1.Study of simple and compound microscopes, including oil immersion objectives

SIMPLE MICROSCOPE :

The microscope is the most indispensible instrument in a microbiology laboratory. Microscopes can be classified into Simple and Compound microscopes based on the number of lenses used.

A simple microscope consists of a single lens,it is also called a dissection microscope and is used in dissection of very minute specimens like plant parts. This microscope is used to observe specimens that are two big for a compound microscope. Even live specimens growing on nutrient medium can be observed. A monocular dissection microscope produces a two-dimensional image of the specimen. Whereas, binocular dissection microscope produces a stereoscopic or 3- dimensional image.

MONOCULAR DISSECTION MICROSCOPE:

A monocular dissection microscope consists of a base/ foot, a vertical limb or pillar, a stage and a lens. The base/ foot supports all the parts of the microscope. The limb or pillar has an attached stage made up of a thick glass plate. A folded arm which can be moved vertically, holds the lense. The movement of the lens is controlled by an adjustment screw provided on the limb. A mirror is attached at the base of the limb to reflect on the specimen.

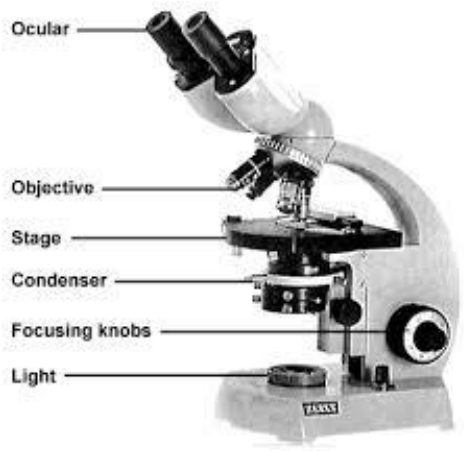
USE OF A DISSECTION MICROSCOPE:

- The optical parts of the microscope consisting of the lens and mirror are cleaned with the help of a lens paper.
- The illumination is adjusted using the mirror such that light is concentrated on the stage.
- The specimen is placed on the stage under the lens and focussed using the adjustment screws to get a sharp image of the specimen.

STUDY OF A COMPOUND MICROSCOPE:

The light compoud microscope or bright field microscope is most commonly used in the microbiology laboratory. The compound microscope consists of a combination of 2 or more lenses; an ***ocular*** or ***eye piece*** and ***objective***.

Construction : The compound microscope basically consists of two parts namely the **mechanical** parts and the **optical** parts.The mechanical parts provide the structural frame work of the microscope and support the optical parts. The optical parts are involved in the magnification of the object or specimen.



PARTS OF A COMPOUND MICROSCOPE : Base, Pillar and Inclination joint.

The microscope is built around a strong base or foot which is U- or V - shaped or horse-shoe shaped and made up of metal and, a vertical pillar.An inclination joint joins the pillar to the arm of the microscope and helps in tilting the arm to any angle.

ARM AND BODY TUBE: The arm is a C- shaped solid piece of metal which is attached at one end to the pillar and at the other end it has a body tube. The body tube is a hollow metallic cylinder to which the optical parts are fixed. The body tube can be moved up and down with the help of a graduated rack and pinion.

DRAW TUBE AND REVOLVING NOSE PIECE: Draw tube is a hollow metallic tube narrower than the body tube and half inserted in the body tube. It holds the ocular or eye piece lens at its upper end. The revolving nose piece is a circular rotating disc attached to the lower end of the body tube.

ADJUSTMENT KNOBS OR SCREWS: There are 2 adjustment screws

1. The ***coarse adjustment screw*** which is used for focusing the object under the low power objective.
2. The ***fine adjustment screw*** used with high power objective and oil immersion objective to get a clearer image of the specimen.

STAGE: It is a small solid square piece of metal with a central circular opening to allow light from the condenser to the object. It is positioned about half- way up the arm and fixed to the

pillar. It is used as a platform to place the object for observation.

MIRROR: The mirror is circular and planoconcave. It is fixed to the pillar below the substage condenser. It collects and reflects light towards the object and can be rotated towards light. The concave side is used when natural light is focussed and plane side is used to focus artificial light. In some microscopes there is in- built illumination instead of the mirror.

