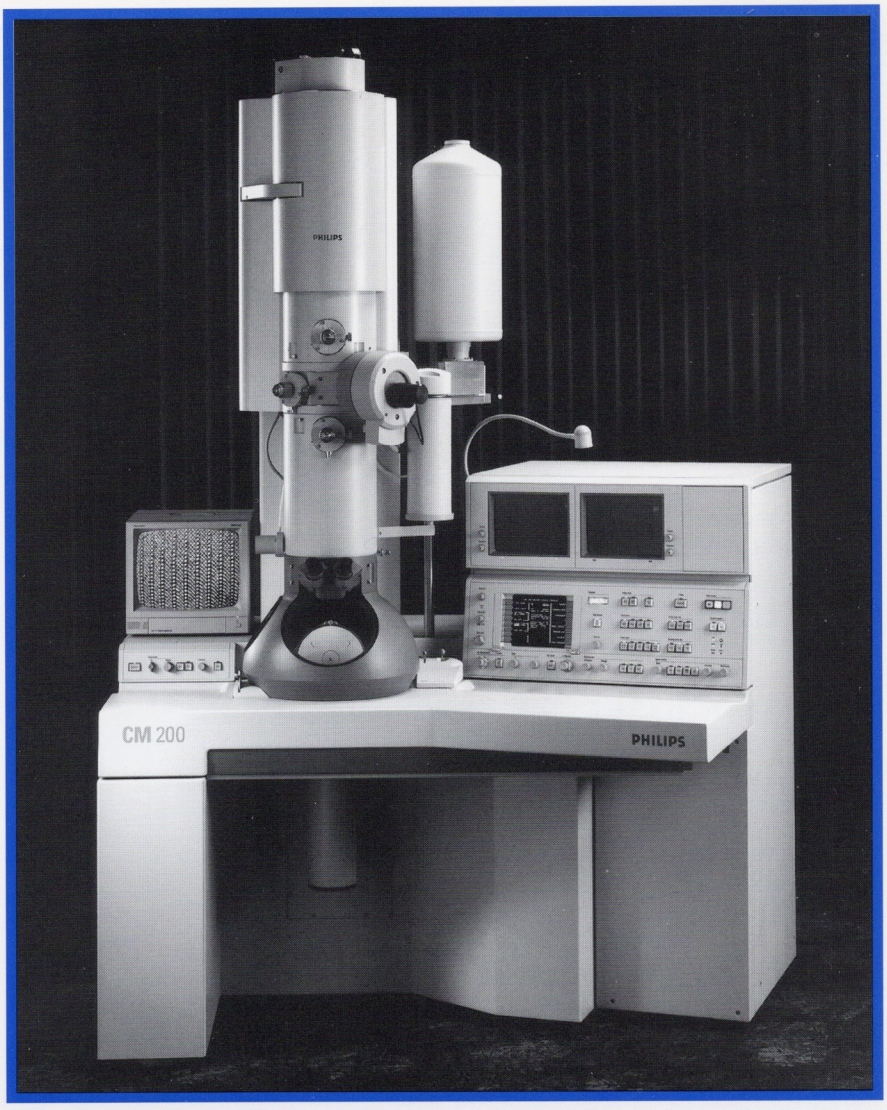


Fig. 4a. A transmission electron microscope dedicated to investigations in the material sciences - the Philips CM200.

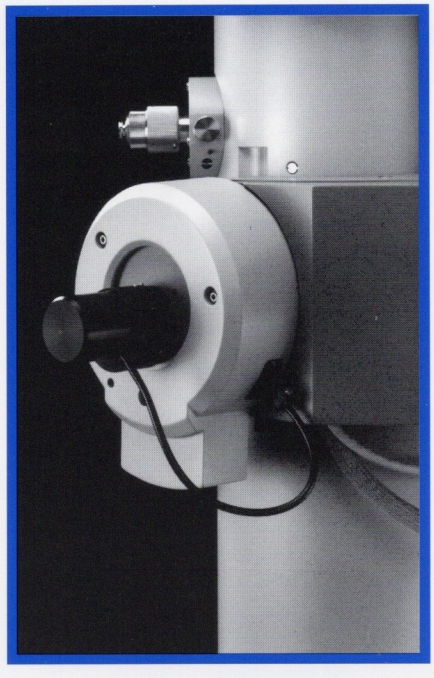


Fig. 4b. Close-up picture of the CompuStage - a highly stable goniometer with motorized movement of the specimen holder along the 5 axes, controlled by a Digital Signal Processor.

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.

Fig. 4c. An example of a modern scanning electron microscope, the Philips XL30.

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, Dr. 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 3 compares the four techniques (transmitted light microscopy, reflected light microscopy, 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 EM 200 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.

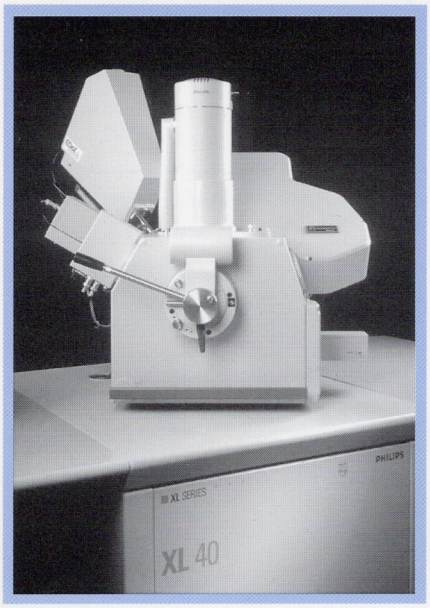


Fig. 4d. XL 40 chamber equipped with detectors.

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.

The Transmission Electron Microscope

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 Philips CM 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. 4a).

