Scanning Electron Microscope (SEM)

The first Scanning **Electron Microscope** was initially made by Mafred von Ardenne in 1937 with an aim to surpass the transmission electron Microscope. He used high-resolution power to scan a small raster using a beam of electrons that were focused on the raster. He also aimed at reducing the problems of chromatic aberrations images produced by the Transmission electron Microscopes. More studies followed by scientists and research institutions such as Cambridge Scientific Instrument Company who eventually developed a fully constructed Scanning electron Microscope, in 1965 and named it a Stereoscan. *The price of the Scanning Electron Microscope (SEM) is approximately $1 million.*

Definition

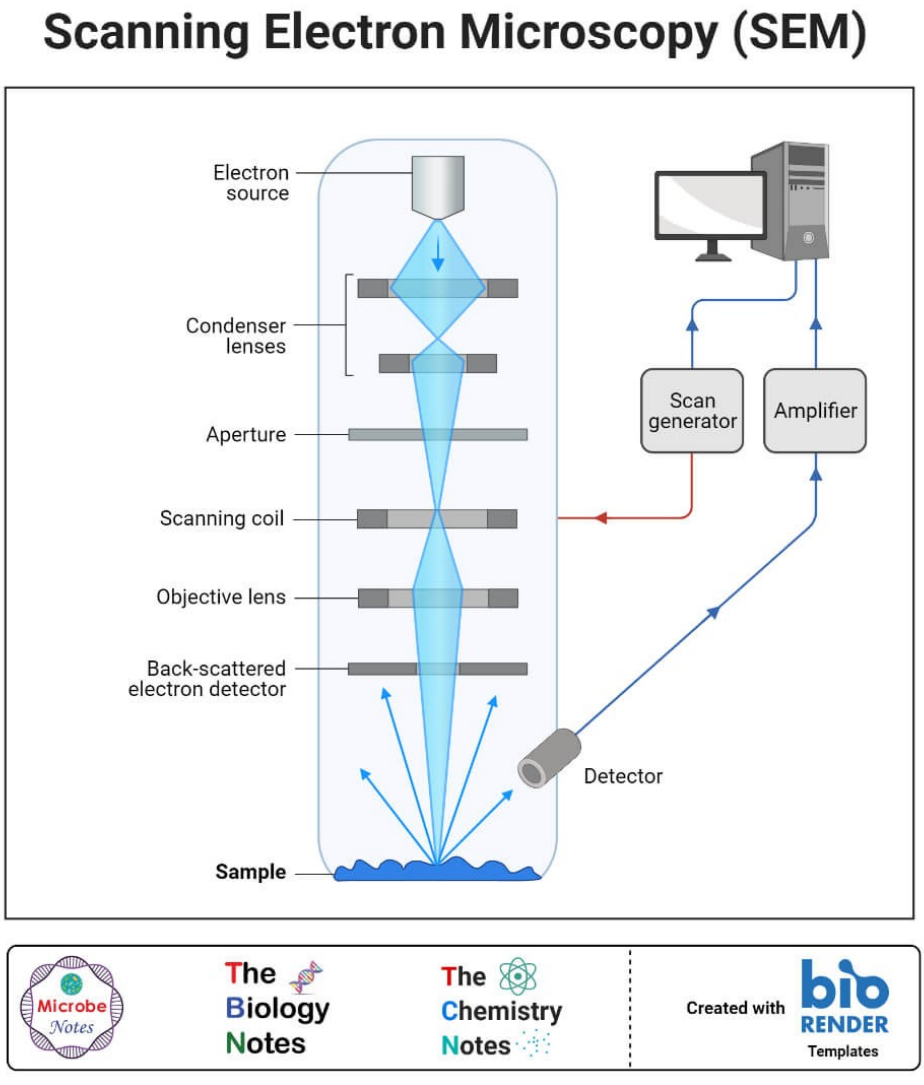
Scanning Electron Microscope (SEM) is a type of electron microscope that scans surfaces of microorganisms that uses a beam of electrons moving at low energy to focus and scan specimens. The development of electron microscopes was due to the inefficiency of the wavelength of light microscopes. electron microscopes have very short wavelengths in comparison to the light microscope which enables better resolution power.

Image Source: JEOL USA, Inc. and Wikipedia.

Principle of Scanning Electron Microscope (SEM)

Unlike the Transmission Electron Microscope which uses transmitted electrons, the scanning electron Microscope uses emitted electrons. The Scanning electron microscope works on the principle of applying kinetic energy to produce signals on the interaction of the electrons. These electrons are secondary electrons, backscattered electrons, and diffracted backscattered electrons which are used to view crystallized elements and photons. Secondary and backscattered electrons are used to produce an image. The secondary electrons are emitted from the specimen play the primary role of detecting the morphology and topography of the specimen while the backscattered electrons show contrast in the composition of the elements of the specimen.

How does the Scanning Electron Microscope work?



