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ABSTRACT
An electron microscope is an instrument, which utilizes short wavelength of electrons as a source of illumination for observing objects at a greater magnification. The major significance of an electron microscope is that it has the highest resolution and magnification. Max Knoll and Ernest Ruska in 1931, developed the first electron microscope. The electron microscope works on the principle similar to that of a light microscope. An electromagnetic field and a beam of electrons act in a way similar to the action of a glass lens and a beam of light. Electron microscope is classified into two types- 1. Scanning electron microscope and 2. Transmission electron microscope. The detail information about above two types covered in the review.

KEYWORDS: Electron microscope, Scanning electron microscope, Transmission electron microscope etc.

INTRODUCTION
The word microscope is derived from the Greek words, mikros (small) and skopeo (look at). From the dawn of science there has been an interest in being able to look at smaller and smaller details of the world around us. Biologists have wanted to examine the structure of cells, bacteria, viruses, and colloidal particles. Materials scientists have wanted to see in homogeneities and imperfections in metals, crystals, and ceramics. In geology, the detailed study of rocks, minerals, and fossils on a microscopic scale provides insight into the origins of our planet and its valuable mineral resources. In the 18th century, technological innovations and design improvements enabled microscopes to gain popularity with scientists and researchers in all areas of human biology, botany, zoology, geology, and materials
science. One hundred years later, in the 1920s, it was discovered that accelerated electrons behave in a vacuum much like light. Furthermore, it was found that electric and magnetic fields could be used to shape the paths followed by electrons similar to the way glass lenses are used to bend and focus visible light. Ernst Ruska at the University of Berlin, along with Max Knoll, combined these characteristics and built the first transmission electron microscope (TEM) in 1931, for which Ruska was awarded the Nobel Prize for Physics in 1986. Electron microscopes are scientific instruments that use a beam of energetic electrons to examine objects on a very fine scale. Electron microscopes were developed due to the limitations of Light Microscopes which are limited by the physics of light. In the early 1930's, this theoretical limit had been reached and there was a scientific desire to see the fine details of the interior structures of organic cells (nucleus, mitochondria...etc.). This required 10,000x plus magnification which was not possible using current optical microscopes. The transmission electron microscope (TEM) was the first type of Electron Microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to "see through" the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931. The first scanning electron microscope (SEM) debuted in 1938 (Von Ardenne) with the first commercial instruments around 1965. Its late development was due to the electronics involved in "scanning" the beam of electrons across the sample. [1, 2, 3]

**Scanning Electron Microscope (Sem)**

**Principle**

The scanning electron microscope differs from transmission electron microscope, as the electron beam is not transmitted through specimen but impinges on its surface from above. A narrow beam of electrons with high velocity originated from an electron gun passes through condenser lenses and other magnetic lenses. They produce and focus an electron beam or probe of 5-10 nm diameter into an intense spot on the specimen surface. The electron probe, scanning over the specimen surface, excite specimen molecule in several form including high energy electrons called “Secondary electrons”. The number of secondary electrons released depends on angle of specimen point with respect to electron beam or probe. These secondary electrons are deflected towards collector and deflector. The successive electric signals are amplified and transmitted to cathode tube. Scanning beam and cathode ray beam are synchronizing. The image can be observed on the T.V. screen.
Interaction of Electron with Samples

The electron interact with the atoms at or close to sample surface produces signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity. The types of signals produced by an SEM include secondary electrons, back-scattered electrons (BSE), characteristic X-rays, light (cathodoluminescence), specimen current and transmitted electrons.

![Diagram of electron interactions](image)

**Fig. 1: Interactions of electrons**

**Secondary electrons**: they are electrons generated as ionization products. They are called 'secondary' because they are generated by other radiation (the primary radiation). This radiation can be in the form of ions, electrons, or photons with sufficiently high energy, i.e. exceeding the ionization potential. Secondary electron detectors are common in all SEMs. A SEM with secondary electron imaging or SEI can produce very high-resolution images of a sample surface, revealing details less than 1 nm in size.