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. 5). 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).

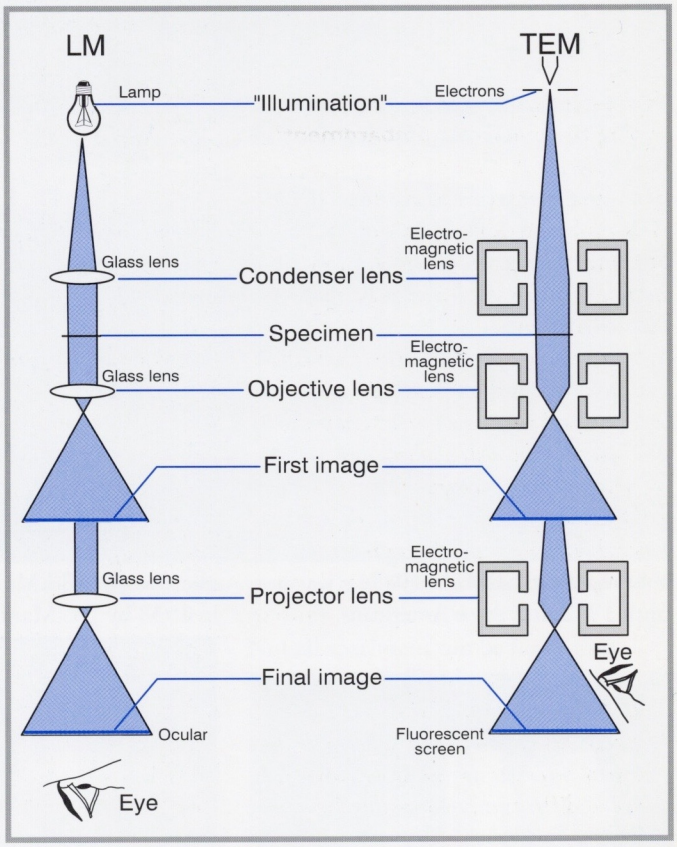


Fig. 5 Ray paths of light in a light microscope (LM) compared with those of electrons in a transmission electron microscope (TEM).

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 below) emerges at the other side. The Wehnelt cylinder which is at a different potential, bunches the electrons into a finely focused point (Fig. 6).

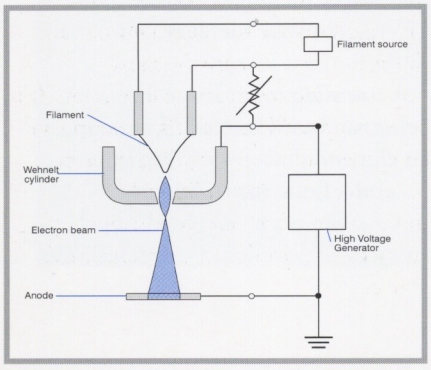


Fig. 6 Schematic cross-section of the electron gun in an electron microscope.

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 of the speed of light).

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.

If the specimen were not thin, the electrons would simply be stopped and no image would be formed (see box "Penetration" on page 4). 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.

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 happen:

1. 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.
2. Other electrons are scattered over small angles, depending on the composition of the specimen; these cause what is called the phase contrast in the image.
3. 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.
4. Some of the impinging electrons are reflected (these are called backscattered electrons).

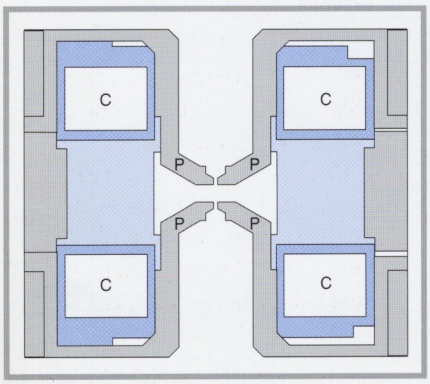


Fig. 7 Cross-section of an electromagnetic lens. C is an electrical coil and P is the soft iron pole piece.

