Electron microscope

An **electron microscope** is a type of microscope that produces an electronically-magnified image of a specimen for detailed observation. The electron microscope (EM) uses a particle beam of electrons to illuminate the specimen and create a magnified image of it. The microscope has a greater resolving power (magnification) than a light-powered optical microscope, because it uses electrons that have wavelengths about 100,000 times shorter than visible light (photons), and can achieve magnifications of up to 1,000,000x, whereas light microscopes are limited to 1000x magnification.

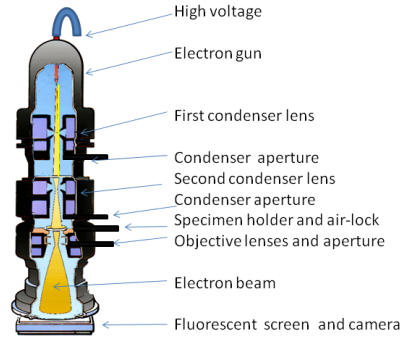


Diagram of a transmission electron microscope

The electron microscope uses electrostatic and electromagnetic "lenses" to control the electron beam and focus it to form an image. These lens are analogous to, but different from the glass lenses of an optical microscope that form a magnified image by focusing light on or through the specimen.

Electron microscopes are used to observe a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals. Industrially, the electron microscope is primarily used for quality control and failure analysis in semiconductor device fabrication.

History

In 1931, the German physicist Ernst Ruska and German electrical engineer Max Knoll constructed the prototype electron microscope, capable of four-hundred-power magnification; the apparatus was a practical application of the principles of electron microscopy.[1] Two years later, in 1933, Ruska built an electron microscope that exceeded the resolution attainable with an optical (lens) microscope.[1] Moreover, Reinhold Rudenberg, the scientific director of Siemens-Schuckertwerke, obtained the patent for the electron microscope in May of 1931. Family illness compelled the electrical engineer to devise an electrostatic microscope, because he wanted to make visible the poliomyelitis virus.

Electron microscope constructed by Ernst Ruska in 1933

In 1937, the Siemens company financed the development work of Ernst Ruska and Bodo von Borries, and employed Helmut Ruska (Ernst's brother) to develop applications for the microscope, especially with biologic specimens.[1] [2] Also in 1937, Manfred von Ardenne pioneered the scanning electron microscope.[3] The first *practical* electron microscope was constructed in 1938, at the University of Toronto, by Eli Franklin Burton and students Cecil Hall, James Hillier, and Albert Prebus; and Siemens produced the first *commercial* Transmission Electron Microscope (TEM) in 1939.[4] Although contemporary electron microscopes are capable of two million-power magnification, as scientific instruments, they remain based upon Ruska's prototype.

Types

Transmission electron microscope (TEM)

The original form of electron microscope, the transmission electron microscope (TEM) uses a high voltage electron beam to create an image. The electrons are emitted by an electron gun, commonly fitted with a tungsten filament cathode as the electron source. The electron beam is accelerated by an anode typically at +100 keV (40 to 400 keV) with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope. The spatial variation in this information (the "image") is viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide. The image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fibre optic light-guide to the sensor of a CCD (charge-coupled device) camera. The image detected by the CCD may be displayed on a monitor or computer.

Resolution of the TEM is limited primarily by spherical aberration, but a new generation of aberration correctors have been able to partially overcome spherical aberration to increase resolution. Hardware correction of spherical aberration for the High Resolution TEM (HRTEM) has allowed the production of images with resolution below 0.5 Ångström (50 picometres)[5] at magnifications above 50 million times.[6] The ability to determine the positions of atoms within materials has made the HRTEM an important tool for nano-technologies research and development.[7]

Scanning electron microscope (SEM)

Unlike the TEM, where electrons of the high voltage beam carry the image of the specimen, the electron beam of the Scanning Electron Microscope (SEM)[8] does not at any time carry a complete image of the specimen. The SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen (raster scanning). At each point on the specimen the incident electron beam loses some energy, and that lost energy is converted into other forms, such as heat, emission of low-energy secondary electrons, light emission (cathodoluminescence) or x-ray emission. The display of the SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated. In the SEM image of an ant shown at right, the image was constructed from signals produced by a secondary electron detector, the normal or conventional imaging mode in most SEMs.

An image of an ant in a scanning electron microscope

Generally, the image resolution of an SEM is about an order of magnitude poorer than that of a TEM. However, because the SEM image relies on surface processes rather than transmission, it is able to image bulk samples up to many centimetres in size and (depending on instrument design and settings) has a great depth of field, and so can produce images that are good representations of the three-dimensional shape of the sample.

Reflection electron microscope (REM)

In the **Reflection Electron Microscope** (REM) as in the TEM, an electron beam is incident on a surface, but instead of using the transmission (TEM) or secondary electrons (SEM), the reflected beam of elastically scattered electrons is detected. This technique is typically coupled with Reflection High Energy Electron Diffraction (RHEED) and *Reflection high-energy loss spectrum (RHELS)*. Another variation is Spin-Polarized Low-Energy Electron Microscopy (SPLEEM), which is used for looking at the microstructure of magnetic domains.[9]

Scanning transmission electron microscope (STEM)

The STEM rasters a focused incident probe across a specimen that (as with the TEM) has been thinned to facilitate detection of electrons scattered *through* the specimen. The high resolution of the TEM is thus possible in STEM. The focusing action (and aberrations) occur before the electrons hit the specimen in the STEM, but afterward in the TEM. The STEMs use of SEM-like beam rastering simplifies annular dark-field imaging, and other analytical techniques, but also means that image data is acquired in serial rather than in parallel fashion.

