4.3 OPTICAL MICROSCOPE

The optical microscope, also referred to as a light microscope, is a type of microscope that commonly uses visible light and a system of lenses to generate magnified images of small objects.

1. PRINCIPLE

The functioning of the light microscope is based on its ability to focus a beam of light through a specimen, which is very small and transparent, to produce an image. The image is then passed through one or two lenses for magnification for viewing. The transparency of the specimen allows easy and quick penetration of light.

2. CONSTRUCTION

The object is placed on a stage and may be directly viewed through one or two eyepieces on the microscope.

3. TYPES OF MICROSCOPE

1. **Bright field microscope**: Transparent objects can be illuminated from below but the solid objects can be illuminated with light coming through and to produce a quality image. It is also known as a compound light microscope. Common types are,

(a) **Simple microscope**: A simple microscope is a microscope that uses only one lens for magnification, and is the original light microscope. It is used to obtain small magnifications. A single biconvex lens magnifies the size of the object to get an enlarged virtual image

(b) **Compound microscope**: The compound microscope uses a set of many lenses in order to maximize magnification. It magnifies the size of the object by a complex system of lens arrangement. It has a series of two lenses; the objective lens and the ocular lens, to magnify the size of the object.

2. **Dark field microscope**: The object is illuminated against a dark background.

3.**Polarized light microscope**: Polarized light may be used to determine crystal orientation of metallic objects.

4. **Phase-contrast microscope:** Phase-contrast imaging can be used to increase image contrast by highlighting small details of differing refractive index.

5. **Fluorescence Microscope**: It is used to view material stained with fluorescent dyes for specific purposes.

6. **Digital microscope**: A digital microscope is a microscope equipped with a digital camera allowing observation of a sample via a computer.

OTHER MICROSCOPE TYPES

There are many variants of the compound optical microscope design for specialized purposes. Some of these are physical design differences allowing specialization for certain purposes

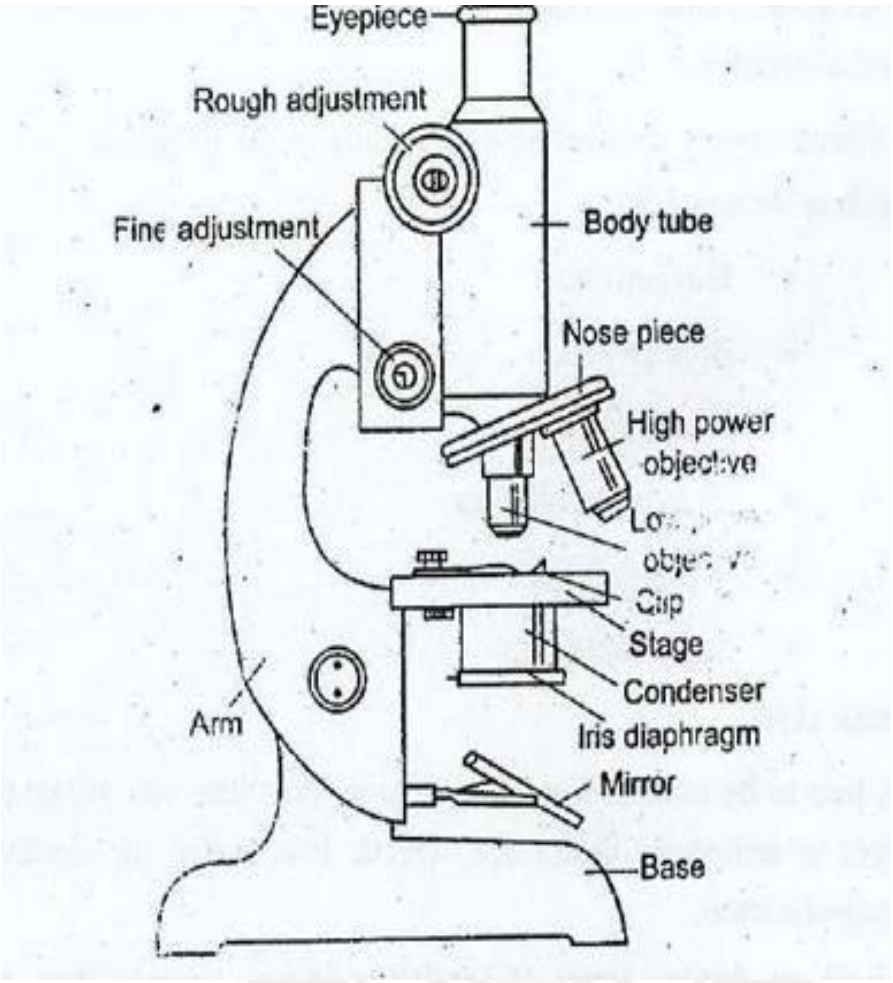
- **Stereo microscope**, a low-powered microscope which provides a stereoscopic view of the sample, commonly used for dissection.