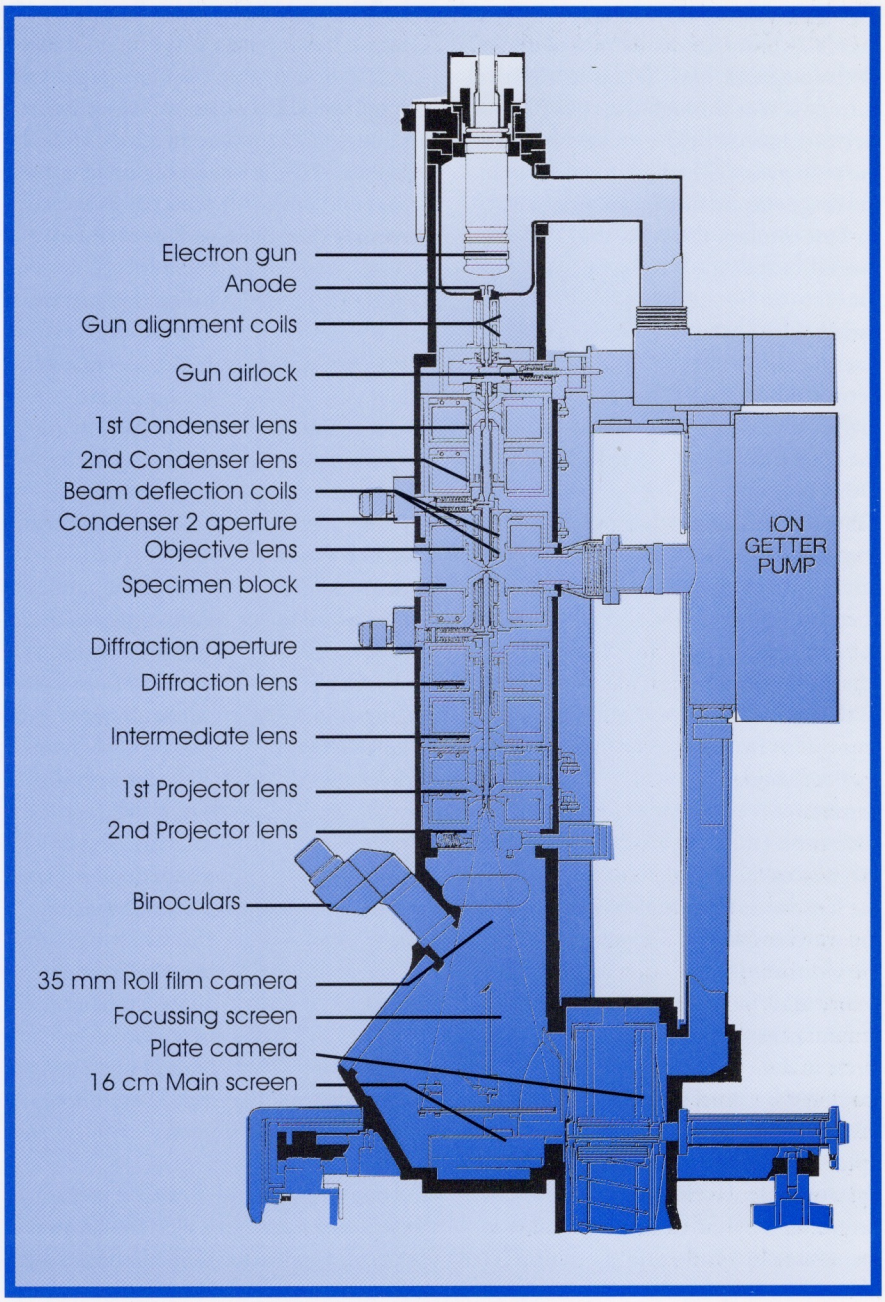


Fig. 8 Cross-section of the column of a modern transmission electron microscope.

5. The impinging electrons can cause the specimen itself to emit electrons (these are called secondary electrons).
6. The impinging electrons cause the specimen to emit X-rays whose energy and wavelength are related to the specimen's elemental composition.
7. The impinging electrons cause the specimen to emit photons (or light); this is called cathodoluminescence.
8. Finally, electrons which have lost an amount of energy because of interaction with the sample 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 7 shows a cross-section of an electromagnetic lens. When an electrical current is sent 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 Philips CM 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 stability and to achieve the highest possible magnification, the lenses in a modern TEM are all water cooled.

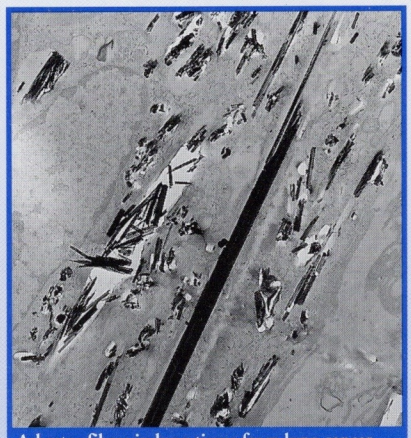
On the way from the filament to the fluorescent screen, the electron beam passes through a series of apertures with different diameters. These apertures stop those electrons which do not contribute to the process of image formation. Using a special holder carrying four different apertures, the diameter 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.

VACUUM

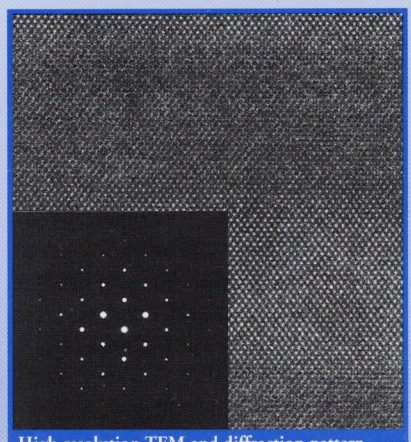
Normal atmospheric air pressure is around 760 mm of mercury. This means that the pressure of the atmosphere is sufficient to support a column of mercury 760 mm high.

Modern physicists use the Pascal (Pa). Normal air pressure = 100 000 Pa; residual pressure in the microscope = 2.5×10^{-5} Pa. At this pressure, the number of gas molecules per litre is about 7×10^{12} and the chance of an electron striking a gas molecule while traversing the column is almost zero.

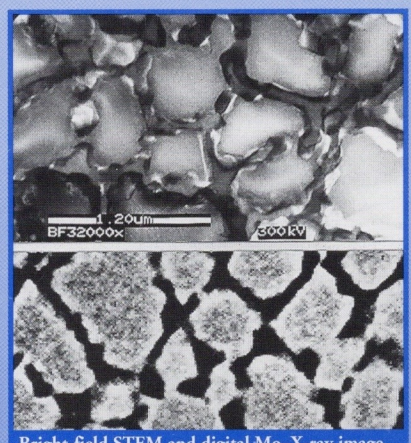
In other words, the mean free path of the electron is several metres – much longer than the trajectory from source to screen.



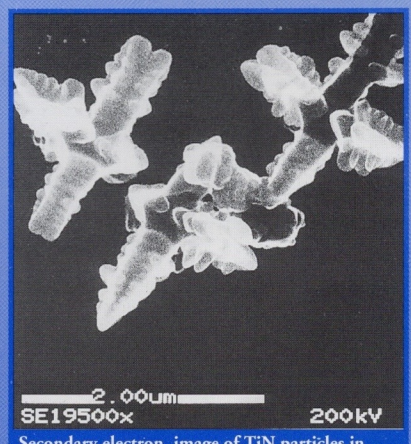
Asbestos fibres in lung tissue from lung cancer patient.