Low voltage electron microscope (LVEM)

The low voltage electron microscope (LVEM) is a combination of SEM, TEM and STEM in one instrument, which operates at relatively low electron accelerating voltage of 5 kV. Low voltage increases image contrast which is especially important for biological specimens. This increase in contrast significantly reduces, or even eliminates the need to stain. Sectioned samples generally need to be thinner than they would be for conventional TEM (20-65nm). Resolutions of a few nm are possible in TEM, SEM and STEM modes. [10] [11]

Sample preparation

Materials to be viewed under an electron microscope may require processing to produce a suitable sample. The technique required varies depending on the specimen and the analysis required:

An insect coated in gold for viewing with a scanning electron microscope.

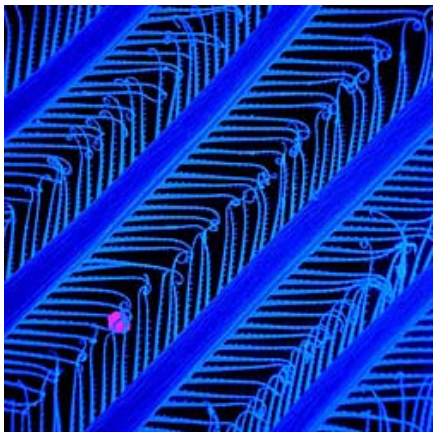
- *Chemical fixation* for biological specimens aims to stabilize the specimen's mobile macromolecular structure by chemical crosslinking of proteins with aldehydes such as formaldehyde and glutaraldehyde, and lipids with osmium tetroxide.
- *Cryofixation* – freezing a specimen so rapidly, to liquid nitrogen or even liquid helium temperatures, that the water forms vitreous (non-crystalline) ice. This preserves the specimen in a snapshot of its solution state. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy of vitreous sections (CEMOVIS), it is now possible to observe samples from virtually any biological specimen close to its native state.
- *Dehydration* – freeze drying, or replacement of water with organic solvents such as ethanol or acetone, followed by critical point drying or infiltration with embedding resins.
- *Embedding, biological specimens* – after dehydration, tissue for observation in the transmission electron microscope is embedded so it can be sectioned ready for viewing. To do this the tissue is passed through a 'transition solvent' such as epoxy propane and then infiltrated with a resin such as Araldite epoxy resin; tissues may also be embedded directly in water-miscible acrylic resin. After the resin has been polymerised (hardened) the sample is thin sectioned (ultrathin sections) and stained - it is then ready for viewing.
- *Embedding, materials* - after embedding in resin, the specimen is usually ground and polished to a mirror-like finish using ultra-fine abrasives. The polishing process must be performed carefully to minimize scratches and

other polishing artifacts that reduce image quality.

- *Sectioning* – produces thin slices of specimen, semitransparent to electrons. These can be cut on an ultramicrotome with a diamond knife to produce ultrathin slices about 60-90 nm thick. Disposable glass knives are also used because they can be made in the lab and are much cheaper.
- *Staining* – uses heavy metals such as lead, uranium or tungsten to scatter imaging electrons and thus give contrast between different structures, since many (especially biological) materials are nearly "transparent" to electrons (weak phase objects). In biology, specimens are can be stained "en bloc" before embedding and also later after sectioning. Typically thin sections are stained for several minutes with an aqueous or alcoholic solution of uranyl acetate followed by aqueous lead citrate.
- *Freeze-fracture or freeze-etch* – a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The fresh tissue or cell suspension is frozen rapidly (cryofixed), then fractured by simply breaking or by using a microtome while maintained at liquid nitrogen temperature. The cold fractured surface (sometimes "etched" by increasing the temperature to about −100 °C for several minutes to let some ice sublime) is then shadowed with evaporated platinum or gold at an average angle of 45° in a high vacuum evaporator. A second coat of carbon, evaporated perpendicular to the average surface plane is often performed to improve stability of the replica coating. The specimen is returned to room temperature and pressure, then the extremely fragile "pre-shadowed" metal replica of the fracture surface is released from the underlying biological material by careful chemical digestion with acids, hypochlorite solution or SDS detergent. The still-floating replica is thoroughly washed from residual chemicals, carefully fished up on fine grids, dried then viewed in the TEM.
- *Ion Beam Milling* – thins samples until they are transparent to electrons by firing ions (typically argon) at the surface from an angle and sputtering material from the surface. A subclass of this is Focused ion beam milling, where gallium ions are used to produce an electron transparent membrane in a specific region of the sample, for example through a device within a microprocessor. Ion beam milling may also be used for cross-section polishing prior to SEM analysis of materials that are difficult to prepare using mechanical polishing.
- *Conductive Coating* – an ultrathin coating of electrically-conducting material, deposited either by high vacuum evaporation or by low vacuum sputter coating of the sample. This is done to prevent the accumulation of static electric fields at the specimen due to the electron irradiation required during imaging. Such coatings include gold, gold/palladium, platinum, tungsten, graphite etc. and are especially important for the study of specimens with the scanning electron microscope. Another reason for coating, even when there is more than enough conductivity, is to improve contrast, a situation more common with the operation of a FESEM (field emission SEM).