- The source of the electrons and the electromagnetic lenses are from tungsten filament lamps that are placed at the top of the column and it is similar to those of the transmission electron Microscope.
- The electrons are emitted after thermal energy is applied to the electron source and allowed to move in a fast motion to the anode, which has a positive charge.

- The beam of electrons activates the emission of primary scattered (Primary) electrons at high energy levels and secondary electrons at low-energy levels from the specimen surface. The beam of electrons interacts with the specimen to produce signals that give information about the surface topography and composition of the specimen.
- The specimen does not need special treatment for visualization under the SEM, even air-dried samples can be examined directly. However, microbial specimens need fixation, dehydration, and drying in order to maintain the structural features of the cells and to prevent collapsing of the cells when exposed to the high vacuum of the microscope.
- The samples are mounted and coated with thin layer of heavy metal elements to allow spatial scattering of electric charges on the surface of the specimen allowing better image production, with high clarity.
- Scanning by this microscope is attained by tapering a beam of electrons back and forth over a thin section of the microscope. When the electrons reach the specimen, the surface releases a tiny staw of electrons known as secondary electrons which are then trapped by a special detector apparatus.
- When the secondary electrons reach and enter the detector, they strike a scintillator (a luminescence material that fluoresces when struck by a charged particle or high-energy photon). This emits flashes of light which get converted into an electric current by a photomultiplier, sending a signal to the cathode ray tube. This produces an image that looks like a television picture that can be viewed and photographed.
- The quantity of secondary electrons that enter the detector is highly defined by the nature of the specimen i.e raised surfaces to receive high quantities of electrons, entering the detector while depressed surfaces have fewer electrons reaching the surface and hence fewer electrons enter the detector.
- Therefore raised surfaces will appear brighter on the screen while depressed surfaces appear darker.

Parts of a Scanning Electron Microscope (SEM)

The major components of the Scanning Electron Microscope include;

- **Electron Source** – This is where electrons are produced under thermal heat at a voltage of 1-40kV. the electrons condense into a beam that is used for the creation of an image and analysis. There are three types of electron sources that can be used i. e Tungsten filament, Lanthanum hexaboride, and Field emission gun (FEG)
- **Lenses** – it has several condenser lenses that focus the beam of electrons from the source through the column forming a narrow beam of electrons that form a spot called a spot size.
- **Scanning Coil** – they are used to deflect the beam over the specimen surface.
- **Detector** – It’s made up of several detectors that are able to differentiate the secondary electrons, backscattered electrons, and diffracted backscattered electrons. The functioning of the detectors highly depends on the voltage speed, the density of the specimen.
- The display device (data output devices)
- Power supply
- Vacuum system

Like the transmission electron Microscope, the Scanning electron microscope should be free from vibrations and any electromagnetic elements.

Applications of the Scanning Electron Microscope (SEM)

It is used in a variety of fields including Industrial uses, nanoscience studies, Biomedical studies, Microbiology

1. Used for spot chemical analysis in energy-Dispersive X-ray Spectroscopy.
2. Used in the analysis of cosmetic components which are very tiny in size.
3. Used to study the filament structures of microorganisms.
4. Used to study the topography of elements used in industries.

Advantages of the Scanning Electron Microscope (SEM)

- They are easy to operate and have user-friendly interfaces.
- They are used in a variety of industrial applications to analyze surfaces of solid objects.
- Some modern SEMs are able to generate digital data that can be portable.
- It is easy to acquire data from the SEM, within a short period of time of about 5 minutes.

Limitations

- They are very expensive to purchase
- They are bulky to carry
- They must be used in rooms that are free of vibrations and free of electromagnetic elements
- They must be maintained with a consistent voltage
- They should be maintained with access to cooling systems

The combination of the working principles of the Scanning Electron Microscope (SEM) and the Transmission Electron Microscope (TEM) formed the **Scanning-Transmission Electron Microscope (STEM).** The Scanning- Transmission Electron Microscope (STEM), uses a convergent beam of electrons to focus on a probe on the specimen, and the probe is then scanned on its surface collecting signals which are then collected as point-to-point to form an image.

Scanning Electron Microscope (SEM) Images

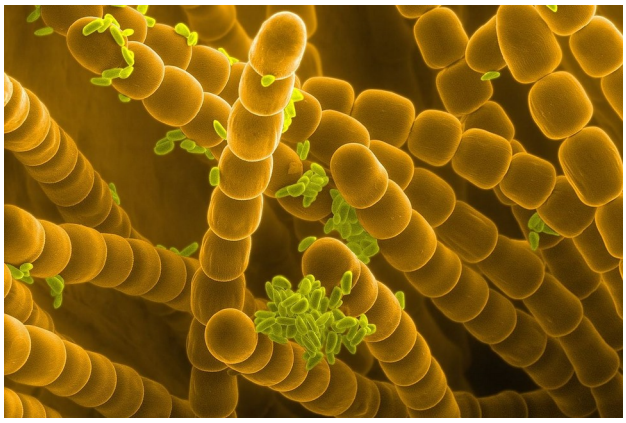


Figure: SEM image of Tradescantia pollen and stamens. Source: Wikipedia

Figure: Low-temperature scanning electron micrograph of soybean cyst nematode and its egg. Magnified 1,000 times. Source: Wikipedia

Figure: Scanning electron microscope image of a hederelloid from the Devonian of Michigan (largest tube diameter is 0.75 mm). Source: Wikipedia.