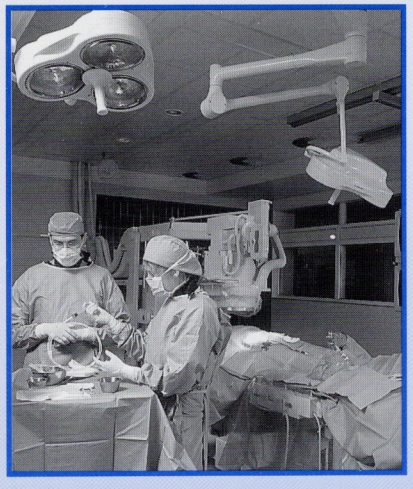
High resolution TEM and diffraction pattern from Si-Si_2Ge multi-layer.

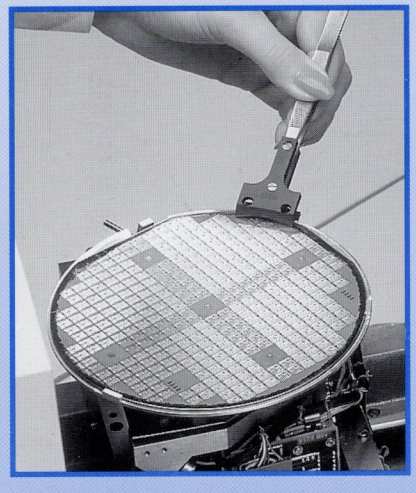


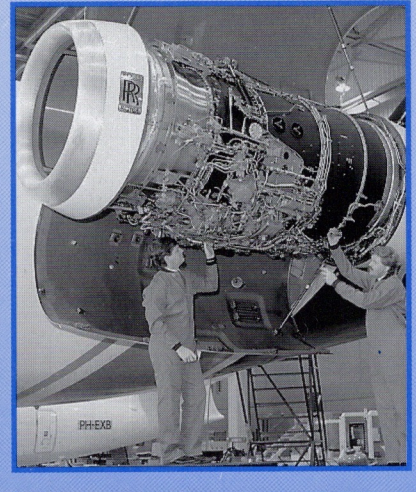
Bright-field STEM and digital Mo–X-ray image from NiMo.

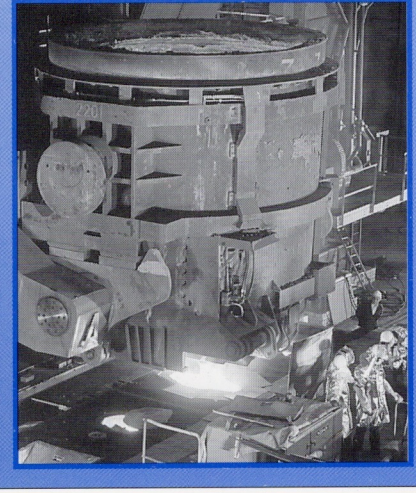


Secondary electron image of TiN particles in steel.









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 von 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. In some microscopes a more economical compact 35 mm film camera can be introduced higher up in the projection chamber (see fig. 8).

If the fluorescent screen is transparent, the image can be observed from the other side by a TV camera. Alternatively, a simple economical TV camera can be mounted on the eye piece of the binocular viewer to produce an image of the focusing screen. 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.

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 (see box). Various levels of vacuum are necessary: the highest vacuum around the specimen and in the gun is obtained with an ion getter pump; the vacuum in the projection chamber and camera chamber is obtained with an oil diffusion pump backed by a rotary pump. Water vapour, which inevitably gets introduced when exchanging a specimen, is trapped by a cryo pump which is a liquid-nitrogen cooled block surrounding the specimen area. The highest vacuum attained is of the order of a ten millionth of a millimetre of mercury.

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.

LIMIT OF DETECTION

The gram atomic molecular (1) weight of an element contains a fixed number of atoms (or molecules). This number is called Avogadro's number after the Italian physicist who discovered the fact. The number is 6×10^{23}. This means that 27 g of aluminium consists of 6×10^{23} atoms of aluminium.

Therefore it is fairly easy to calculate that 10^{-12} g of aluminium consists of 2.2×10^{10} atoms. Scientists working at the extreme limits of what is possible in X-ray detection (using extremely finely focussed intense beams of electrons) have been able to detect the presence of 10^{-18} g of an element which means a mere 10^5 to 10^6 atoms are being detected.

