Microscope

Microscope, instrument that produces enlarged images of small objects, allowing the observer an exceedingly close view of minute structures at a scale convenient for examination and analysis. The magnifying power of a microscope is an expression of the number of times the object being examined appears to be enlarged and is a dimensionless ratio. It is usually expressed in the form 10× (for an image magnified 10-fold). The resolution of a microscope is a measure of the smallest detail of the object that can be observed. Resolution is expressed in linear units, usually micrometres (μm).

The most familiar type of microscope is the optical, or light, microscope, in which glass lenses are used to form the image. Optical microscopes can be simple, consisting of a single lens, or compound, consisting of several optical components in line. The hand magnifying glass can magnify about 3 to 20×. Single-lensed simple microscopes can magnify up to 300×—and are capable of revealing bacteria—while compound microscopes can magnify up to 2,000×.

The most important is the electron microscope, which uses a beam of electrons in its image formation. The transmission electron microscope (TEM) has magnifying powers of more than 1,000,000×. TEMs form images of thin specimens, typically sections, in a near vacuum. A scanning electron microscope (SEM), which creates a reflected image of relief in a contoured specimen, usually has a lower resolution than a TEM but can show solid surfaces in a way that the conventional electron microscope cannot.

Parts of Microscope

1. Arm

It is in the back of the microscope and supports the objectives and ocular. Also, it is the part that we use to carry or lift it.

2. Base

It's the bottom of the scope. In addition, it houses the light source and the back section of base acts as a handle to carry the scope.

3. Course Focusing Knob

We use it to adjust the position of objective lenses. Also, this should be done keeping in mind that the objective should not hit the slide. In addition, it should be stopped when the object is completely visible through the ocular.

4. Fine Focusing Knob

We use it to bring the specimen in perfect focus once the specimen is visible through the course-focusing knob. Also, focus slowly to avoid contact between the objective and the specimen.

5. Illuminator

It is the light source of the microscope.

6. Numerical Aperture or Objective lens

It is found in a compound scope and is the lens that is closest to the specimen.

7. Ocular Lens

This is the lens closest to the viewer in a compound light microscope.

8. Oil immersion Lens

This is a 100x (100 times) objective lens. Also, this lens is small in order to attain high resolution and magnification. Furthermore, due to its size, it is important for the lens to get as much light as possible.

Moreover, by immersion of lens in oil it eliminates the refraction of light, it happens because the glass and oil have almost the same refractive index. Most noteworthy, in this way the light is maximized and gives the clearest image. Besides, It oil immersion lens is used without oil then the produced Image will become unclear and has a poor resolution.

The simple microscope Principles

The simple microscope consists of a single lens traditionally called a loupe.

Light microscopes

Most student microscopes are classified as **light microscopes**. In a light microscope, visible light passes through the specimen (the biological sample you are looking at) and is bent through the lens system, allowing the user to see a magnified image. A benefit of light microscopy is that it can often be performed on living cells, so it's possible to watch cells carrying out their normal behaviors (e.g., migrating or dividing) under the microscope.

Student lab microscopes tend to be **brightfield** microscopes, meaning that visible light is passed through the sample and used to form an image directly, without any modifications. Slightly more sophisticated forms of light microscopy use optical tricks to enhance contrast, making details of cells and tissues easier to see.

Another type of light microscopy is **fluorescence microscopy**, which is used to image samples that fluoresce (absorb one wavelength of light and emit another). Light of one wavelength is used to excite the fluorescent molecules, and the light of a different wavelength that they emit is collected and used to form a picture. In most cases, the part of a cell or tissue that we want to look at isn't naturally fluorescent, and instead must be labeled with a fluorescent dye or tag before it goes on the microscope.

Electron microscopes

An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination. It is a special type of microscope having a high resolution of images, able to magnify objects in nanometres, which are formed by controlled use of electrons in a vacuum captured on a phosphorescent screen. Ernst Ruska (1906-1988), a German engineer and academic professor, built the first Electron Microscope in 1931.

Electron microscopes differ from light microscopes in that they produce an image of a specimen by using a beam of electrons rather than a beam of light. Electrons have much a shorter wavelength than visible light, and this allows electron microscopes to produce higher-resolution images than standard light microscopes. Electron microscopes can be used to examine not just whole cells, but also the subcellular structures and compartments within them.

One limitation, however, is that electron microscopy samples must be placed under vacuum in electron microscopy (and typically are prepared via an extensive fixation process). This means that live cells cannot be imaged. The bacteria show up as tiny purple dots in the light microscope image, whereas in the electron micrograph, you can clearly see their shape and surface texture, as well as details of the human cells they're trying to invade.

Working Principle of Electron microscope

Electron microscopes use signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology, and composition.