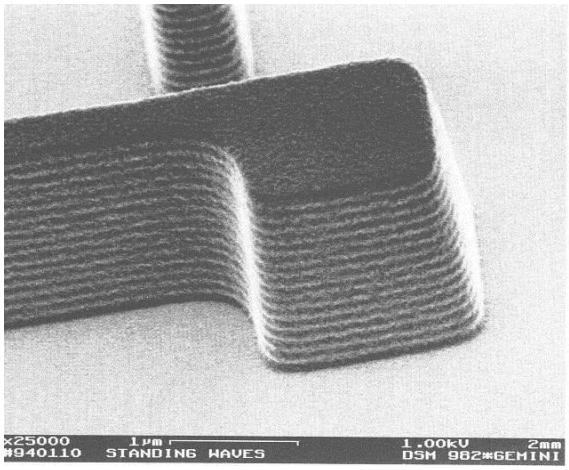


Figure: Photoresist SEM micrograph (1995) SEM= DSM 982 Gemini from Zeiss. Source: Wikipedia.

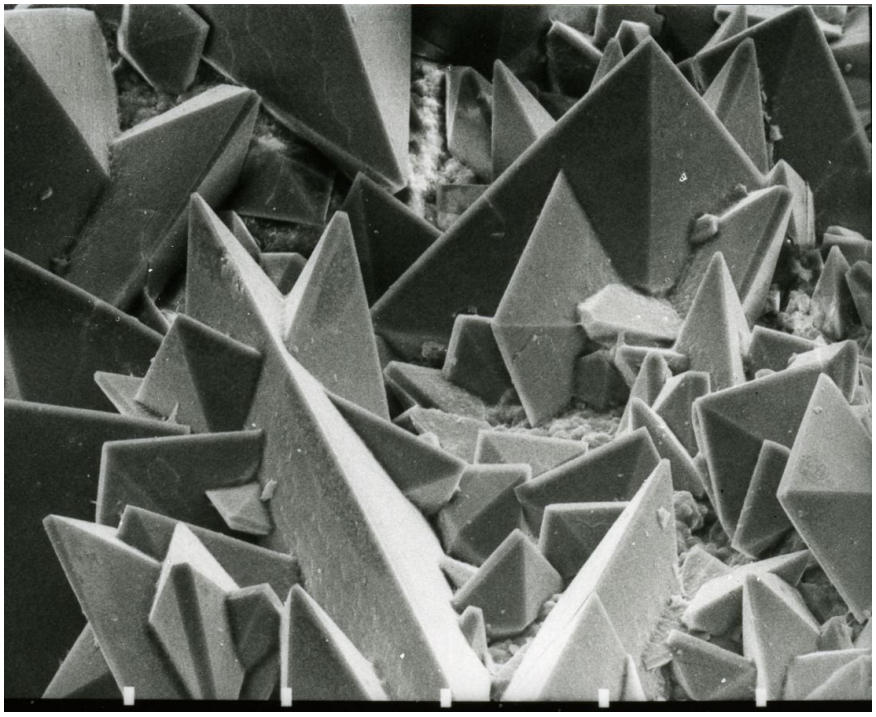


Figure: Scanning Electron Micrograph of the surface of a kidney stone showing tetragonal crystals of Weddellite (calcium oxalate dihydrate) emerging from the amorphous central part of the stone. Source: Wikipedia.