**Back-scattered electrons (BSE)**: they are beam electrons that are reflected from the sample by elastic scattering. BSE are often used in analytical SEM along with the spectra made from the characteristic X-rays. Because the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images can provide information about the distribution of different elements in the sample.

**Characteristic X-rays**: they are emitted when the electron beam removes an inner shell electron from the sample, causing a higher energy electron to fill the shell and release energy. These characteristic X-rays are used to identify the composition and measure the abundance of elements in the sample.
Auger Electrons: Auger electrons are produced following the ionization of an atom by the incident electron beam and the falling back of an outer shell electron to fill an inner shell vacancy. The excess energy released by this process may be carried away by an Auger electron. This electron has a characteristic energy and can therefore be used to provide chemical information. Because of their low energies, Auger electrons are emitted only from near the surface. They have escape depths of only a few nanometers and are principally used in surface analysis.

Cathodoluminescence: Cathodoluminescence is another mechanism for energy stabilization following beam specimen interaction. Certain materials will release excess energy in the form of photons with infrared, visible, or ultraviolet wavelengths when electrons recombine to fill holes made by the collision of the primary beam with the specimen. These photons can be detected and counted by using a light pipe and photomultiplier similar to the ones utilized by the secondary electron detector. The best possible image resolution using this approach is estimated at about 50 nm.

Instrumentation [5, 6, 7]

![Instrumentation of SEM](image)

Fig. 2 Instrumentation of SEM

Electron Guns
Modern SEM systems require that the electron gun produces a stable electron beam with high current, small spot size, adjustable energy, and small energy dispersion. Several types of electron guns are used in SEM system and the qualities of electrons beam they produced vary considerably. The first SEM systems generally used tungsten “hairpin” or lanthanum
hexaboride (LaB6) cathodes, but for the modern SEMs, the trend is to use field emission sources, which provide enhanced current and lower energy dispersion. Emitter lifetime is another important consideration for selection of electron sources.

**Tungsten Electron Guns**

Tungsten electron guns have been used for more than 70 years, and their reliability and low cost encourage their use in many applications, especially for low magnification imaging and x-ray microanalysis [5]

![Schematic of the self-biased thermionic tungsten electron gun](image)

The most widely used electron gun is composed of three parts: a V-shaped hairpin tungsten filament (the cathode), a Wehnelt cylinder, and an anode. The tungsten filament is about 100 µm in diameter. The V-shaped filament is heated to a temperature of more than 2,800 K by applying a filament current so that the electrons can escape from the surface of the filament tip. A negative potential, which is varied in the range of 0.1–30 kV, is applied on the tungsten and Wehnelt cylinder by a high voltage supply. As the anode is grounded, the electric field between the filament and the anode plate extracts and accelerates the electrons toward the anode. In thermionic emission, the electrons have widely spread trajectories from the filament tip. A slightly negative potential between the Wehnelt cylinder and the filament, referred to “bias,” provides steeply curved equipotentials near the aperture of the Wehnelt cylinder, which produces a crude focusing of electron beam. The focusing effect of Wehnelt cylinder on the electron beam.
Lanthanum Hexaboride Guns
An alternative for tungsten filament is the LaB6 filament. This material has a lower work function (2.4 eV) than tungsten (4.5 eV). This means LaB6 can provide stronger emission of electrons at the same heating temperature. Therefore, LaB6 electron guns provide 5 to 10× greater brightness and a longer lifetime compared with conventional tungsten guns [6]

Field Emission Guns
Thermionic sources depend on a high temperature to overcome the work function of the metal so that the electrons can escape from the cathode.

Fig. 4 (a) SEM image of LaB6 electron gun and (b) a higher magnification image, small contamination spots are easily recognized.

Though they are inexpensive and the requirement of vacuum is relatively low, the disadvantages, such as short lifetime, low brightness, and large energy spread, restrict their applications. For modern electron microscopes, field emission electron guns (FEG) are a good alternative for thermionic electron guns.