- **Comparison microscope**, which has two separate light paths allowing direct comparison of two samples via one image in each eye.

- **Inverted microscope**, for studying samples om below; useful for cell cultures in liquid, or for metallography.

- **Epifluorescence microscope**, designed for analysis of samples which include fluoro phores.

- **Confocal microscope**, & widely used variant of epifluorescent illumination which uses a scanning laser to illuminate a sample for fluorescence.



Typical cross section of optical microscope

4. PREPARATION OF SPECIMEN

Basically preparation of specimen for optical microscopy is done by respective processes

- ❖ Cut required part of specimen
- ❖ Mount the specimen in mounting press.
- ❖ Grind the specimen as per to requirements.
- ❖ Polish the specimen.
- ❖ Etching.

5. COMPONENTS

- **Eyepiece (ocular lens):** It is a cylinder containing two or more lenses; itsfunction is to bring the image into focus for the eye.
- **Objective turret** (or) revolver or revolving nose piece (to hold multiple objective lenses)
- **Objective lenses**: There will be around three objective lenses screwed. These arrangements are designed to be parfocal, which means that when one changes from one lens to another on a microscope, the sample stays in focus.
- **Diaphragm and condenser**: The condenser is a lens designed to focus light from the illumination source onto the sample.
- **Focus knobs** (to move the stage)
  - Coarse adjustment knob
  - Fine adjustment knob
- **Stage** (to hold the specimen)
- **Light source** (a light or a mirror)

6. **WORKING**

- The stage moves up and down when you turn a thumb wheel on the side of the microscope. By raising and lowering the stage, you move the lenses closer to or further away from the object you're examining, adjusting the focus of the image to see.

- The slide is held in place by two metal clips, one on either side.

- Light traveling up from the mirror passes through the glass slide, specimen, and cover slip to the objective lens (the one closest to the object). This makes the first magnification; it works by spreading out light rays from the specimen so they appear to come from a bigger

object. The objective "lens" usually consists of more than one lens.

- A selection of other objective lenses can be used to magnify the specimen by more or less.

- The eyepiece lens (the one closest to your eye) magnifies the image from the objective lens, rather like a magnifying glass.

7. **MAGNIFICATION**

- The maximum magnification power of optical microscopes is typically limited to around 1000x because of the limited resolving power of visible light.
- The magnification of a compound optical microscope is the product of the magnification of the eyepiece (say 10X) and the lens (say 100x), to give a total magnification of 1,000X.

8. **ADVANTAGES**

- Measuring microscopes are used for precision meast

- It is relatively easy to use.

- It is small and lightweight.

- It offers high levels of observational quality.

- It is unaffected by electromagnetic fields.

- It does not require radiation to operate.
- It requires very little training.
- It allow you to observe living organisms.
- It have a minor maintenance cost compared to other med
- It can use fluorescent lights to display a sample visuall
- It is fully adjustable to the comfort level of the user.

9. **DISADVANTAGES**

- Resolution limit of optical microscopes Due to diffraction, even the best classic optical microscope is limited to a resolution of 12 micro meters.
- Low magnification
- Separate sample Preparation
- Poor surface view
- Light microscopes cannot operate in darkness.
- Light microscopes cannot provide three-dimensional renderings.

10. **APPLICATION**

- Optical microscopy is used extensively in microelectronics, nanophysics, biotechnology, pharmaceutical research, mineralogy and microbiology. Optical microscopy is used for medical diagnosis.

- In industrial use, binocular microscopes are common.

- In certain applications, long-working-distance or long-focus microscopes are beneficial.

- An item may need to be examined behind a window, or industrial subjects may be a hazard to the objective.