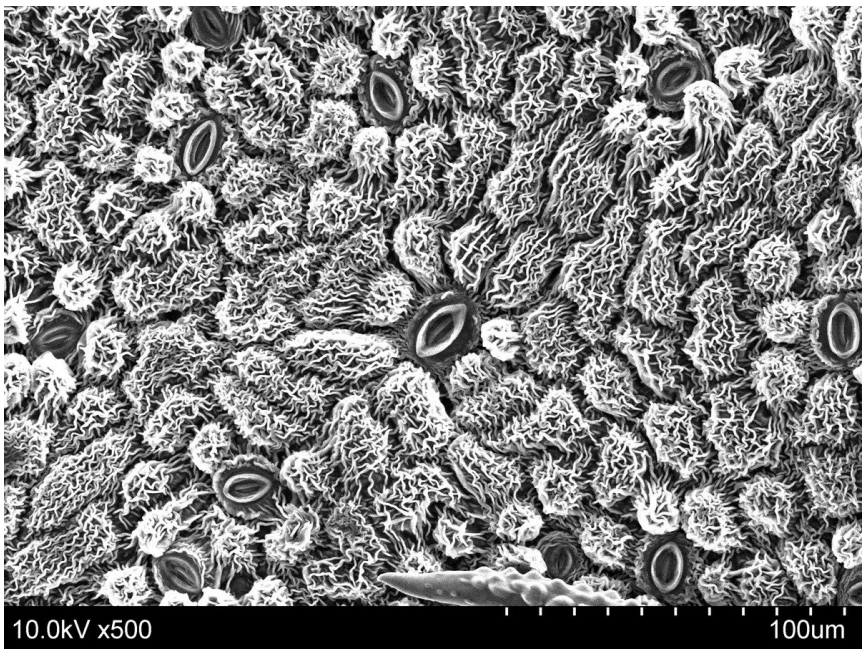
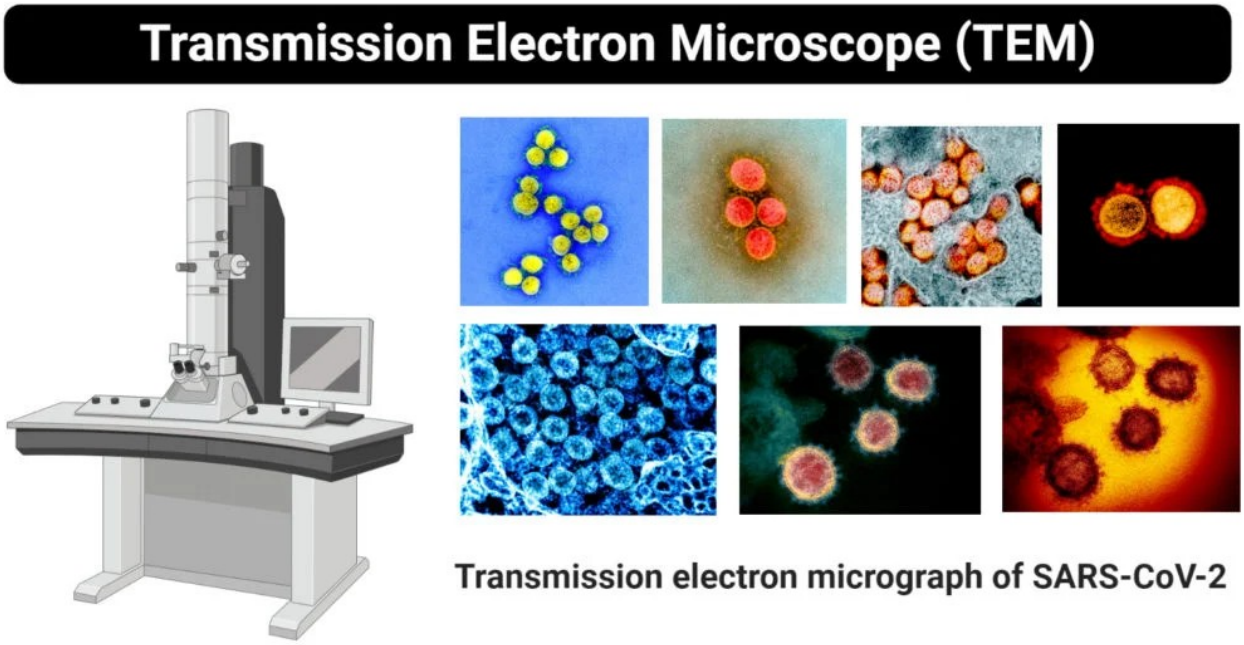


Figure: SEM image of stomata on the lower surface of a leaf. Source: Wikipedia.

Transmission Electron Microscope (TEM)

- This is a powerful electron microscope that uses a beam of electrons to focus on a specimen producing a highly magnified and detailed image of the specimen.
- The magnification power is over 2 million times better than that of the **light microscope**, producing the image of the specimen which enables easy characterization of the image in its morphological features, compositions and crystallization information is also detailed.
- Early discovery of cathode rays like electrons by Louis de Broglie in the early 1920s, paved way into the development of an electron microscope where they used a beam of electrons creating a form of wave motion.
- Magnetic fields were used as lenses for the electrons. With these discoveries, the first electron microscope was later developed by Ernst Ruska and Max Knolls in 1931 and modified into a Transmission Electron Microscope (TEM) by Ernst Ruska along with the Sieman's company, in 1933.
- This TEM microscope has several advantages compared to the light microscope with its efficiency also being very high.
- Among all microscopes both light and electron microscopes, TEM are the most powerful microscopes used in laboratories. It can magnify a mall particle of about 2nm, and therefore they have a resolution limit of 0.2um.

Principle of Transmission Electron Microscope



The working principle of the Transmission Electron Microscope (TEM) is similar to the light microscope. The major difference is that light microscopes use light rays to focus and produce an image while the TEM uses a beam of electrons to focus on the specimen, to produce an image.

Electrons have a shorter wavelength in comparison to light which has a long wavelength. The mechanism of a light microscope is that an increase in resolution power decreases the wavelength of the light, but in the TEM, when the electron illuminates the specimen, the resolution power

increases increasing the wavelength of the electron transmission. The wavelength of the electrons is about 0.005nm which is 100,000X shorter than that of light, hence TEM has better resolution than that of the light microscope, of about 1000times.