There are three types of FEGs that are used in the SEM systems [7]. One is the cold field emission (CFE) sources. The “cold field” means the electron sources operate at room temperature. The second class is thermal field emission (TFE) sources, which is operated in elevated temperature. Beside CFE and TFE sources, Schottky emitters (SE) sources are also used in modern SEM systems. The performances of SE and CFE sources are superior to thermionic sources in the case of brightness, source size, and lifetime.
**Electron Lenses**

Electron beams can be focused by electrostatic or magnetic field. But electron beam controlled by magnetic field has smaller aberration, so only magnetic field is employed in SEM system. Coils of wire, known as “electromagnets,” are used to produce magnetic field, and the trajectories of the electrons can be adjusted by the current applied on these coils.

**Condenser Lenses**

The electron beam will diverge after passing through the anode plate from the emission source. By using the condenser lens, the electron beam is converged and collimated into a relatively parallel stream. A condenser aperture, generally, is associated with the condenser lens, and the focal point of the electron beam is above the aperture.

![Diagram](image-url)

**Fig. 5** A diagram showing how the electrons travel through the condenser lens and condenser aperture.

**Objective Lenses**

The electron beam will diverge below the condenser aperture. Objective lenses are used to focus the electron beam into a probe point at the specimen surface and to supply further demagnification.

Three designs of objective lenses are shown-

1. The asymmetric pinhole lens -is the most common objective lens. There is only a small bore on the pole piece, and this keeps the magnetic field within the lens and provides a field-free region above the specimen for detecting the secondary electrons.
Fig. 6 a) asymmetric pinhole lens

2. Symmetric immersion lens -the specimen is placed inside the lens, which can reduce the focal length significantly.

Fig. 6 b) symmetric immersion lens.

3. The Snorkel lens (Fig. 1.14c) produces a strong magnetic field that extends to the specimen. This kind of lens possesses the advantages of the pinhole lens and the immersion lens, combining low lens aberration with permission of large specimen.

Fig. 6 c) snorkel lens.
Scanning coils

Scanning coils are used to deflect the electron beam so that it can scan on the specimen surface along x- or y-axis. Several detectors are used to detect different signals: solid state BSE detectors for BSEs; the ET detector for secondary and BSEs; energy-dispersive x-ray spectrometer and wavelength-dispersive x-ray spectrometer for the characteristic x-rays; and photomultipliers for cathodoluminescence. The detected signal is also processed and projected on the CRT screen or camera. The scanning process of CRT or camera is synchronized with the electron beam by the scanning signal generator and hence a point-to-point image for the scanning area is produced.

![Image formation system in a typical scanning electron microscope](image)

**Fig. 7 Image formation system in a typical scanning electron microscope**

Detection of secondary electrons

The most common imaging mode collects low-energy (<50 eV) secondary electrons that are ejected from the k-shell of the specimen atoms by inelastic scattering interactions with beam electrons. Due to their low energy, these electrons originate within a few nanometers from the sample surface. The electrons are detected by an Everhart-Thornley detector, which is a type of scintillator-photomultiplier system. The secondary electrons are first collected by attracting them towards an electrically biased grid at about +400 V, and then further accelerated towards a phosphor or scintillator positively biased to about +2,000 V. The accelerated secondary electrons are now sufficiently energetic to cause the scintillator to emit flashes of light (cathodoluminescence), which are conducted to a photomultiplier outside the SEM column via a light pipe and a window in the wall of the specimen chamber. The amplified electrical signal output by the photomultiplier is displayed as a two-dimensional intensity distribution that can be viewed and photographed on an analogue video display, or subjected to analog-to-digital conversion and displayed and saved as a digital image. This process relies on a raster-scanned primary beam. The brightness of the signal depends on the
number of secondary electrons reaching the detector. If the beam enters the sample perpendicular to the surface, then the activated region is uniform about the axis of the beam and a certain number of electrons "escape" from within the sample. As the angle of incidence increases, the "escape" distance of one side of the beam will decrease, and more secondary electrons will be emitted. Thus steep surfaces and edges tend to be brighter than flat surfaces, which results in images with a well-defined, three-dimensional appearance. Using the signal of secondary electrons image resolution less than 0.5 nm is possible. [8, 9]

![Fig. 8 Comparison of SEM techniques](image)

Top: backscattered electron analysis – composition
Bottom: secondary electron analysis – topography

