MICROSCOPY

Microbiology – I

BVOC- HCT - 1 semester

Dr. Angelina Stanley

Introduction

- Microorganisms are so small they cannot be seen distinctly with the unaided eye. Microscopes enable us to view them.

- Based on principles of **refraction of light** - lenses bend and focus light to form images.

- Our eyes cannot focus on objects nearer than about **25 cm or 10 inches.**

- This limitation may be overcome by using a **convex lens** as a simple magnifier (or microscope) and holding it close to an object.



The Microscope:

- The optical microscope often referred to as the **light microscope.**

- Type of microscope that uses visible light and a system of lenses to magnify images of small subjects.

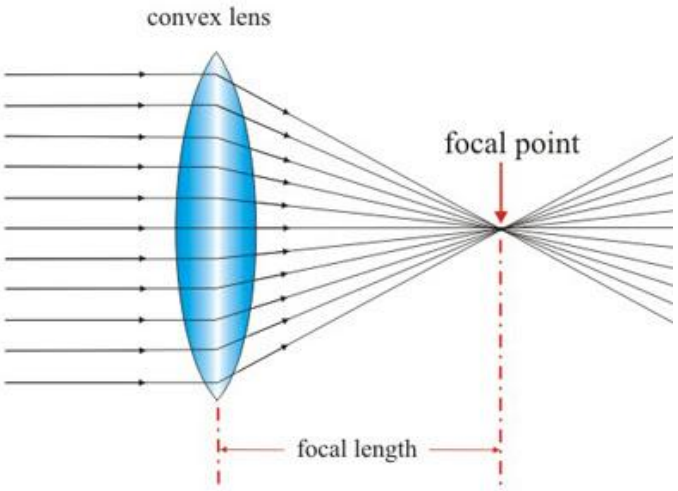
There are two basic types of optical microscopes:

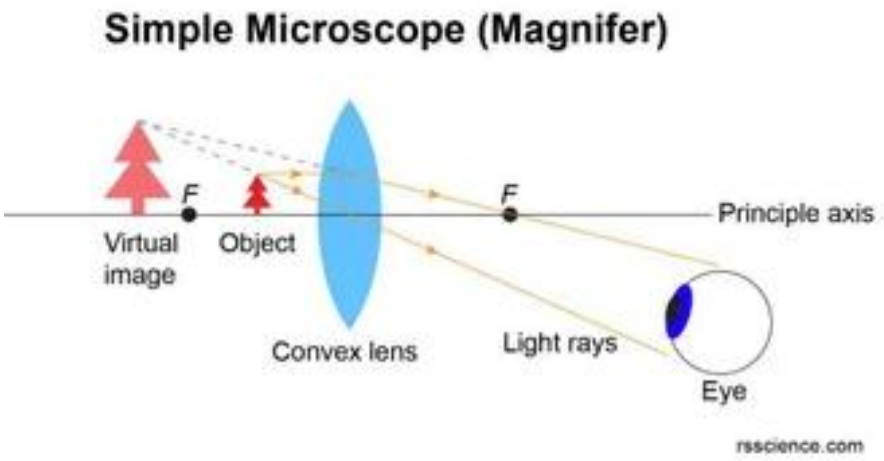
1. Simple microscopes
2. Compound microscopes.

Simple microscope

- **Definition:** A simple microscope is one that uses a **single lens** for magnification.
- It is a **convex lens** of small focal length, which is used for seeing the magnified images of small objects.
- Ex. magnifying glass

- It uses a lens to enlarge an object through angular magnification alone, giving the viewer an **erect enlarged virtual image**.
- Used in simple magnification devices : **magnifying glass**, loupes, and eyepieces for telescopes and **microscopes**.





Simple magnification devices

Magnifying glass

Loupes

Microscope

Telescope

Principle:

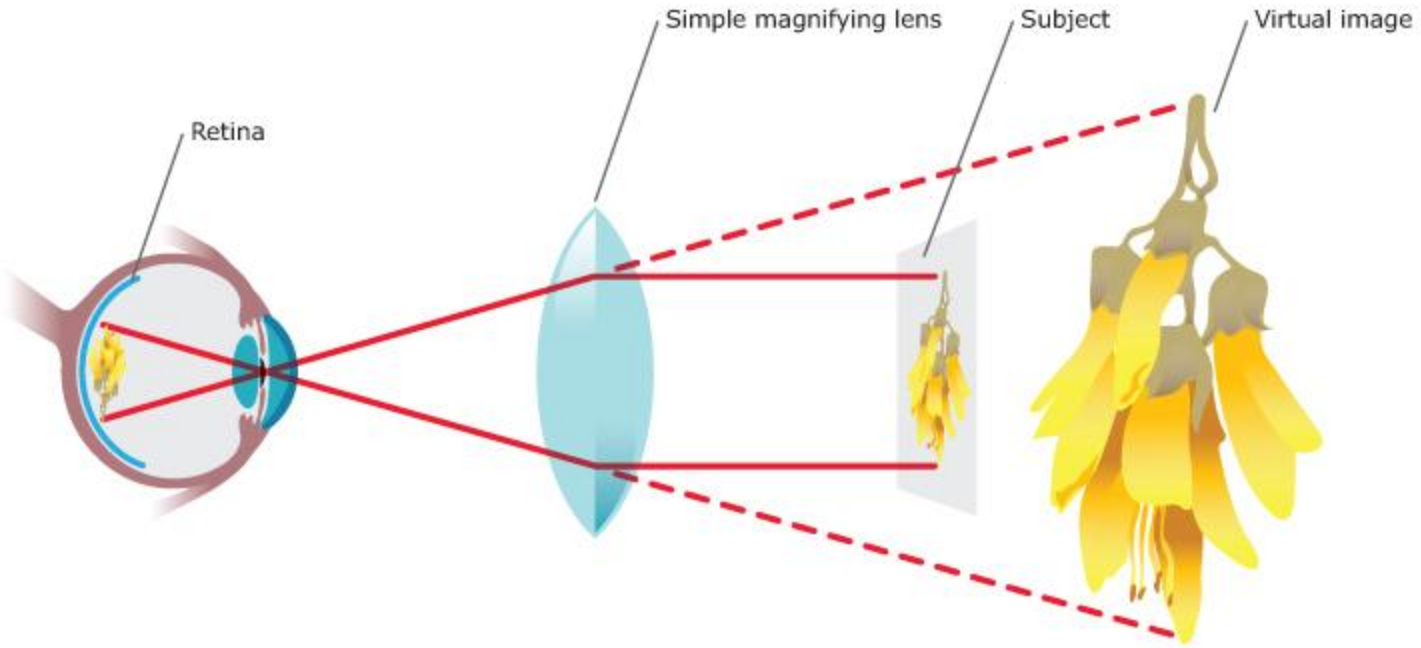
- When a tiny object is placed within its focus, a **virtual, erect and magnified image** of the object is formed at the **least distance** of distinct vision from the eye held close to the lens.