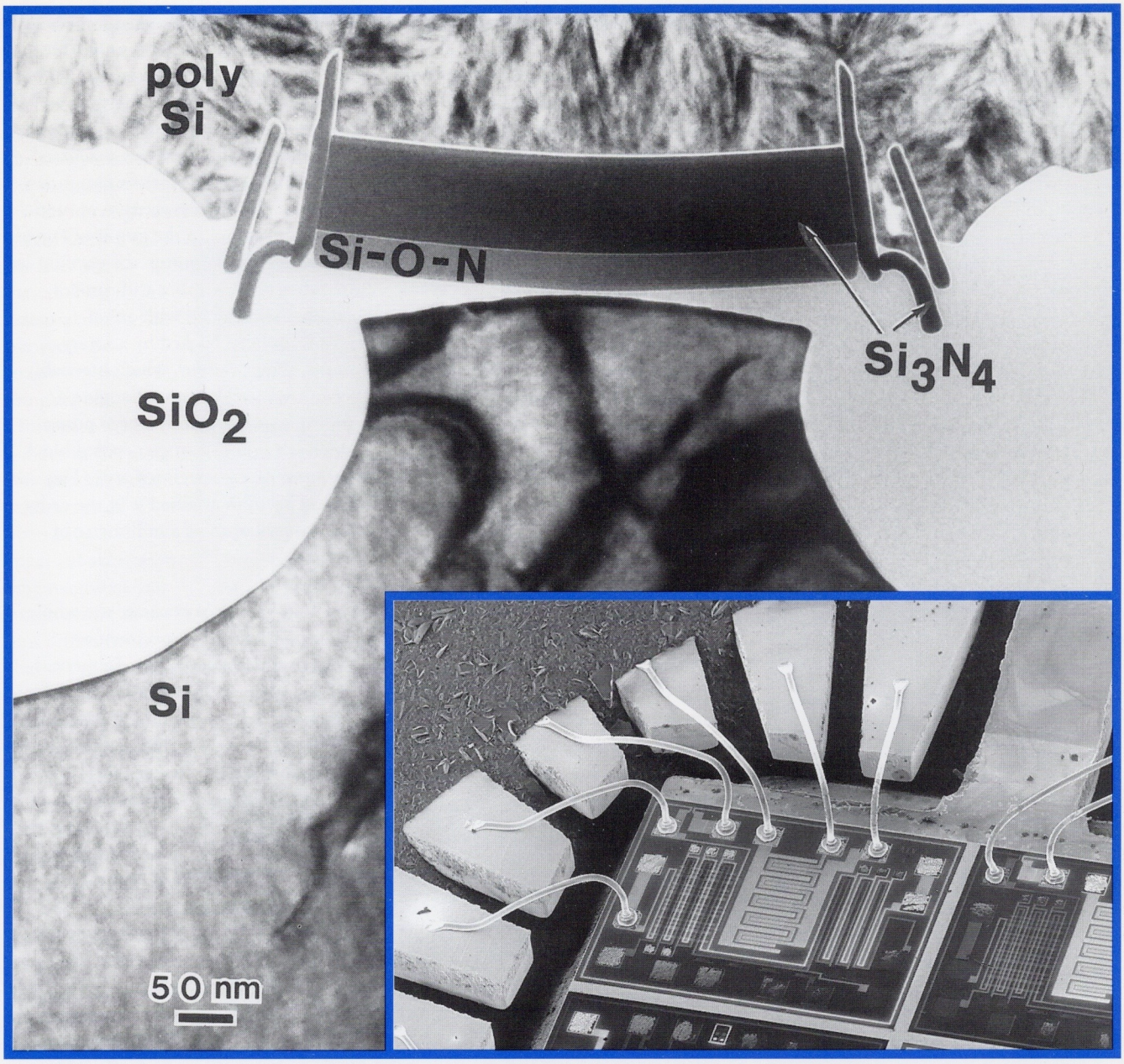


Fig. 9 TEM image of a test device used for investigation of sidewall masking isolation in semiconductors. The insert shows an SEM micrograph of an IC device. Leadframes and bonds to chip are clearly visible.

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. The result of employing a microprocessor-based Microcontroller in the Philips CM series of TEMs is noticeable in the clear layout of the control panel: the number of control knobs is dramatically reduced with respect to previous models and the operating condition of the whole instrument, including that of the vacuum system, can be checked at any time on a monitor on the operating desk.

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 the highest.

The goniometer itself provides 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.

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 page 9). 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.

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. 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 is discussed later.

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 a 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 the electrons to pass through.
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.

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 page 5). While scanning the room to determine its topography, the viewer would also be aware of the coiour 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 speci men to be removed from its orbit (if the atom is near the surface, it can become a secondary electron). The atom remains 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. Finally the outermost missing electron is replaced by one from the free electrons always pre sent 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 transmissions) or a light quantum (in the case of free electron to outer orbit transfers). We can see that diffirent materials produce diffirent colours by examining a colour television screen with a magnifying glass. Diffirent phosphors produce diffirent colours of light under the impact of the scanning electron beam.

The Scanning Electron Microscope

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.

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. 10. The functioning of a SEM is very similar to that of the TV monitor with which it is connected. 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.

All the components of a SEM are usually housed in one unit (Fig. 4c page 5). 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 an 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 signal from which is amplified and used to modulate the brightness of a cathode ray tube (CRT) which is scanning in synchronism with the primary beam in the column (see box). So there is a correspondence between the brightness of an image point on the CRT and the number of secondary electrons emitted by a point on the specimen. The ratio of the size of the screen of the viewing monitor (CRT) and the size of the area scanned on the specimen is the magnification. Increasing the magnification is achieved by reducing the size of the area scanned on the specimen (see box next page). Recording is done by photographing the monitor screen (or, more usually, a separate high resolution screen), or making videoprints.

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.

The most important differences between TEM and SEM are:

A. the beam is not static as in the TEM: with the aid of an electromagnetic field, produced by the scanning coils, the beam is scanned line by line over an extremely small area of the specimen's surface (see box bottom left);

B. the accelerating voltages are much lower than in TEM because it is no longer necessary to penetrate the specimen; in a SEM they range from 200 to 30 000 volts.

C. specimens need no complex preperations.

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).

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 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.