**Detection of backscattered electrons**

Backscattered electrons (BSE) consist of high-energy electrons originating in the electron beam, that are reflected or back-scattered out of the specimen interaction volume by elastic scattering interactions with specimen atoms. Since heavy elements (high atomic number) backscatter electrons more strongly than light elements (low atomic number), and thus appear brighter in the image, BSE are used to detect contrast between areas with different chemical compositions. [8] The Everhart-Thornley detector, which is normally positioned to one side of the specimen, is inefficient for the detection of backscattered electrons because few such electrons are emitted in the solid angle subtended by the detector, and because the positively biased detection grid has little ability to attract the higher energy BSE. Dedicated backscattered electron detectors are positioned above the sample in a "doughnut" type arrangement, concentric with the electron beam, maximizing the solid angle of collection.
BSE detectors are usually either of scintillator or of semiconductor types. When all parts of the detector are used to collect electrons symmetrically about the beam, atomic number contrast is produced. However, strong topographic contrast is produced by collecting back-scattered electrons from one side above the specimen using an asymmetrical, directional BSE detector; the resulting contrast appears as illumination of the topography from that side. Semiconductor detectors can be made in radial segments that can be switched in or out to control the type of contrast produced and its directionality. Backscattered electrons can also be used to form an electron backscatter diffraction (EBSD) image that can be used to determine the crystallographic structure of the specimen [9].

**SEM Advantages** [10]

1. Advantages of a Scanning Electron Microscope include its wide-array of applications, the detailed three-dimensional and topographical imaging and the versatile information garnered from different detectors.
2. SEMs are also easy to operate with the proper training and advances in computer technology and associated software make operation user-friendly.
3. This instrument works fast, often completing SEI, BSE and EDS analyses in less than five minutes. In addition, the technological advances in modern SEMs allow for the generation of data in digital form.
4. Although all samples must be prepared before placed in the vacuum chamber, most SEM samples require minimal preparation actions.

**SEM Disadvantages** [10]

1. The disadvantages of a Scanning Electron Microscope start with the size and cost.
2. SEMs are expensive, large and must be housed in an area free of any possible electric, magnetic or vibration interference.
3. Maintenance involves keeping a steady voltage, currents to electromagnetic coils and circulation of cool water.
4. Special training is required to operate an SEM as well as prepare samples.
5. The preparation of samples can result in artifacts. The negative impact can be minimized with knowledgeable experience researchers being able to identify artifacts from actual data as well as preparation skill. There is no absolute way to eliminate or identify all potential artifacts.
6. In addition, SEMs are limited to solid, inorganic samples small enough to fit inside the vacuum chamber that can handle moderate vacuum pressure.

7. Finally, SEMs carry a small risk of radiation exposure associated with the electrons that scatter from beneath the sample surface.

8. The sample chamber is designed to prevent any electrical and magnetic interference, which should eliminate the chance of radiation escaping the chamber. Even though the risk is minimal, SEM operators and researchers are advised to observe safety precautions.

**SEM Applications**\(^{[1, 2, 10]}\)

1. SEMs have a variety of applications in a number of scientific and industry-related fields, especially where characterizations of solid materials is beneficial.

2. In addition to topographical, morphological and compositional information, a Scanning Electron Microscope can detect and analyze surface fractures, provide information in microstructures, examine surface contaminations, reveal spatial variations in chemical compositions, provide qualitative chemical analyses and identify crystalline structures.

3. SEMs can be as essential research tool in fields such as life science, biology, gemology, medical and forensic science, metallurgy.

4. In addition, SEMs have practical industrial and technological applications such as semiconductor inspection, production line of miniscule products and assembly of microchips for computers.