This can accurately be stated that the TEM can be used to detail the internal structures of the smallest particles like a virion particle.

Parts of Transmission Electron Microscope

Their working mechanism is enabled by the high-resolution power they produce which allows it to be used in a wide variety of fields. It has three working parts which include:

1. Electron gun
2. Image producing system
3. Image recording system

Electron gun

- This is the part of the Transmission Electron Microscope responsible for producing electron beams.
- Electrons are produced by a cathode that is a tungsten filament that is V-shaped and it is normally heated. The tungsten filament is covered by a control grid known as a Wehnelt cylinder made up of a central hole which lies columnar to the tube. The cathode lies on top of or below the cylindrical column hole. The cathode and the control grid are negatively charged with an end of the anode which is disk-shaped that also has an axial hole.
- When electrons are transmitted from the cathode, they pass through the columnar aperture (hole) to the anode at high voltage with constant energy, which is efficient for focusing the specimen to produce an accurately defined image.
- It also has the condenser lens system which works to focus the electron beam on the specimen by controlling the energy intensity and the column hole of the electron gun. The TEM uses two condenser lenses to converge the beam of electrons to the specimen. The two condenser lens each function to produce an image i.e the first lens which has strong magnification, produces a smaller image of the specimen, to the second condenser lens, directing the image to the objectives.

Image- Producing system

- Its made up of the objective lens, a movable stage or holding the specimen, intermediate and projector lenses. They function by focusing the passing electrons through the specimen forming a highly magnified image.
- The objective has a short focal length of about 1-5mm and it produces an intermediate image from the condenser which are transmitted to the projector lenses for magnification.
- The projector lenses are of two types, i.e the intermediate lens which allows great magnification of the image and the projector lens which gives a generally greater magnification over the intermediate lens.
- To produce efficient high standard images, the objectives and the projector lenses need high power supplies with high stability for the highest standard of resolution.

Image-Recording System

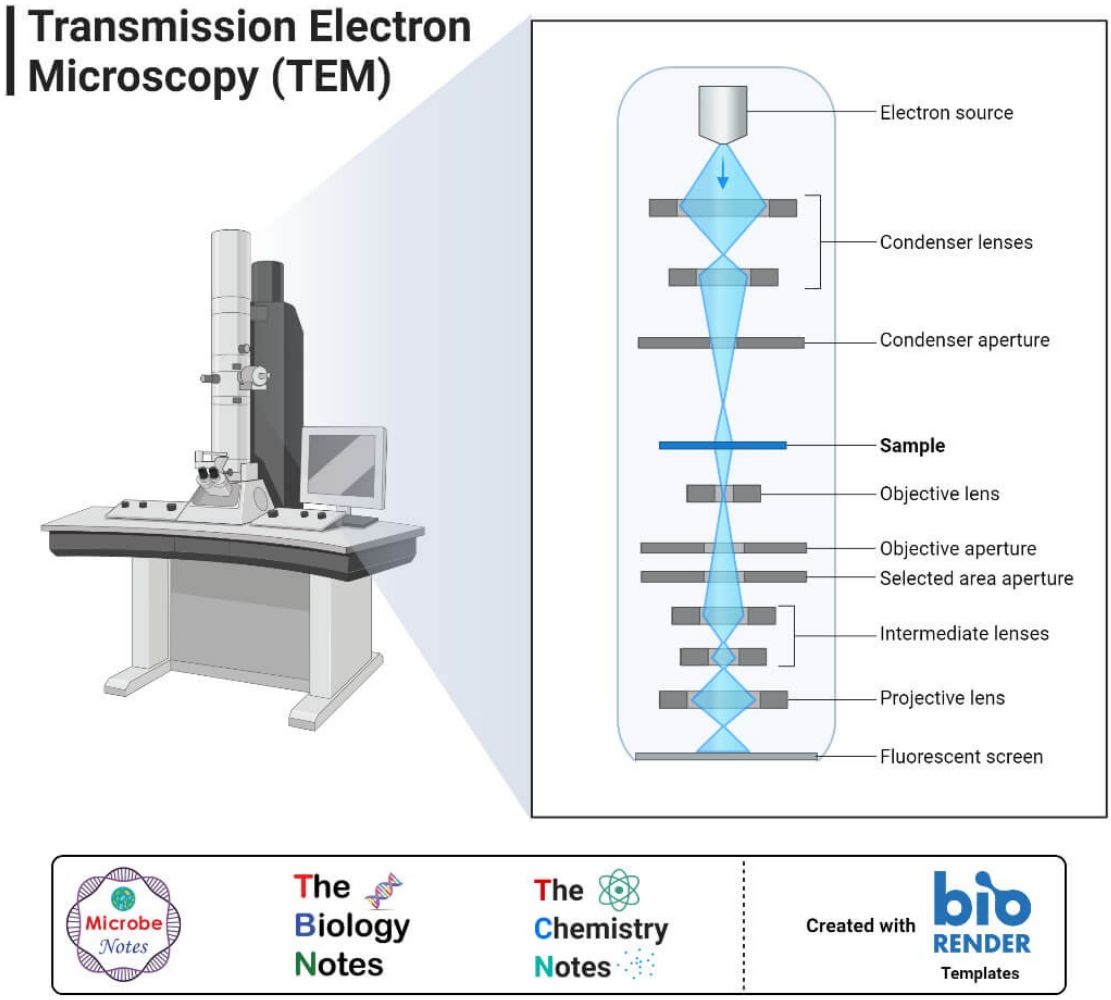
- Its made up of the fluorescent screen used to view and to focus on the image. They also have a digital camera that permanently records the images captured after viewing.
- They have a vacuum system that prevents the bombardment or collision of electrons with air molecules disrupting their movement and ability to focus. A vacuumed system facilitates the straight movement of electrons to the image.
- The vacuumed system is made up of a pump, gauge, valves and a power supply.
- The image that is formed is called a monochromatic image, which is greyish or black and white. The image must be visible to the human eye, and therefore, the electrons are allowed to pass through a fluorescent screen fixed at the base of the microscope.
- The image can also be captured digitally and displayed on a computer and stored in a JPEG or TIFF format. During the storage, the image can be manipulated from its monochromatic state to a colored image depending on the recording apparatus eg use of pixel cameras can store the image in color.
- The presence of colored images allows easy visualization, identification, and characterization of the images.