SUBSTAGE CONDENSER AND IRIS DIAPHRAGM: The condenser consists of a condensing lens which converges lights and focuses it on the object. The iris diaphragm is made up of overlapping circular metallic discs. The opening or aperture of the diaphragm can be controlled by means of a lever which helps in regulating the amount of light reaching the condenser.

OPTICAL PARTS: The two sets of lenses are **eyepiece** or **ocular**, and the **objective lenses.**

THE EYE PIECE OR OCULAR: The eye piece is the lens closer to the eye and it is fixed on the upper end of the draw tube. It magnifies the image of the object formed by the objective. Microscopes with single ocular are called **monocular** and with two oculars are called **binocular**. The eye piece may have 5X, 10X or 15X magnification.

OBJECTIVE LENS: The objective lens is the lens closer to the object and may have 10X,40X,60X or 100X magnification. The objective lenses are attached to the revolving nose piece and they form a real magnified image of the specimen. The total magnification of the compound microscope is calculated by the product of the magnifications of eye piece and objective lenses.

HANDLING OF COMPOUND MICROCOPE:

1. All parts of the microscope are cleaned before and after use. The lenses and mirror are cleaned with lens paper.
2. The microscope should not be pulled to the edge of the table and while lifting it both hands must by used ,one hand holding the base.
3. The slides to be observed must be clean and dry.
4. The microscope should be covered when not in use.

WORKING PRINCIPLE OF A COMPOUND MICROSCOPE

All the light microscope function on the same principle of magnification .the source of energy for magnification is light .The light rays reflected by a plano-concave mirror enter the condenser .The condenser lens focuses a cone of light on the specimen through the stage hole .Some of the light rays in this holes forms the background of the bright light .Those light rays which strikes the specimen will bend and pass through the objective lens to form the image of the object within the body tube .This image is further enlarged by the ocular lens system .Thus the image that we see is magnified two times .

Therefore magnification of microscope=Ocular lens magnification X Objective lens magnification

IMAGE FORMATION IN COMPOUND MICROSCOPE

Image formation is done by dual lens system .The objective lens produces a real ,inverted ,intermediate image which lies within the principle focus of the eye piece .The intermediate image act as an object for the eye piece .The eyepiece produces the final image .Thus the final image is inverted ,magnified and it is formed in the eye.

RELATIONSHIP BETWEEN WORKING DISTANCE OF OBJECTIVE LEN AND ADUJUSTMENT OF IRIS DIAPHRAGM

The cone of light which enters the objective of the microscope differs with each objective as the magnification of the objective increases the working distance decreases .The working distance is the distance between the specimen and the objective lens .The size of the light cone is controlled by iris diaphragm place just below the sub stage condenser.

When 10X , 45X objectives are used the diaphragm is not opened fully .When oil immersion is used the diaphragm is opened fully .As oil immersion objective requires maximum light .Oil immersion objective magnifies the object 100 times with the least working distance.

OIL IMMERSION OBJECTIVE:

The oil immersion objective is of 90X – 100X magnification. It usually has a black or coloured line painted around it. It has a numerical aperture greater than 1.0. It produces a clear ,highly magnified image of the specimen when it is immersed in a drop of oil over the specimen mounted on the microscope slide . The immersion oil (cedar wood oil) between the slide and the objective improves the resolution because it has a refractive index of around 1.5,the same as that of glass. The oil does not allow scattering off light and all the light from the specimen enters into the objective. The image formed by the oil immersion object has a high resolution because the working distance between the object and the objective is very much reduced when compared to the high dry objective.

Use of Oil immersion objective : The specimen on the microscope slide is first focused on the low power objective and then under high dry objective . The resolving nose piece is turned half way through to the oil immersion objective. A small drop of immersion oil is placed just above the specimen on the microscope slide. The oil immersion objective is turned gently into place and immersed in the drop of oil and the specimen is focused using only the fine adjustment knob, because the oil immersion objective is now very close to the specimen.

