

## **Experiment: Study of microscope and its parts**

**Aim:** To study the parts of microscope

**Objective:**

- ❖ To study the principles and operation of a compound microscope
- ❖ To study the different types of microscopes

### **COPMPOUND MICROSCOPE**

The word microscope has been derived from two Greek words: Mikros meaning small and Skopein means to see. A microscope is an optical instrument used for magnifying and resolving the image of any object, which cannot be seen by a naked eye. The first operational microscope was developed by Z. Janseen and H. Janseen of Holland in 1590. It is designed to obtain both magnification and resolving power; it utilizes visible light of wave length ranging from 380 nm to 740 nm as the source of illumination.

### **PARTS OF MICROSCOPE**

1.  **Eyepiece Lens:** the lens at the top that you look through. They are usually 10X or 15X power.
2.  **Body Tube:** A hollow cylindrical tube that connects the eyepiece to the objective lenses and through which light passes from objective lens to the eye piece at the top.
3.  **Arm:** it is a curved back that supports the upper parts of the microscope. It is useful to carry the microscope.
4.  **Base or foot:** The bottom of the microscope that is U shaped and gives stability to the instrument.
5.  **Illuminator:** A steady light source (110 volts) used in place of a mirror.
6.  **Stage:** The flat platform where you place your specimen to be observed. Stage clips hold the slides in place.
7.  **Revolving Nose Piece or Turret:** This is the saucer shaped plate that holds two or more objective lenses and this can be rotated easily to change power.
8.  **Objective Lens:** Usually you will find 3 or 4 objective lenses on a microscope. They consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X, 400X and 1000X respectively. The shortest lens is the one having lowest power; the longest one is the lens with the greatest power. The high-power objective lenses are retractable (i.e. 40XR). This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby

protecting the lens and the slide.

9. **Iris Diaphragm:** Many microscopes have a rotating disk above the condenser and below the stage. Iris regulates the amount of light reaching the specimen.
10. **Condenser Lens:** The purpose of the condenser lens is to collect the light reflected by the mirror and focus the light onto the specimen. Microscopes with a condenser lens render sharper image than those with no lens.
11. **OBJECTIVE LENS SYSTEM:** The lens system near the object is called objective lens system. The objective is mounted on revolving nose piece and objective is the primary magnifying mechanism.
  - There are 3 types of objective lens are generally used  
LowPower-10X  
Higher power-40X  
Oil immersions Objective-100X
12. **Eye piece / ocular lens:** Placed on the top of body tube and magnifies the image formed by objective lens.
13. **FOCUSING KNOB:** Two types of adjustable knobs are useful to move the body tube upward and downwards in order to focus
  - A. **Coarse adjustment knob:** It is always used first for rough focusing. Coarse adjustment is done only with the low power objective.
  - B. **Fine adjustment knob:** Used after coarse adjustment for fine focusing. It is used with the high- power objective to bring the specimen into better focus.

For higher magnification (400X) an oil immersion objective lens (100X) can be used. The light gathering properties of lens are greatly increased by placing a fluid between the objective lens and coverslip. The fluid must have same refractive index as the lens itself so the fluid generally used is cedar Oil.

**MAGNIFICATION:** Magnification is an apparent increase in the size of an object achieved with the help of a lens.

**Total magnification** = magnification of eye piece x magnification of objective.

(a) **Effective Magnification (EM):** It is the minimum magnification to view a particular object.

**Effective Magnification**=Resolving power of a healthy human eye/resolving power of microscope

**(b) Useful Magnification:** It is 2-4 times of the effective magnification

**(c) Empty/ Useless Magnification:** It is over & above the useful magnification. It is than four times of effective magnification.

**RESOLVING POWER:** It is defined as the power of an instrument to distinguish two points or two lines that are close together. Greater the resolving power greater is the efficiency of microscope.

$$\text{RESOLVING POWER} = 0.61 \times \lambda / \text{Numerical Aperture}$$

Where, NA is the numerical aperture of objective. Smaller the numerical aperture, smaller will be the resolving power.

0.61 is the constant i.e., minimum detectable reference in contrast.

$\lambda$  is wavelength of illumination. Shorter the wavelength of light, higher will be the resolving power.

**Numerical Aperture:** It is the light gathering/collecting capacity of objective lens. It indicates resolving power of lens.  $\text{NA} = n \sin \theta$

Here 'n' is the index of refraction of the medium in which the lens is working (1.00 for air, 1.33 for pure water, and typically 1.6 for immersion oil); and

$\theta$  is the angle of the marginal rays formed with the axis..

### **TYPES OF MICROSCOPES:**

Depending up on the source of illumination & principle of magnification, microscopes are of following types.

1. **Compound Microscope:** A compound microscope works by illuminating the slide from underneath with a light bulb. The specimen is then magnified by a series of lenses, one near the slide (called the objective lens) and one near the top (called the eyepiece). This results in a two-dimensional image. Other advantages of compound microscopes are that they can go up to a high magnification and are affordable for amateurs, students, and scientists. A

disadvantage is that they have a lower resolution, so your image will never be as crisp and sharp as some more advanced types of microscopes (as described below).

2. **Dark-field microscope:** This microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. A light object is seen on a dark background. Live organisms such as spirochetes can be seen with this microscope.
3. **Phase-contrast microscope:** This microscope also contains special condensers that throw light “out of phase” and cause it to pass through the object at different speeds. It is commonly used to observe living cells without staining them. Internal cell parts such as mitochondria, lysosomes, and the Golgi body can be seen with this instrument.
4. **The fluorescent microscope** uses ultraviolet light as its light source. When ultraviolet light hits an object, it excites the electrons of the object, and they give off light in various shades of color. Since ultraviolet light is used, the resolution of the object increases. Fluorescent dyes are used to help identify unknown bacteria.
5. **Electron microscopy.** The energy source used in the **electron microscope** is a beam of electrons. Since the beam has an exceptionally short wavelength, it strikes most objects in its path and increases the resolution of the microscope significantly. Viruses and some large molecules can be seen with this instrument. The electrons travel in a vacuum to avoid contact with deflecting air molecules, and magnets focus the beam on the object to be viewed. An image is created on a monitor and viewed by the technologist. TEM and SEM are types of electron microscope.
6. **Scanning Electron Microscope (SEM):** The scanning electron microscope, or SEM, uses electrons rather than light for image formation. Samples are scanned in vacuum, so they must be specially prepared by first undergoing dehydration and then being coated with a thin layer of a conductive material, such as gold or palladium. After the item is prepared and placed in the chamber, the densely coated parts of the specimen deflect the electron beam, and both dark and light areas of image are seen on a computer screen. Although this microscope gives lower magnifications than the TEM, the SEM permits three-dimensional views of microorganisms and other objects.
7. **Transmission Electron Microscope (TEM):** Like the scanning electron microscope, the transmission electron microscope (TEM) uses electrons in creating a magnified image. To use this instrument, one places ultra thin slices of microorganisms or viruses on a wire grid and then stains them with gold or palladium to obtain a 2-D view of specimens. A TEM offers a high degree of both magnification and resolution than SEM.

**Problems:**

1. Draw a neatly labelled diagram of a compound microscope and its working mechanism.
  
2. Calculate the resolving power of microscopes with the following data:
  - a.  $\lambda = 1500$  angstroms and numerical aperture = 1.6
  
  - b.  $\lambda = 2000$  angstroms and numerical aperture = 1.6
  
  - c.  $\lambda = 4200$  angstroms and numerical aperture = 1.0



**Fig.: Schematic diagram of compound microscope**