How does a Transmission Electron Microscope (TEM) work?

From the instrumentation described, the working mechanism is a sequential process of the parts of the TEM mentioned above. To mean:

- A heated tungsten filament in the electron gun produces electrons that get focus on the specimen by the condenser lenses.
- Magnetic lenses are used to focus the beam of electrons of the specimen. By the assistance offered by the column tube of the condenser lens into the vacuum creating a clear image, the vacuum allows electrons to produce a clear image without collision with any air molecules which may deflect them.
- On reaching the specimen, the specimen scatters the electrons focusing them on the magnetic lenses forming a large clear image, and if it passes through a fluorescent screen it forms a polychromatic image.
- The denser the specimen, the more the electrons are scattered forming a darker image because fewer electron reaches the screen for visualization while thinner, more transparent specimens appear brighter.

NOTE: If the screen is moved aside, a photographic image can be captured in pixels forming a permanent image.



Preparation of specimen for visualization by TEM

The specimen to be viewed under the TEM must undergo a special preparation technique to enable visualization and creation of a clear image.

- Electrons are easily absorbed and easily scattered on solid elements, showing poor visualization for thick specimens. And therefore, very thin specimens are used for accurate and clear visualization forming a clear image as well. The specimen should be about 20-100nm thin and 0.025-0.1nm diameter, as small as that of a bacterial cell. Thin specimens allow interaction with electrons in a vacuumed space, are able to maintain their innate structure.
- To get thin slice specimens, the specimen is first fixed on a plastic material with glutaraldehyde or osmium tetraoxide. These chemical agents stabilize the structure of the cell and maintain its originality. The addition of an organic solvent like alcohol such as ethanol will dehydrate the cell completely for embedding the specimen to the plastics.
- The specimen is then permeated by adding an unpolymerized liquid epoxy plastic making it hardened like a solid block. This is where thin sections are cut from using a glass knife with a piece of special equipment known as an ultramicrotome.
- The specimen is then stained appropriately (with the appropriate stain) for the uniform scattering of electrons. The thin sections are then soaked in heavy metallic elements such as

lead citrate and uranyl acetate allowing the lean and aluminum ions to bind to the cell structures. This forms an opaque layer against the electrons on the cell structures to increase contrast.

- The stained thin sections are then mounted on copper grids for viewing.
- The primary staining techniques that are applied for viewing under the TEM is Negative staining coupled with heavy metallic elements coating. The metallic coating scatters electrons which appears on the photographic film while uncoated sections and used to study bacterial, viral cell morphologies and structures.

Applications of Transmission Electron Microscope (TEM)

TEM is used in a wide variety of fields From Biology, Microbiology, Nanotechnology, forensic studies, etc. Some of these applications include:

1. To visualize and study cell structures of bacteria, viruses, and fungi
2. To view bacteria flagella and plasmids
3. To view the shapes and sizes of microbial cell organelles
4. To study and differentiate between plant and animal cells.
5. Its also used in nanotechnology to study nanoparticles such as ZnO nanoparticles
6. It is used to detect and identify fractures, damaged microparticles which further enable repair mechanisms of the particles.