The Dark-Field Microscope

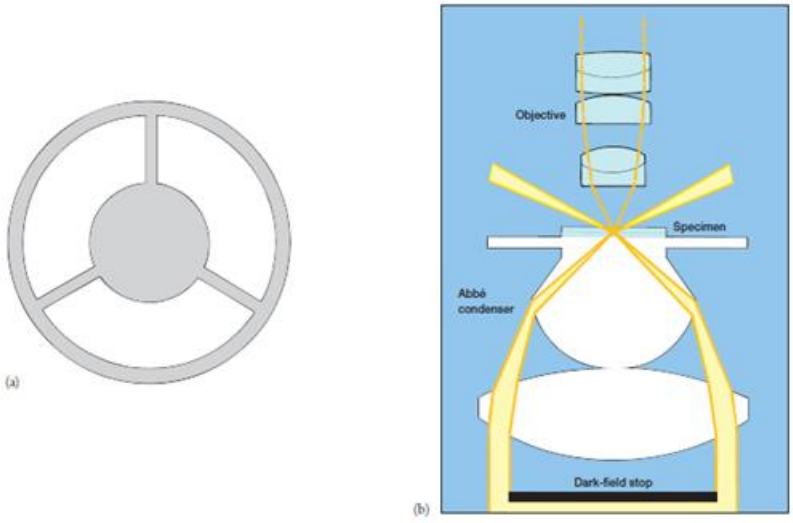
The dark field microscope is essentially a light microscope,but with a different condenser. The normally used abbe condenser is replaced by dark field condenser. In a dark field condenser, the centre of the top lens is opaque, so that none of the central rays of light can pass through, and the specimen on the stage is illuminated only with the peripheral oblique rays.

Living, unstained cells and organisms can be observed by simply changing the way in which they are illuminated. A hollow cone of light is focused on the specimen in such a way that unreflected and unrefracted rays do not enter the objective. Only light that has been reflected or refracted by the specimen forms an image. The field surrounding a specimen appears black,

while the object itself is brightly illuminated because the background is dark, this type of microscopy is called dark-field microscopy. Considerable internal structure is often visible in larger eucaryotic microorganisms . The dark-field microscope is used to identify bacteria like the thin and distinctively shaped *Treponema pallidum*, the causative agent of syphilis.

Uses: 1.It is used for examination of live unstained preparations of microorganisms.

2. The motility of microorganisms can also be observed since they are in a moist living condition.



Dark-Field Microscopy. The simplest way to convert a microscope to dark-field microscopy is to place (**a**) a dark-field stop underneath (**b**) the condenser lens system. The condenser then produces a hollow cone of light so that the only light entering the objective comes from the specimen.

The Phase-Contrast Microscope

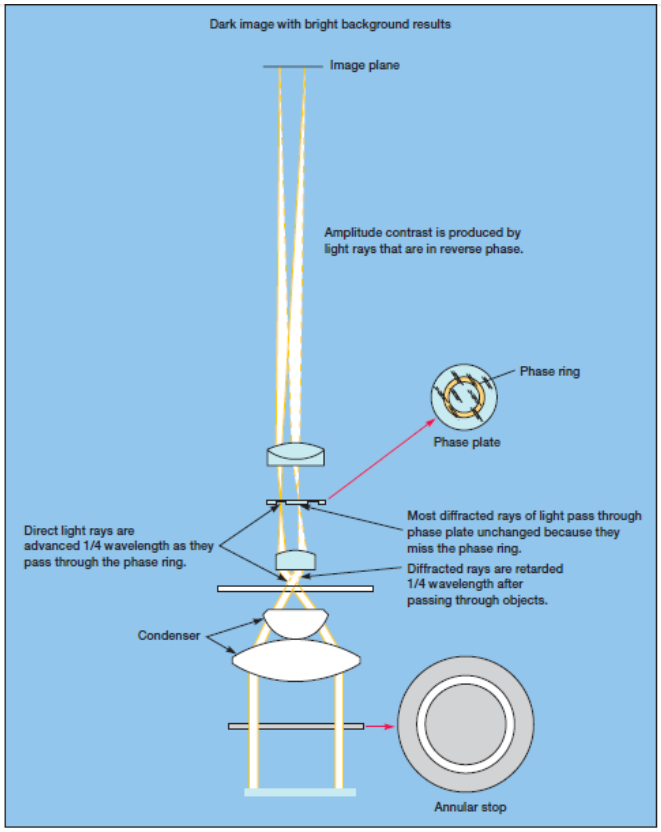
The dasic construction of a phase contrast microscope is like a bright field microscope only except for two special attachments. These are: 1. A special type condenser and 2. A phase plate.