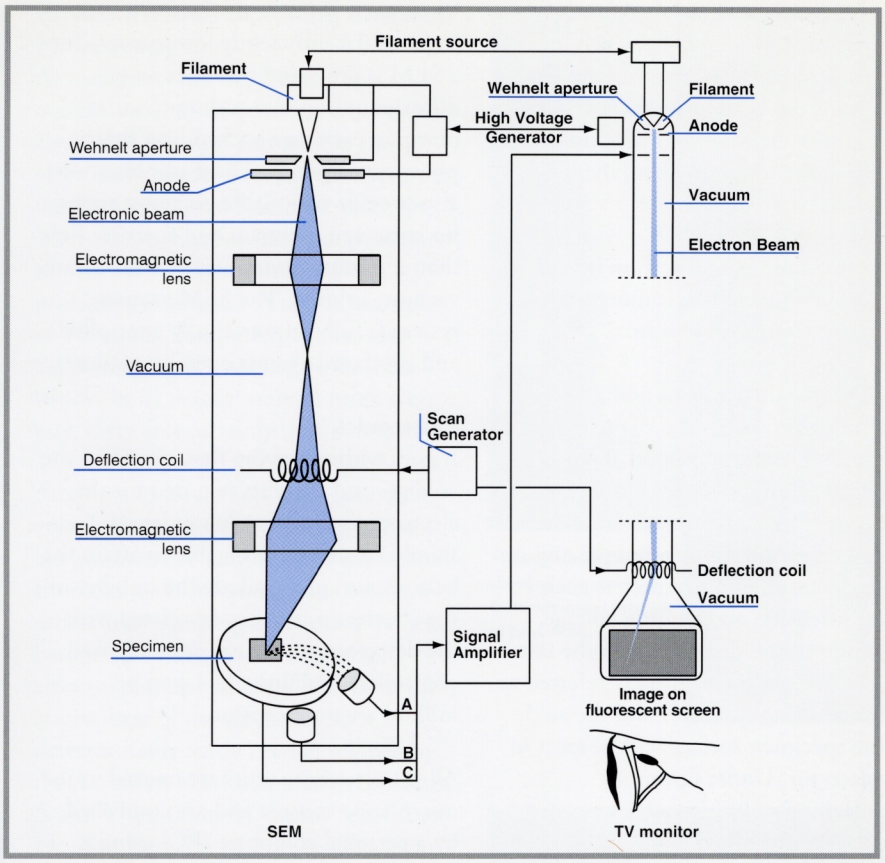


Fig. 10 The functioning of a SEM compared with that of a TV monitor.

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.

RESOLUTION IN SEM

The resolution (determined by the size of the scanning spot) means that magnifications of the order of 300 000x can be realised. If the viewing screen is, say, 300 mm wide, then the full width of the scanned area is only 1 micrometres.

ELECTRON INTERACTIONS WITH MATTER

In the modern view of matter, an atom consists of a heavy positively charged nucleus surrounded by a number of orbiting electrons. The incoming electron can interact with the nucleus and be backscattered with virtually undiminished energy (just as a space probe is deviated by the gravity of a planet during a fly-past). Or it can interact with the orbiting electrons: an electron may be ejected from the atom, (this is a secondary electron), and the atom with one electron short restores the status quo by emitting its excess energy in the form of an X-ray quantum or a light photon.

$$\textit{SEM magnification} = \frac{\textit{length of one line "written" by the electron beam on the monitor}}{\textit{length of one "track" of the electron beam on the specimen}}$$

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).

The maximum magnification is usually about 300 000x which is more than sufficient for the resolution.

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 currently be attained.

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 70 mm or Polaroid).

To facilitate the observation and correct choice of the parameters mentioned above, Philips XL Series 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 can also be stored as electronic information on computer (hard) disks.

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.

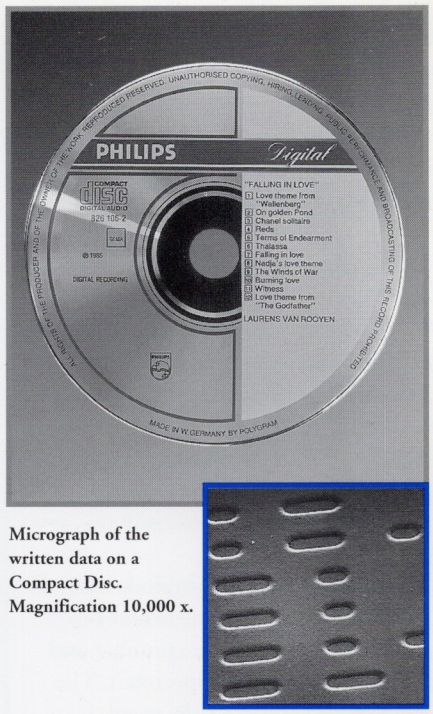
All the electronic units are housed in the microscope console and are controlled by a personal computer (PC) using a keyboard and mouse.

Specimen orientation and manipulation

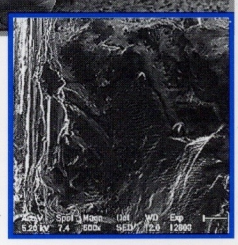
As has been mentioned, the quality of the image in a SEM depends on the orientation and the distance of the specimen from the detectors and the final lens. The specimen stage allows the specimen to be moved in a horizontal plane (X and Y direction) and up and down (Z direction) and rotated and tilted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

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.

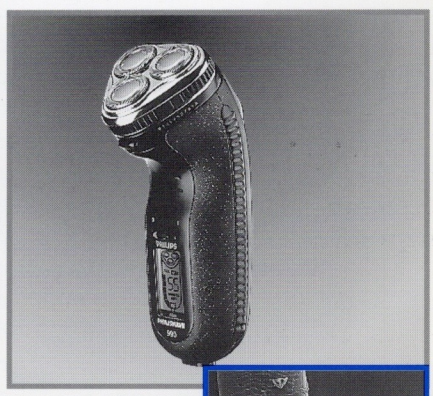
The simplest model, the XL20, accepts specimens of a few cm in diameter and can move them 20 mm in the X and Y directions. The largest chamber, that of the XL40, accepts samples up to 200 mm in diameter and can move them 150 mm in each direction. All models allow samples to be tilted up to high angles and rotated through 360 degrees.