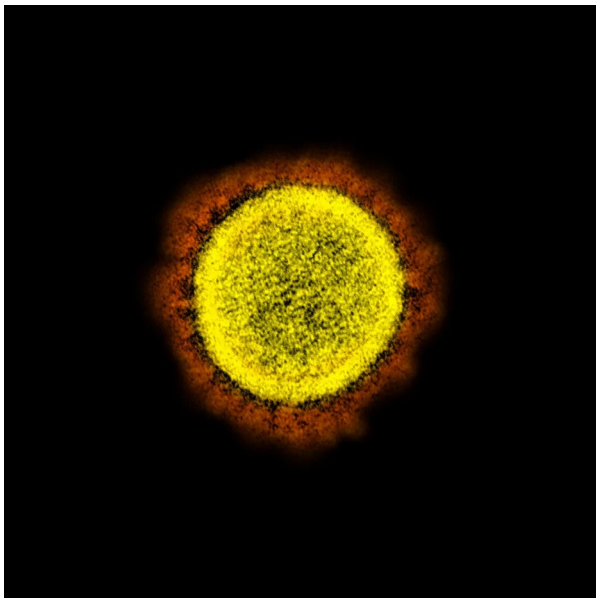
Advantages of Transmission Electron Microscope (TEM)

1. It has a very powerful magnification of about 2 million times that of the Light microscope.
2. It can be used for a variety of applications ranging from basic Biology to Nanotechnology, to education and industrial uses.
3. It can be used to acquire vast information on compounds and their structures.
4. It produces very efficient, high-quality images with high clarity.
5. It can produce permanent images.
6. It is easy to train and use the Transmission Electron Microscope

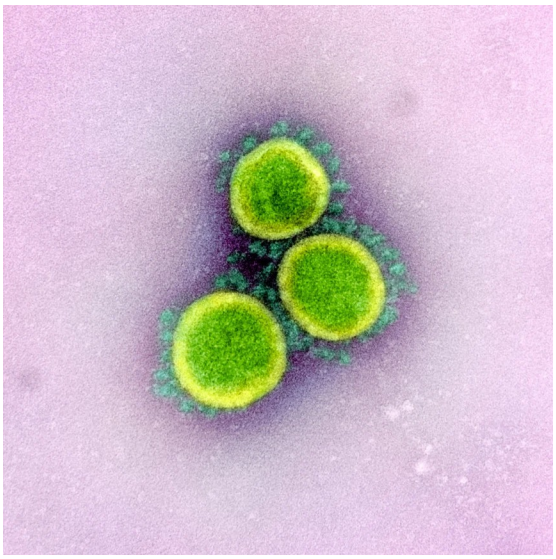
Limitations of Transmission Electron Microscope (TEM)

1. Generally, the TEMs are very expensive to purchase
2. They are very big to handle.
3. The preparation of specimens to be viewed under the TEM is very tedious.
4. The use of chemical fixations, dehydrators, and embedments can cause the dangers of artifacts.
5. They are laborious to maintain.
6. It requires a constant inflow of voltage to operate.
7. They are extremely sensitive to vibrations and electro-magnetic movements hence they are used in isolated areas, where they are not exposed.
8. It produces monochromatic images, unless they use a fluorescent screen at the end of visualization.

Transmission Electron Microscope (TEM) Images



Transmission electron micrograph of SARS-CoV-2 virus particles, isolated from a patient. Image captured and color-enhanced at the NIAID Integrated Research Facility (IRF) in Fort Detrick, Maryland. Credit: NIAID.



Atomic Force Microscope (AFM)

The atomic force **microscope** (AFM) is a type of scanning probe microscope whose primary roles include measuring properties such as magnetism, height, friction. The resolution is measured in a nanometer, which is much more accurate and effective than the optical diffraction limit. It uses a probe for measuring and collection of data involves touching the surface that has the probe. An image is formed when the scanning probe microscope raster-scans the probe over a section of the sample, measuring its local properties concurrently. They also have piezoelectric elements, which are electric charges that accumulate in selected solid materials like **DNA**, biological proteins, crystal, etc, to enable tiny accurate and precise movement during scanning upon an electric command. The Atomic Force Microscope was invented in 1982, by scientists working in IBM, just after the invention of the Scanning tunneling Microscope in 1980 by Gerd Binnig and Heinrich Rohler by IBM Research in Zurich. That is when Binnig later invented the Atomic Force Microscope, and it was first used experimentally in 1986. It was put on the market for commercial sale in 1989.