**Transmission Electron Microscope (Tem)**

**Principle**

The source of illumination in TEM is electron beam. It is previously mentioned that resolution of microscope depends on wavelength of light. Use of light is with low wavelengths is responsible for increasing resolution. The electron beam used in this microscope has wavelength 5 nm. (Compared to wavelength of light rays – 280 nm to 800 nm). If the system gives highest resolution, the magnification can be increased by \(10^5\). Transmission electron microscope is so called, as the electron beam transmits through this specimen. The rest of principle is somewhat similar to that of light microscope.
INSTRUMENTATION

Transmission electron microscope (TEM), type of electron microscope that has three essential systems: (1) **an electron gun**, which produces the electron beam, and the condenser system, which focuses the beam onto the object, (2) **the image-producing system**, consisting of the objective lens, movable specimen stage, and intermediate and projector lenses, which focus the electrons passing through the specimen to form a real, highly magnified image, and (3) **the image-recording system**, which converts the electron image into some form perceptible to the human eye. The image-recording system usually consists of a fluorescent screen for viewing and focusing the image and a digital camera for permanent records. In addition, a **vacuum system**, consisting of pumps and their associated gauges and valves, and power supplies are required.

The electron gun and condenser system

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**Fig. 9 Instrumentation of TEM**

**Fig. 10 Electron gun**
The source of electrons, the cathode, is a heated V-shaped tungsten filament or, in high-performance instruments, a sharply pointed rod of a material such as lanthanum hexaboride. The filament is surrounded by a control grid, sometimes called a Wehnelt cylinder, with a central aperture arranged on the axis of the column; the apex of the cathode is arranged to lie at or just above or below this aperture. The cathode and control grid are at a negative potential equal to the desired accelerating voltage and are insulated from the rest of the instrument. The final electrode of the electron gun is the anode, which takes the form of a disk with an axial hole. Electrons leave the cathode and shield, accelerate toward the anode, and, if the stabilization of the high voltage is adequate, pass through the central aperture at a constant energy. The control and alignment of the electron gun are critical in ensuring satisfactory operation.

The intensity and angular aperture of the beam are controlled by the condenser lens system between the gun and the specimen. A single lens may be used to converge the beam onto the object, but, more commonly, a double condenser is employed. In this the first lens is strong and produces a reduced image of the source, which is then imaged by the second lens onto the object. Such an arrangement is economical of space between the electron gun and the object stage and is more flexible, because the reduction in size of the image of the source (and hence the final size of illuminated area on the specimen) may be varied widely by controlling the first lens. The use of a small spot size minimizes disturbances in the specimen due to heating and irradiation.

The image-producing system
The specimen grid is carried in a small holder in a movable specimen stage. The objective lens is usually of short focal length (1–5 mm [0.04–0.2 inch]) and produces a real intermediate image that is further magnified by the projector lens or lenses. A single projector lens may provide a range of magnification of 5:1, and by the use of interchangeable pole pieces in the projector a wider range of magnifications may be obtained. Modern instruments employ two projector lenses (one called the intermediate lens) to permit a greater range of magnification and to provide a greater overall magnification without a commensurate increase in the physical length of the column of the microscope. For practical reasons of image stability and brightness, the microscope is often operated to give a final magnification of 1,000–250,000× on the screen. If a higher final magnification is required, it may be obtained by photographic or digital enlargement. The quality of the final image in the
electron microscope depends largely upon the accuracy of the various mechanical and electrical adjustments with which the various lenses are aligned to one another and to the illuminating system. The lenses require power supplies of a high degree of stability; for the highest standard of resolution, electronic stabilization to better than one part in a million is necessary. The control of a modern electron microscope is carried out by a computer, and dedicated software is readily available.

The Image recording system
Imaging systems in a TEM consist of a phosphor screen, which may be made of fine (10–100 μm) particulate zinc sulphide, for direct observation by the operator. Optionally, an image recording system such as film based or doped YAG screen coupled CCDs.[12] Typically these devices can be removed or inserted into the beam path by the operator as required.