The condenser has a special diaphragm consisting of an annular stop. This annular stop can be compared to a solid rod kept loosely at the centre of a cylinder . The annular stop allows only a hallow cone of light rays to pass through the condenser and light the object on the slide.

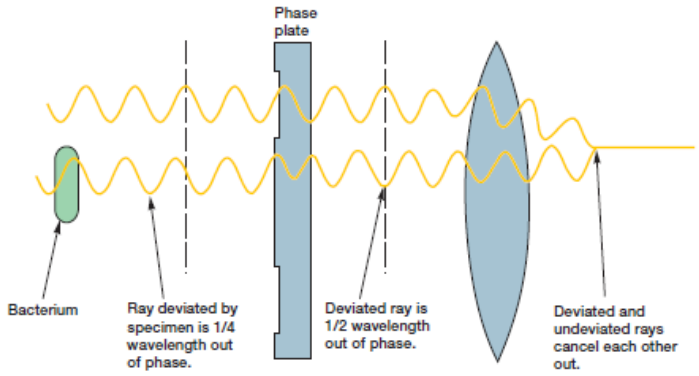
The phase plate is a special optical disc located in the rear focal plane of the objective. It has a special phase ring coated with a material that can either advance or retard the direct rays depending on its construction.

Unpigmented living cells are not clearly visible in the bright field microscope because there is little difference in contrast between the cells and water. Thus microorganisms often must be fixed and stained before observation to increase contrast and create variations in color between cell structures. A **phase-contrast microscope** converts slight differences in refractive index and cell density into easily detected variations in light intensity and is an excellent way to observe living cells.The condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light . As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about 1/4 wavelength. The deviated light is focused to

form an image of the object. Undeviated light rays strike a phase ring in the phase plate, a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate. If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by 1/4 wavelength, the deviated and undeviated waves will be about 1/2 wavelength out of phase and will cancel each other when they come together to form an image. The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined. This type of microscopy is called **dark-phase-contrast microscopy.** Color filters often are used to improve the image . Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial components such as endospores and inclusion bodies that contain poly- -hydroxybutyrate, polymetaphosphate, sulfur, or other substances.These are clearly visible because they have refractive indexes markedly different from that of water. Phase contrast microscopes also are widely used in studying eukaryotic cells.



Phase-Contrast Microscopy. The optics of a dark-phase-contrast microscope



The Production of Contrast in Phase Microscopy. The behavior of deviated and undeviated or undiffracted light rays in the dark phase-contrast microscope. Because the light rays tend to cancel each other out, the image of the specimen will be dark against a brighter background.

Stereo Microscope (The Differential Interference Contrast Microscope)

The **differential interference contrast (DIC) microscope** is similar to the phase-contrast microscope in that it creates an image by detecting differences in refractive indices and thickness. Two beams of plane polarized light at right angles to each other are generated by prisms. In one design, the object beam passes through the specimen, while the reference beam passes through a clear area of the slide. After passing through the specimen, the two beams are combined and interfere with each other to form an image. A live, unstained specimen appears brightly colored and three-dimensional . Structures such as cell walls, endospores, granules, vacuoles, and eucaryotic nuclei are clearly visible.