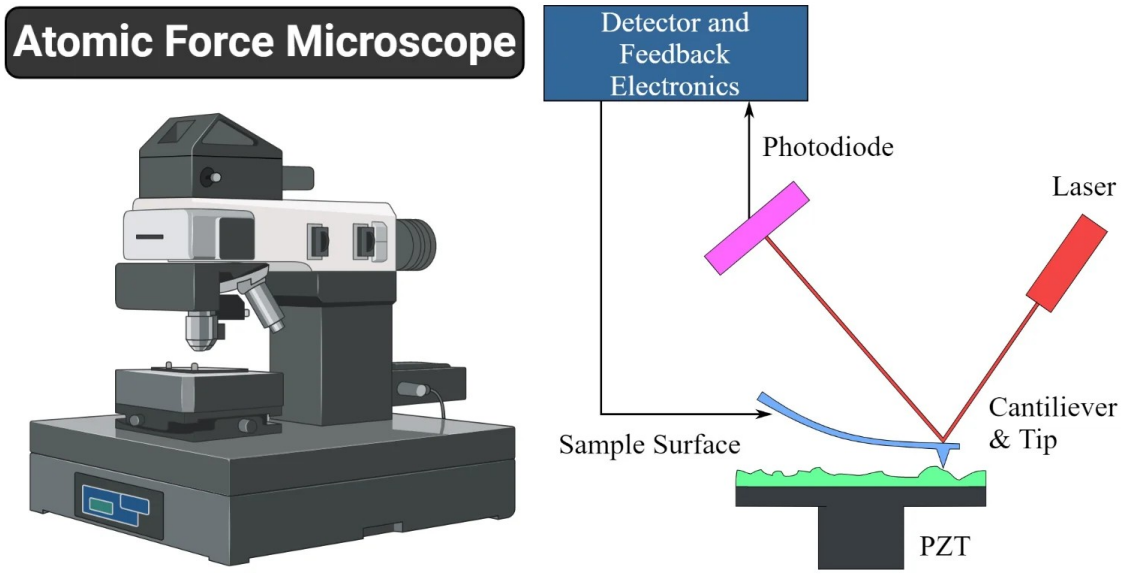


Figure: Atomic Force Microscope (AFM)

Principle of Atomic Force Microscope

The Atomic Force Microscope works on the principle measuring intermolecular forces and sees atoms by using probed surfaces of the specimen in nanoscale. Its functioning is enabled by three of its major working principles that include Surface sensing, Detection, and Imaging.

1. The Atomic Force Microscope (AFM) performs surface sensing by using a cantilever (an element that is made of a rigid block like a beam or plate, that attaches to the end of support, from which it protrudes making a perpendicularly flat connection that is vertical like a wall). The cantilever has a sharp tip that scans over the sample surface, by forming an attractive force between the surface and the tip when it draws closer to the sample surface. When it draws very close making contact with the surface of the sample, a repulsive force gradually takes control making the cantilever avert from the surface.

2. During the deflection of the cantilever away from the sample surface, there is a change in direction of reflection of the beam, and a laser beam detects the aversion, by reflecting off a beam from the flat surface of the cantilever. Using a positive-sensitive photo-diode (PSPD- a component that is based on silicon PIN diode technology and is used to measure the position of the integral focus of an incoming light signal), it tracks these changes of deflection and change in direction of the reflected beam and records them.
3. The Atomic Force Microscope (AFM) takes the image of the surface topography of the sample by force by scanning the cantilever over a section of interest. Depending on how raised or how low the surface of the sample is, it determines the deflection of the beam, which is monitored by the Positive-sensitive photo-diode (PSDP). The microscope has a feedback loop that controls the length of the cantilever tip just above the sample surface, therefore, it will maintain the laser position thus generating an accurate imaging map of the surface of the image.

Parts of Atomic Force Microscope

Atomic Force Microscopes have several techniques for measuring force interactions such as van der Waals, thermal, electrical and magnetic force interactions for these interactions done by the AFM, it has the following parts that assist in controlling its functions.

- Modified tips which are used to detect the sample surface and undergo deflections
- Software adjustments used to image the samples.
- Feedback loop control – they control the force interactions and the tip positions using a laser deflector. the laser reflects from the back of the cantilever and the tip and while the tip interacts with the surface of the sample, the laser's position on the photodetector is used in the feedback loop for tracking the surface of the sample and measurement.
- Deflection – The Atomic Force Microscope is constructed with a laser beam deflection system. The laser is reflected from the back of the AFM lever to the sensitive detector. They are made from silicon compounds with a tip radius of about 10nm.
- Force measurement – the AFM works and depends highly on the force interactions, they contribute to the image produced. The forces are measured by calculation of the deflection lever when the stiffness of the cantilever is known. This calculation is defined by Hooke's law, defined as follows: **F= -kz**, where F is the force, k is the stiffness of the lever, and z is the distance the lever is bent.

Applications of Atomic Force Microscope

This type of microscopy has been used in various disciplines in natural science such as solid-state physics, semiconductor studies, molecular engineering, polymer chemistry, surface chemistry, molecular biology, cell biology, medicine, and physics.

Some of these applications include:

1. Identifying atoms from samples
2. Evaluating force interactions between atoms
3. Studying the physical changing properties of atoms

4. Studying the structural and mechanical properties of protein complexes and assembly, such as microtubules.
5. used to differentiate cancer cells and normal cells.
6. Evaluating and differentiating neighboring cells and their shape and cell wall rigidity.

Advantages of Atomic Force Microscope

1. Easy to prepare samples for observation
2. It can be used in vacuums, air, and liquids.
3. Measurement of sample sizes is accurate
4. It has a 3D imaging
5. It can be used to study living and nonliving elements
6. It can be used to quantify the roughness of surfaces
7. It is used in dynamic environments.

Disadvantages of Atomic Force Microscope

1. It can only scan a single nanosized image at a time of about 150x150nm.
2. They have a low scanning time which might cause thermal drift on the sample.
3. The tip and the sample can be damaged during detection.
4. It has a limited magnification and vertical range.