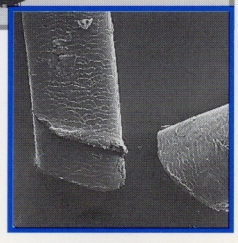


Micrograph of the written data on a Compact Disc. Magnification 10,000 x.



A fractured aluminium holder mounted in a SEM shows the ribbed structure of fatigue marks.





This SEM image shows two beard hairs to investigate the so-called "lift and cut" technique for the Philishave. Magnification 320 x.

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.

More information from the specimen

In a SEM characteristic X-rays are produced when the electron beam strikes the specimen (see box on page 11). These can be exploited by placing a suitable detector in the specimen chamber and thus very small quantities of elements can be detected (see page 7 under TEM).

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.

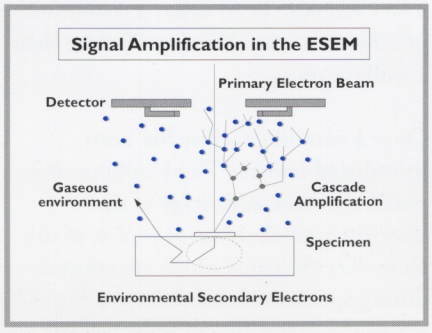
Environmental Scanning Electron Microscopy

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. A classic example of a material that poses problems for SEM imaging is wool or cotton tissue which, in its natural state, is often wet, oily, dirty and certainly non-conductive.

Attempts to view a specimen containing volatile components by placing it in a environmental chamber (see box) isolated from the main column by one or more differential pumping apertures have been hampered by the lack of a suitable electron detector which can work in the atmosphere of the chamber. Such a detector has recently been developed by the American SEM manufacturer Electro Scans. The patented Gaseous Secondary Electron Detector makes use of cascade amplification (see box) not only to enhance the secondary electron signal but also to produce positive ions which are attracted by negative charge on the insulated specimen surface and effectively suppress charging artifacts.

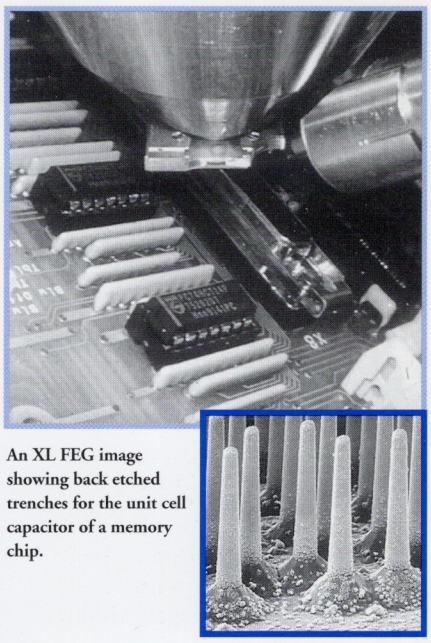
ENVIRONMENTAL CHAMBER

The pressure-temperature phase diagram for H_2O indicates that true "wet" conditions only exist at pressures of at least 600 Pa at 0 deg C (environmental microscopists usually refer to 4.6 Torr = 4.6 mm of mercury - see box page 8). In the range 650 to 1300 Pa (5-10 Torr) therefore the specimen may be observed whilst at equilibrium with water.

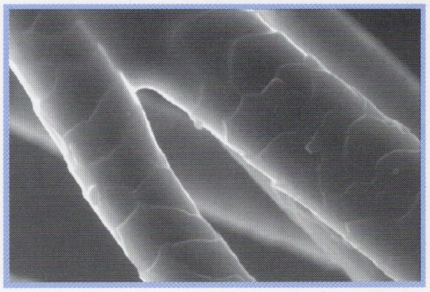


Gas ionisation amplifies the secondary electron signal and, in a nonconductive specimen, positive ions are attracted to the sample surface as charge accumulates on the specimen surface.

SE image on an XL SEM showing desposition of materials due to precence of hard water.



An XL FEG image showing back etched trenches for the unit cell capacitor of a memory chip.



Wool fibres seen in their natural state: uncoated and wet. A water droplet can be seen in the left upper corner connecting the two fibres.

Additional Techniques

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 combination of SEM and TEM techniques is called Scanning Transmission Electron Microscopy (STEM).