1. The electron gun generates electrons.
2. Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam.
3. To move electrons down the column, an accelerating voltage (mostly between 100 kV-1000 kV) is applied between the tungsten filament and anode.
4. The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope. Ultra-thin sections of 20-100 nm are cut which is already placed on the specimen holder.
5. The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen.
6. The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter.
7. The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image.
8. The ocular lenses then produce the final further magnified image.

Parts of Electron Microscope

Electron Microscope is in the form of a tall vacuum column that is vertically mounted. It has the following components:

1. Electron gun

- The electron gun is a heated tungsten filament, which generates electrons.

2. Electromagnetic lenses

- **The condenser lens** focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam.
- The electron beam coming out of the specimen passes down the second of magnetic coils called the **objective lens**, which has high power and forms the intermediate magnified image.
- The third set of magnetic lenses called **projector (ocular) lenses** produce the final further magnified image.
- Each of these lenses acts as an image magnifier all the while maintaining an incredible level of detail and resolution.

3. Specimen Holder

- The specimen holder is an extremely thin film of carbon or collodion held by a metal grid.

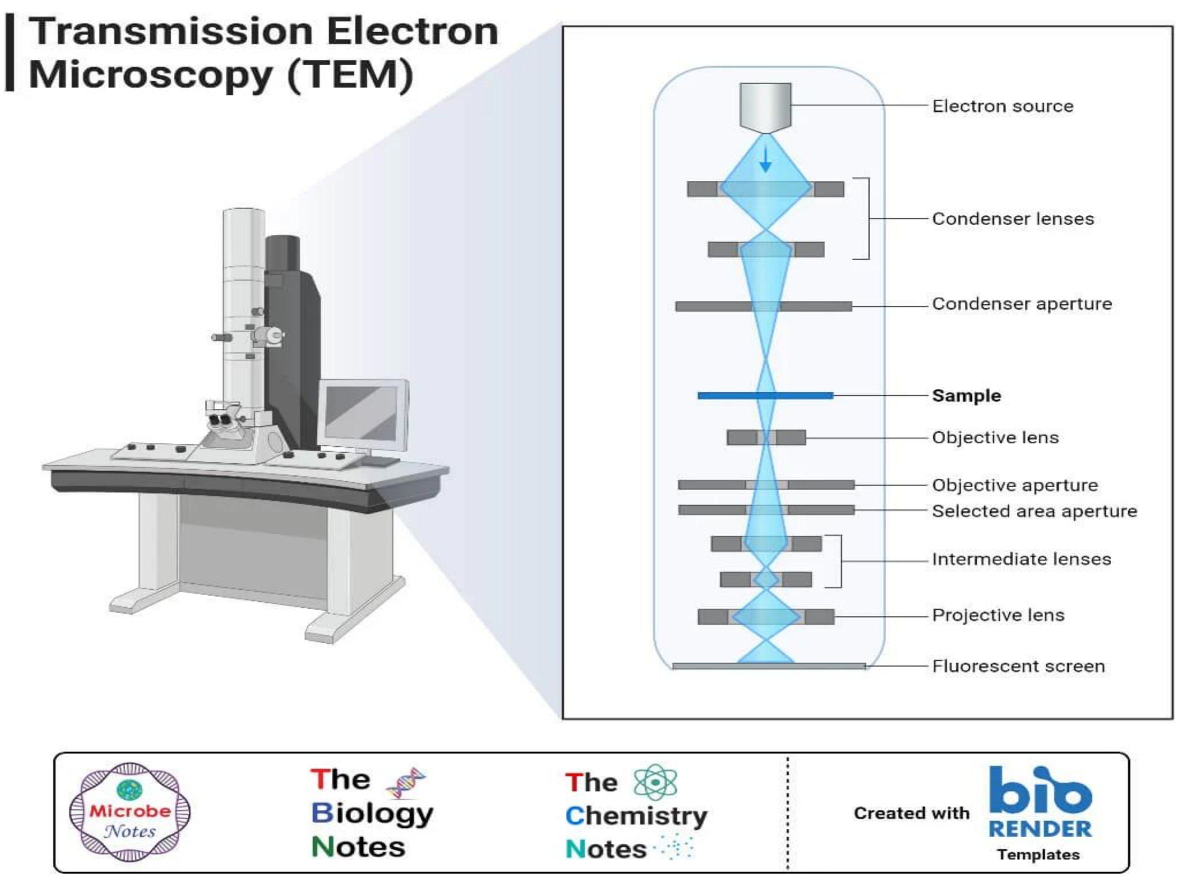
4. Image viewing and Recording System

- The final image is projected on a fluorescent screen.
- Below the fluorescent screen is a camera for recording the image.