The Fluorescence Microscope

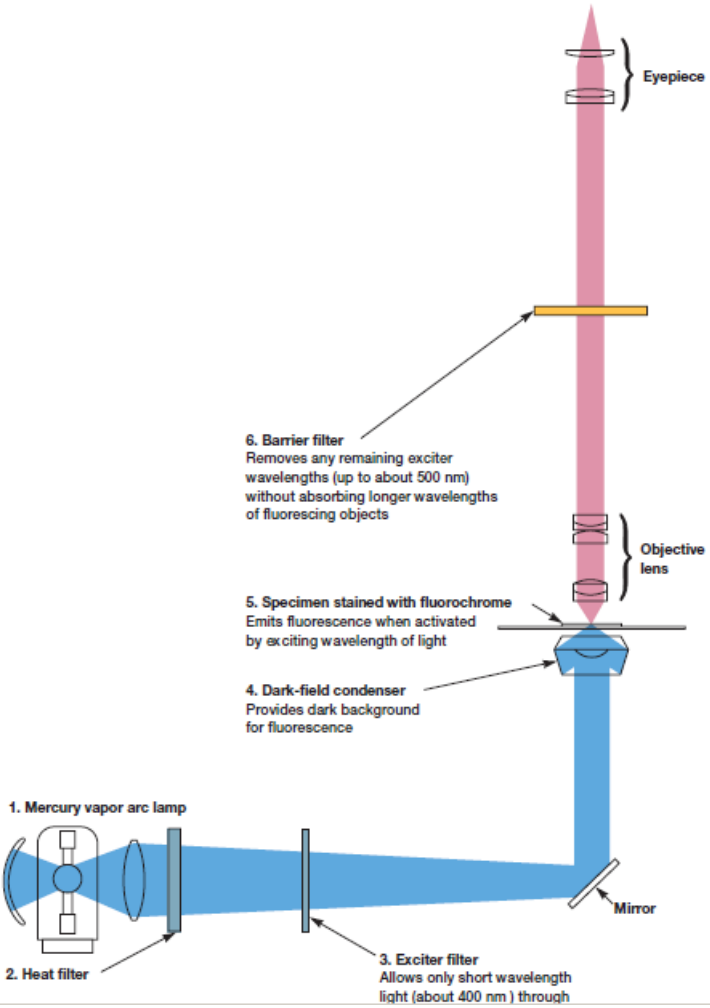
A fluorescence microscope differs from an ordinary light microscope in the following respects.

1. Light source is a mercury vapour lamp

2. A dark field condenser is used instead of the normal abbe condenser
3. Three sets of filters are employed to alter the light rays that pass through the instrument and reach the eye.-Heat filter, Exciter filter and Barrier filter

The microscopes thus far considered produce an image from light that passes through a specimen. An object also can be seen because it actually emits light, and this is the basis of fluorescence microscopy. When some molecules absorb radiant energy, they become excited and later release much of their trapped energy as light. Any light emitted by an excited molecule will have a longer wavelength (or be of lower energy) than the radiation originally absorbed. **Fluorescent light** is emitted very quickly by the excited molecule as it gives up its trapped energy and returns to a more stable state. The **fluorescence microscope** exposes a specimen to ultraviolet, violet, or blue light and forms an image of the object with the resulting fluorescent light. A mercury vapor arc lamp or other source produces an intense beam, and heat transfer is

limited by a special infrared filter. The light passes through an exciter filter that transmits only the desired wavelength. A darkfield condenser provides a black background against which the fluorescent objects glow. Usually the specimens have been stained with dye molecules, called **fluorochromes,** that fluoresce brightly upon exposure to light of a specific wavelength, but some microorganisms are autofluorescing. The microscopefrom the light emitted when they fluoresce. A barrier filter positioned after the objective lenses removes any remaining ultraviolet light, which could damage the viewer's eyes, or blue and violet light, which would reduce the image's contrast. The fluorescence microscope has become an essential tool in medical microbiology and microbial ecology. Bacterial pathogens (e.g., *Mycobacterium tuberculosis,* the cause of tuberculosis) can be identified after staining them with fluorochromes or specifically labeling them with fluorescent antibodies using immunofluorescence procedures. In ecological studies the fluorescence microscope is used to observe microorganisms stained with fluorochrome-labeled probes or fluorochromes such as acridine orange and DAPI (diamidino-2-phenylindole, a DNA-specific stain). The stained organisms will fluoresce orange or green and can be detected even in the midst of other particulate material. It is even possible to distinguish live bacteria from dead bacteria by the color they fluoresce after treatment with a special mixture of stains. Thus the microorganisms can be viewed and directly counted in a relatively undisturbed ecological niche.



Fluorescence Microscopy. The principles of operation of a fluorescence microscope

ELECTRON MICROSCOPE:

The electron microscope was invented by Ruska and Knoll in 1930. In this microscope an electron beam, produced by an electron gun made of a heated tungsten filament , is used as a source of illumination . The modern electron microscope has a high resolving power with the limit of resolution being 5-10 nm as compared to 0.2μm of light microscope. Therefore ultra fine structure of bacterial cells and structure of viruses can be studied only under an electron microscope. The electron microscope consists of the electron gun, electomagnetic lenses and condensers and a fluorescent screen for viewing the image of the specimen. The microscope is operated under a high voltage and high vacuum. There are two types of electron microscope; 1. ***Transmission electron microscope*** and 2. ***Scanning electron microscope.***

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 (TEM):

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 (TEM): 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.