Vacuum system
To increase the mean free path of the electron gas interaction, a standard TEM is evacuated to low pressures, typically on the order of $10^{-4}$ Pa.[13] The need for this is twofold: first the allowance for the voltage difference between the cathode and the ground without generating an arc, and secondly to reduce the collision frequency of electrons with gas atoms to negligible levels—this effect is characterised by the mean free path. TEM components such as specimen holders and film cartridges must be routinely inserted or replaced requiring a system with the ability to re-evacuate on a regular basis. As such, TEMs are equipped with multiple pumping systems and airlocks and are not permanently vacuum sealed. The vacuum system for evacuating a TEM to an operating pressure level consists of several stages. Initially a low or roughing vacuum is achieved with either a rotary vane pump or diaphragm pumps bringing the TEM to a sufficiently low pressure to allow the operation of a turbomolecular or diffusion pump which brings the TEM to its high vacuum level necessary for operations. To allow for the low vacuum pump to not require continuous operation, while continually operating the turbomolecular pumps, the vacuum side of a low-pressure pump may be connected to chambers which accommodate the exhaust gases from the turbomolecular pump.[14] Sections of the TEM may be isolated by the use of pressure-limiting apertures, to allow for different vacuum levels in specific areas, such as a higher vacuum of $10^{-4}$ to $10^{-7}$ Pa or higher in the electron gun in high-resolution or field-emission TEMs. High-voltage TEMs require ultra-high vacuums on the range of $10^{-7}$ to $10^{-9}$ Pa to prevent...
generation of an electrical arc, particularly at the TEM cathode.\textsuperscript{[15]} As such for higher voltage TEMs a third vacuum system may operate, with the gun isolated from the main chamber either by use of gate valves or by the use of a differential pumping aperture. The differential pumping aperture is a small hole that prevents diffusion of gas molecules into the higher vacuum gun area faster than they can be pumped out. For these very low pressures either an ion pump or a getter material is used. Poor vacuum in a TEM can cause several problems, from deposition of gas inside the TEM onto the specimen as it is being viewed through a process known as electron beam induced deposition, or in more severe cases damage to the cathode from an electrical discharge.\textsuperscript{[16]} Vacuum problems due to specimen sublimation are limited by the use of a cold trap to adsorb sublimated gases in the vicinity of the specimen.\textsuperscript{[14]}

**Sample Preparation**

Sample preparation in TEM can be a complex procedure.\textsuperscript{[17]} TEM specimens are required to be at most hundreds of nanometers thick, as unlike neutron or X-Ray radiation the electron beam interacts readily with the sample, an effect that increases roughly with atomic number squared ($z^2$).\textsuperscript{[16]} High quality samples will have a thickness that is comparable to the mean free path of the electrons that travel through the samples, which may be only a few tens of nanometers. Preparation of TEM specimens is specific to the material under analysis and the desired information to obtain from the specimen. As such, many generic techniques have been used for the preparation of the required thin sections. Materials that have dimensions small enough to be electron transparent, such as powders or nanotubes, can be quickly prepared by the deposition of a dilute sample containing the specimen onto support grids or films. In the biological sciences in order to withstand the instrument vacuum and facilitate handling, biological specimens can be fixated using either a negative staining material such as uranyl acetate or by plastic embedding. Alternately samples may be held at liquid nitrogen temperatures after embedding in vitreous ice.\textsuperscript{[18]} In material science and metallurgy the specimens tend to be naturally resistant to vacuum, but still must be prepared as a thin foil, or etched so some portion of the specimen is thin enough for the beam to penetrate. Constraints on the thickness of the material may be limited by the scattering cross-section of the atoms from which the material is comprised.

**Tissue sectioning**

By passing samples over a glass or diamond edge, small, thin sections can be readily obtained using a semi-automated method.\textsuperscript{[19]} This method is used to obtain thin, minimally deformed
samples that allow for the observation of tissue samples. Additionally inorganic samples have been studied, such as aluminium, although this usage is limited owing to the heavy damage induced in the less soft samples. To prevent charge build-up at the sample surface, tissue samples need to be coated with a thin layer of conducting material, such as carbon, where the coating thickness is several nanometers. This may be achieved via an electric arc deposition process using a sputter coating device.

**Sample staining**
Details in light microscope samples can be enhanced by stains that absorb light; similarly TEM samples of biological tissues can utilize high atomic number stains to enhance contrast. The stain absorbs electrons or scatters part of the electron beam which otherwise is projected onto the imaging system. Compounds of heavy metals such as osmium, lead, uranium or gold (in immunogold labeling) may be used prior to TEM observation to selectivity deposit electron dense atoms in or on the sample in desired cellular or protein regions, requiring an understanding of how heavy metals bind to biological tissues.