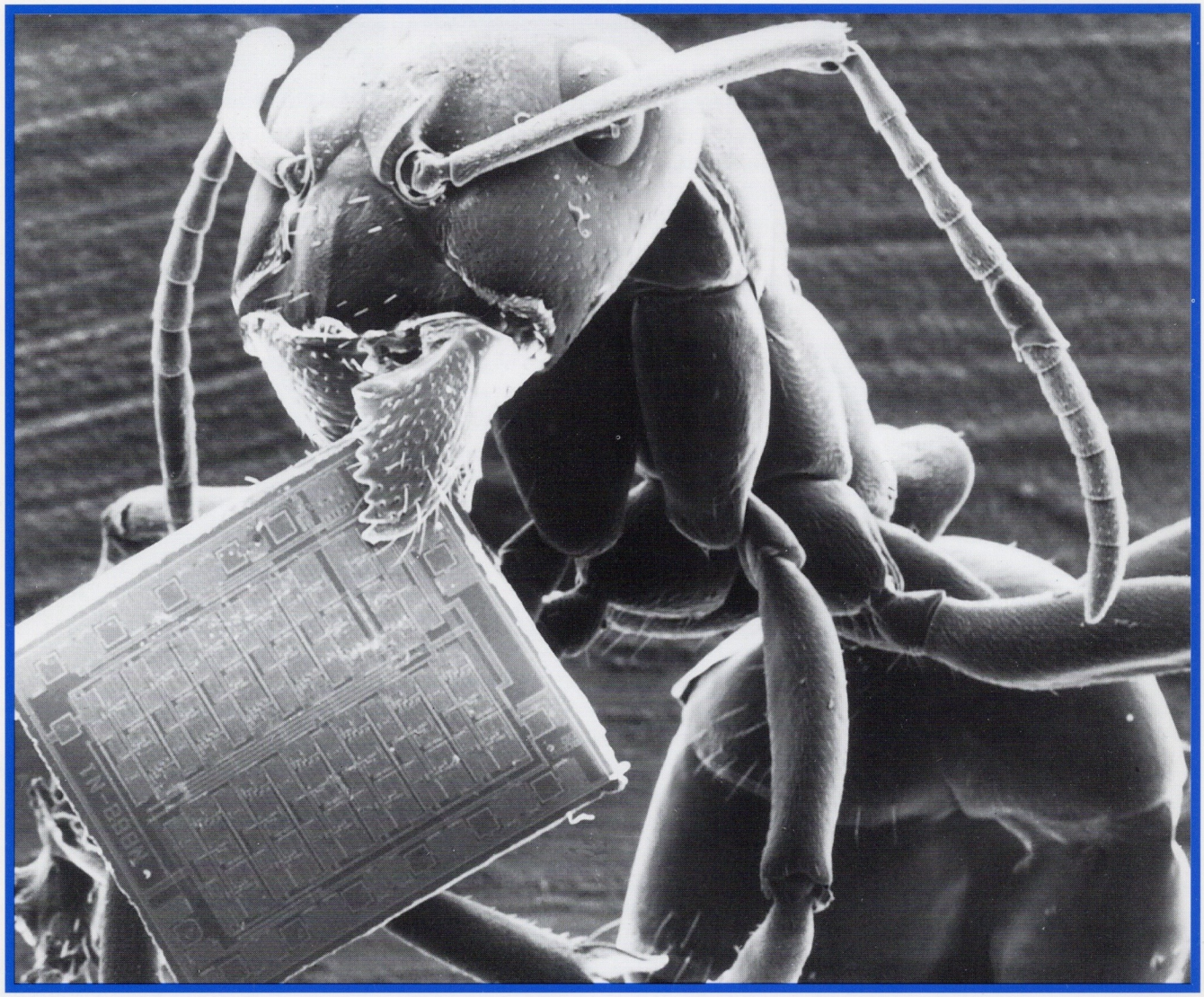


Fig. 11 Scanning electron micrograph (not a photo montage!) of an ant with a "chip" in its mandibles (30x). This award winning micrograph which was published many million times all over the world was made in the Application Laboratory for EM, Philips Electron Optics Eindhoven. The size of the chip is approx 2 x 2 mm.

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 Philips 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 CM200/STEM 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. 12 shows the very different information provided by these two signals.

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 life time 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 (LaB_6) 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.

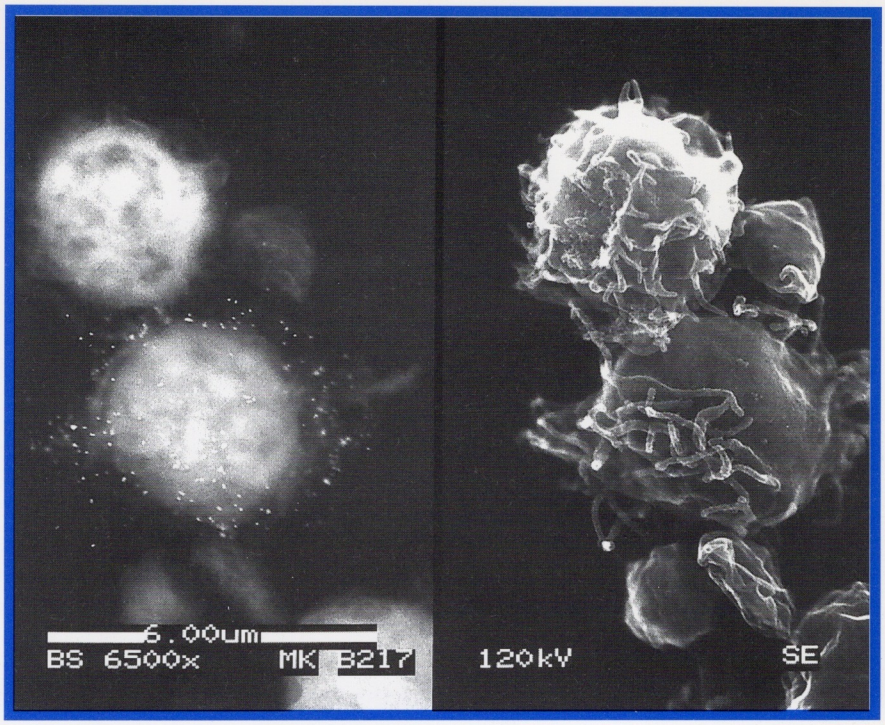


Fig. 12 Backscattered (left) and secondary-electron (right) images of peripheral blood mononuclear cells from a Human Immunodeficiency Virus carrier (aids), showing the presence of viral antigens after immunogold labelling. Specimen courtesy of Dr. M.I. Herrera, Instituto de Salud Carlos III, Madrid, Spain.

Other "microscopes"

TEMs and SEMs and their combinations are not the only devices without glass lenses that include the word "microscope" in their name. They have however had the widest areas of application. Other types are:

- thermal emission microscope
- field emission microscope
- field ion microscope
- mirror electron microscope
- scanning acoustic microscope
- scanning laser acoustic microscope
- X-ray microscope
- the recently developed scanning tunnelling microscope and atomic force microscope.

So far these have had, for one reason or another, only a limited range of application.

Section of high resolution TEM image from $Si\text{-}Si_2Ge$ multi-layer (see page 8).

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