Working of Transmission Electron Microscope (TEM) :

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 beca
- 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.

Freeze-itching treatment:

To reduce the possible dangers of artifacts, freeze-itching is used especially for the treatment of microbial cells, unlike chemical fixation, dehydration, and embedding, where most specimens get contaminated.

- Microbial cell organelles undergo special treatment known as Freeze-itching whereby the specimens are prepared with liquid nitrogen and then warmed at -100°C in a vacuum chamber.
- The sections are then cut with a precooled knife in liquid nitrogen at -196°C. After warming up the sectioned specimen in a high vacuum for about 2 minutes, it can then coated ith platinum and carbon layer forming replicas.
- These are then be viewed under the TEM displaying more detailed internal structures of the cell in 3D.
- This step of treatment with Liquid nitrogen is known as freeze-itching.

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.

SCANNING ELECTRON MICROSCOPE (SEM):

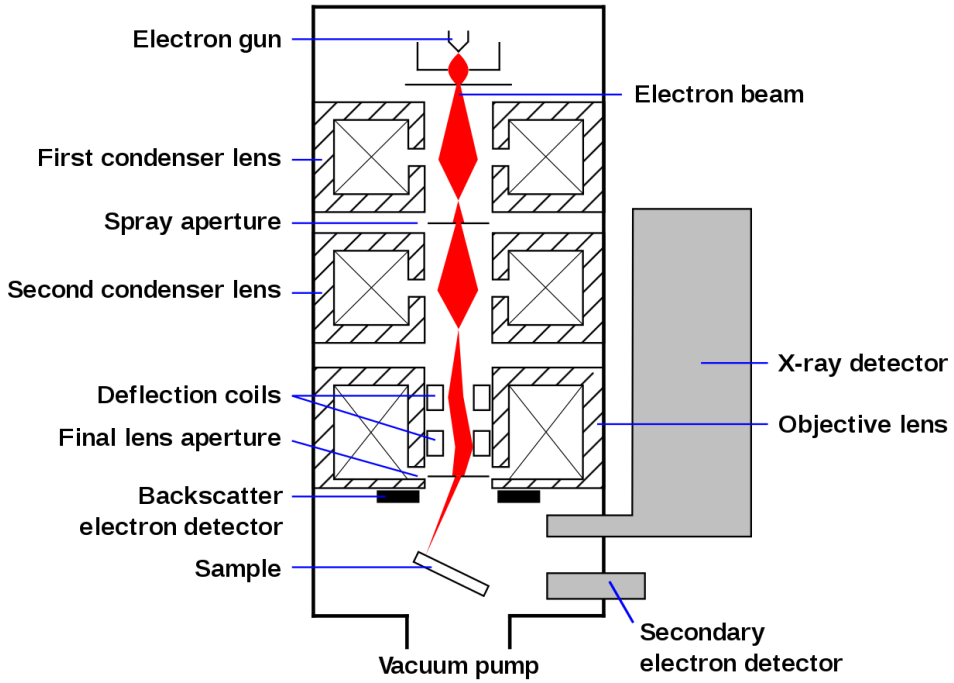
The first Scanning **Electron Microscope** was initially made by Mafred von Ardenne in 1937 with an aim to surpass the transmission electron Microscope. He used high-resolution power to scan a small raster using a beam of electrons that were focused on the raster. He also aimed at reducing the problems 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 development of electron microscopes was due to the inefficiency of the wavelength of the light microscopes. electron microscopes have vert short wavelengths in comparison to the light microscope which enables better resolution power.

The Principle of the Scanning Electron Microscope:

Unlike the Transmission Electron Microscope which uses transmitted electrons, the scanning electron Microscope used 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.

Working of Scanning Electron Microscope (SEM)



- 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 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 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 the condense into a beam that is used for the creation of ana 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 – Its 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 has 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.

REFERENCE:

Prescott, Harley, and Klein's Microbiology. Seventh Edition, 2008

R. P. Singh, Microbiology, 2016