**Mechanical milling**
Mechanical polishing may be used to prepare samples. Polishing needs to be done to a high quality, to ensure constant sample thickness across the region of interest. A diamond, or cubic boron nitride polishing compound may be used in the final stages of polishing to remove any scratches that may cause contrast fluctuations due to varying sample thickness. Even after careful mechanical milling, additional fine methods such as ion etching may be required to perform final stage thinning.

**Chemical etching**
Certain samples may be prepared by chemical etching, particularly metallic specimens. These samples are thinned using a chemical etchant, such as an acid, to prepare the sample for TEM observation. Devices to control the thinning process may allow the operator to control either the voltage or current passing through the specimen, and may include systems to detect when the sample has been thinned to a sufficient level of optical transparency.

**Ion etching**
Ion etching is a sputtering process that can remove very fine quantities of material. This is used to perform a finishing polish of specimens polished by other means. Ion etching uses an inert gas passed through an electric field to generate a plasmastream that is directed to the
sample surface. Acceleration energies for gases such as argon are typically a few kilovolts. The sample may be rotated to promote even polishing of the sample surface. The sputtering rate of such methods is on the order of tens of micrometers per hour, limiting the method to only extremely fine polishing. More recently focused ion beam methods have been used to prepare samples. FIB is a relatively new technique to prepare thin samples for TEM examination from larger specimens. Because FIB can be used to micro-machine samples very precisely, it is possible to mill very thin membranes from a specific area of interest in a sample, such as a semiconductor or metal. Unlike inert gas ion sputtering, FIB makes use of significantly more energetic gallium ions and may alter the composition or structure of the material through gallium implantation.\[^{22}\]

**Advantages of TEM**\[^{23}\]

A Transmission Electron Microscope is an impressive instrument with a number of advantages such as:

1. TEMs offer the most powerful magnification, potentially over one million times or more
2. TEMs have a wide-range of applications and can be utilized in a variety of different scientific, educational and industrial fields
3. TEMs provide information on element and compound structure
4. Images are high-quality and detailed
5. TEMs are able to yield information of surface features, shape, size and structure
6. They are easy to operate with proper training

**Disadvantages of TEM**\[^{23}\]

Some cons of electron microscopes include:

1. TEMs are large and very expensive
2. Laborious sample preparation
3. Potential artifacts from sample preparation
4. Operation and analysis requires special training
5. Samples are limited to those that are electron transparent, able to tolerate the vacuum chamber and small enough to fit in the chamber
6. TEMs require special housing and maintenance
7. Images are black and white
8. Electron microscopes are sensitive to vibration and electromagnetic fields and must be housed in an area that isolates them from possible exposure.
9. A Transmission Electron Microscope requires constant upkeep including maintaining voltage, currents to the electromagnetic coils and cooling water.

Applications Of Tem \cite{1, 2, 23}

1. A Transmission Electron Microscope is ideal for a number of different fields such as life sciences, nanotechnology, medical, biological and material research, forensic analysis, gemology and metallurgy as well as industry and education.
2. TEMs provide topographical, morphological, compositional and crystalline information.
3. The images allow researchers to view samples on a molecular level, making it possible to analyze structure and texture.
4. This information is useful in the study of crystals and metals, but also has industrial applications.
5. TEMs can be used in semiconductor analysis and production and the manufacturing of computer and silicon chips.
6. Technology companies use TEMs to identify flaws, fractures and damages to micro-sized objects; this data can help fix problems and/or help to make a more durable, efficient product.
7. Colleges and universities can utilize TEMs for research and studies.
8. Although electron microscopes require specialized training, students can assist professors and learn TEM techniques.
9. Students will have the opportunity to observe a nano-sized world in incredible depth and detail.

CONCLUSION

It can help Scanning Electron Microscope users and nonmaterial’s researchers to master the basic techniques to study nonmaterials in a short time. With the understanding of the basics and knowing the configurations of the microscope. Electron microscope can also be used to study internal as well as external structure of the sample. In pharmaceutical field, scanning electron microscopy is very useful in studies associated with the surface characteristics of drug particles and morphological studies of antibiotic producing microorganisms and their pores.

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