Disadvantages

Electron microscopes are expensive to build and maintain, but the capital and running costs of confocal light microscope systems now overlaps with those of basic electron microscopes. They are dynamic rather than static in their operation, requiring extremely stable high-voltage supplies, extremely stable currents to each electromagnetic coil/lens, continuously-pumped high- or ultra-high-vacuum systems, and a cooling water supply circulation through the lenses and pumps. As they are very sensitive to vibration and external magnetic fields, microscopes designed to achieve high resolutions must be housed in stable buildings (sometimes underground) with special services such as magnetic field cancelling systems. Some desktop low voltage electron microscopes have TEM capabilities at very low voltages (around 5 kV) without stringent voltage supply, lens coil current, cooling water or vibration isolation requirements and as such are much less expensive to buy and far easier to install and maintain, but do not have the same ultra-high (atomic scale) resolution capabilities as the larger instruments.



False-color SEM image of the filter setae of an Antarctic krill. (Raw electron microscope images carry no color information.)
Pictured: First degree filter setae with V-shaped second degree setae pointing towards the inside of the feeding basket. The purple ball is 1 μm in diameter.

The samples largely have to be viewed in vacuum, as the molecules that make up air would scatter the electrons. One exception is the environmental scanning electron microscope, which allows hydrated samples to be viewed in a low-pressure (up to 20 Torr/2.7 kPa), wet environment.

Scanning electron microscopes usually image conductive or semi-conductive materials best. Non-conductive materials can be imaged by an environmental scanning electron microscope. A common preparation technique is to coat the sample with a several-nanometer layer of conductive material, such as gold, from a sputtering machine; however, this process has the potential to disturb delicate samples.

Small, stable specimens such as carbon nanotubes, diatom frustules and small mineral crystals (asbestos fibres, for example) require no special treatment before being examined in the electron microscope. Samples of hydrated materials, including almost all biological specimens have to be prepared in various ways to stabilize them, reduce their thickness (ultrathin sectioning) and increase their electron optical contrast (staining). These processes may result in *artifacts*, but these can usually be identified by comparing the results obtained by using radically different specimen preparation methods. It is generally believed by scientists working in the field that as results from various preparation techniques have been compared and that there is no reason that they should all produce similar artifacts, it is reasonable to believe that electron microscopy features correspond with those of living cells. In addition, higher-resolution work has been directly compared to results from X-ray crystallography, providing independent confirmation of the validity of this technique. Since the 1980s, analysis of cryofixed, vitrified specimens has also become increasingly used by scientists, further confirming the validity of this technique.[12] [13] [14]

Applications

Semiconductor and data storage

- Circuit edit
- Defect analysis
- Failure analysis

Biology and life sciences

- Diagnostic electron microscopy
- Cryobiology
- Protein localization
- Electron tomography
- Cellular tomography
- Cryo-electron microscopy
- Toxicology
- Biological production and viral load monitoring
- Particle analysis
- Pharmaceutical QC
- Structural biology
- 3D tissue imaging
- Virology
- Vitrification

Research

- Electron beam-induced deposition
- Materials qualification
- Materials and sample preparation
- Nanoprototyping
- Nanometrology
- Device testing and characterization

Industry

- High-resolution imaging
- 2D & 3D micro-characterization
- Macro sample to nanometer metrology
- Particle detection and characterization
- Direct beam-writing fabrication
- Dynamic materials experiments
- Sample preparation
- Forensics
- Mining (mineral liberation analysis)
- Chemical/Petrochemical

See also

- Category:Electron microscope images
- Field emission microscope
- HiRISE
- Scanning tunneling microscope
- Transmission Electron Aberration-corrected Microscope

External links

- Science Aid: Electron Microscopy [15] High School (GCSE, A Level) resource
- Cell Centered Database - Electron microscopy data [16]

General

- Nanohedron.com|Nano image gallery [17] beautiful images generated with electron microscopes.
- electron microscopy [18] Website of the ETH Zurich: Very good graphics and images, which illustrate various procedures.
- Environmental Scanning Electron Microscope (ESEM) [19]
- X-ray element analysis in electron microscope [20] – Information portal with X-ray microanalysis and EDX contents

History

- John H.L. Watson: Very early Electron Microscopy in the Department of Physics, the University of Toronto – A personal recollection [21]
- Rubin Borasky Electron Microscopy Collection, 1930-1988 [22] Archives Center, National Museum of American History, Smithsonian Institution.

Other

- The Royal Microscopical Society, Electron Microscopy Section (UK) [23]
- Albert Lleal micrograph. Scanning Electron Micrograph Coloured SEM [24]

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