There are two major types of electron microscopy.

1. **Transmission Electron Microscope (TEM)**

In **transmission electron microscopy** (**TEM**), in contrast, the sample is cut into extremely thin slices before imaging, and the electron beam passes through the slice rather than skimming over its surface. TEM is often used to obtain detailed images of the internal structures of cells.

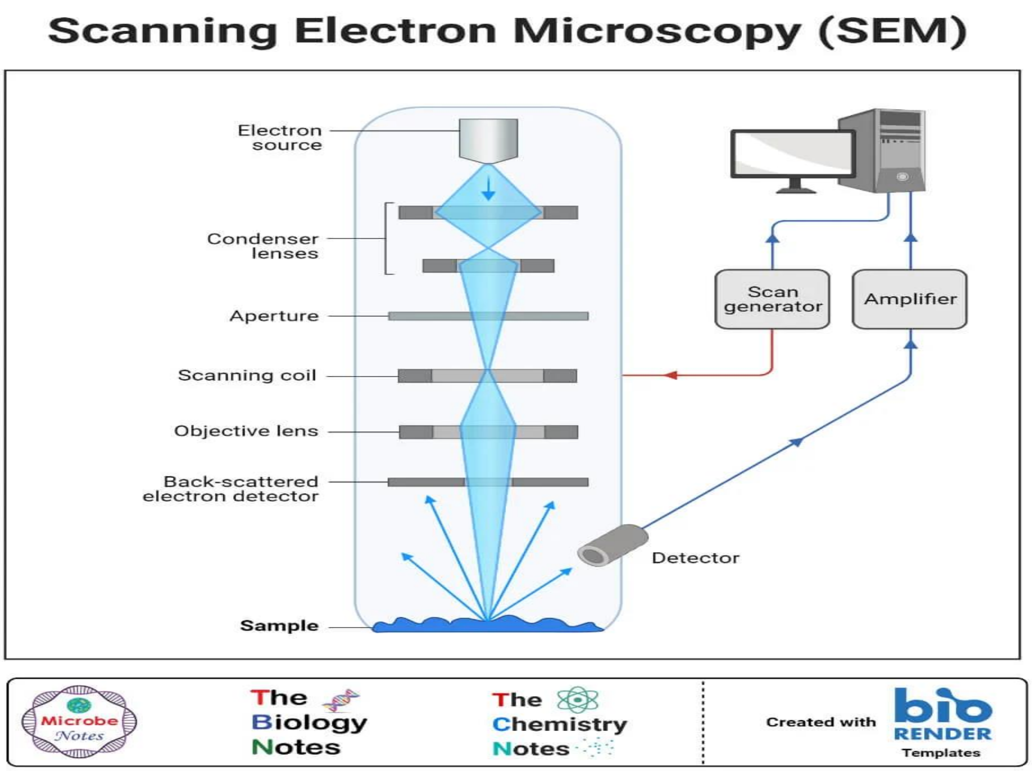


- The transmission electron microscope is used to view thin specimens through which electrons can pass generating a projection image.
- The TEM is analogous in many ways to the conventional (compound) light microscope.
- TEM is used, among other things, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture).

2. Scanning Electron Microscope (SEM)

- Conventional scanning electron microscopy depends on the emission of secondary electrons from the surface of a specimen.
- Because of its great depth of focus, a scanning electron microscope is the EM analog of a stereo light microscope.
- It provides detailed images of the surfaces of cells and whole organisms that are not possible by TEM. It can also be used for particle counting and size determination, and for process control.
- It is termed a scanning electron microscope because the image is formed by scanning a focused electron beam onto the surface of the specimen in a raster pattern.

- In **scanning electron microscopy** (**SEM**), a beam of electrons moves back and forth across the surface of a cell or tissue, creating a detailed image of the 3D surface.



Applications of Electron microscope

- Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals.
- Industrially, electron microscopes are often used for quality control and failure analysis.
- Modern electron microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the images.
- The science of microbiology owes its development to the electron microscope. The study of microorganisms like bacteria, virus, and other pathogens have made the treatment of diseases very effective.

Advantages of Electron microscope

- Very high magnification
- Incredibly high resolution
- Material rarely distorted by preparation
- It is possible to investigate a greater depth of field
- Diverse applications

Limitations of Electron microscope

- The live specimen cannot be observed.
- As the penetration power of the electron beam is very low, the object should be ultra-thin. For this, the specimen is dried and cut into ultra-thin sections before observation.
- As the EM works in a vacuum, the specimen should be completely dry.
- Expensive to build and maintain
- Requiring researcher training
- Image artifacts resulting from specimen preparation.
- This type of microscope is large, cumbersome extremely sensitive to vibration and external magnetic fields.