Magnification:

- The magnifying power of a simple microscope is given by:
- **M = 1 + D/F**
- Where D = the least distance of distinct vision
  F = focal length of the convex lens

- The focal length of the convex lens should be small
- **Smaller** the focal length - greater will be its **magnifying** power.
- The maximum magnification is about **10x.**
- The object will appear 10 times **larger.**

Magnification in Simple Microscope



The parts of a simple microscope:

1. **Mechanical parts**: Foot, stand, vertical limb, stage, adjustment screw, clip, folded arm.
2. **Optical parts:**

Lens & mirror.

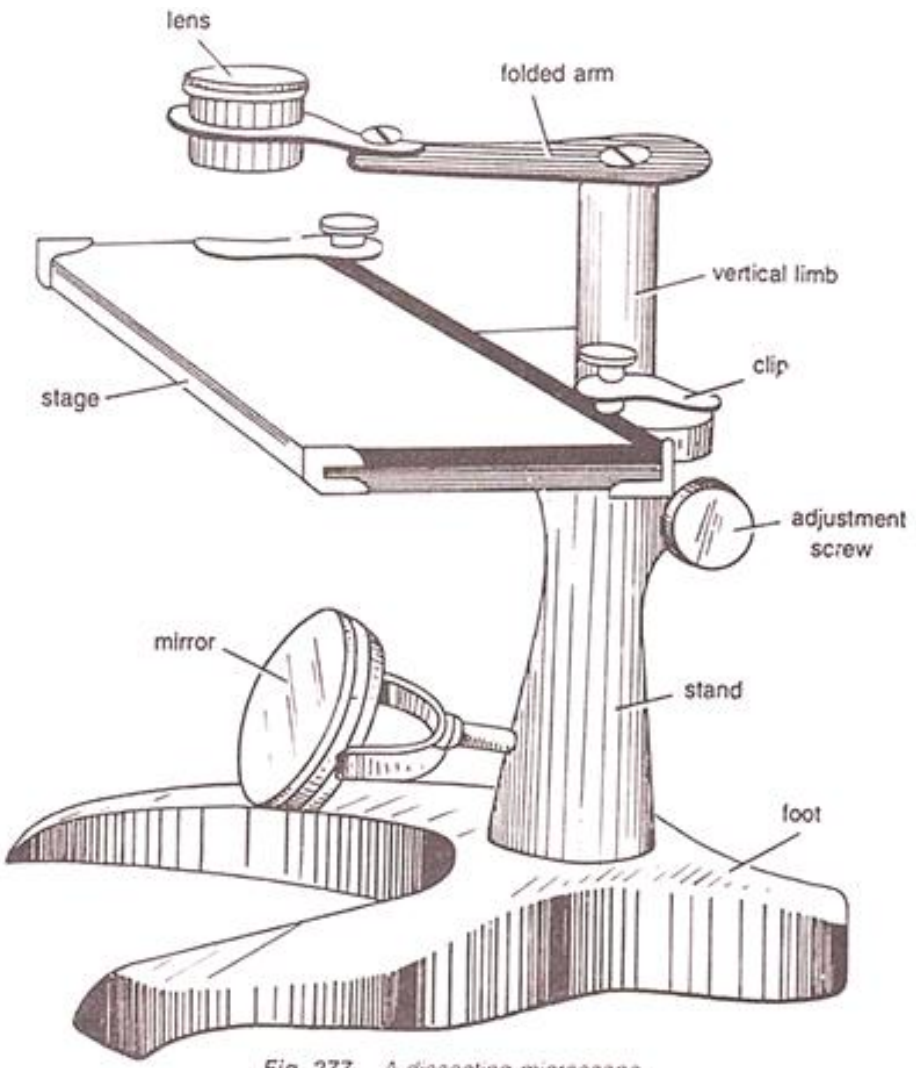


Fig. 277. A dissecting microscope.

Mechanical parts

- They include the following components:

Foot or Base:

- It is the basal, horse-shoe shaped or circular part of dissecting microscope.
- It is made of heavy material.
- It provides support to other parts of microscope.

Stand:

- It is short but strong, hollow cylindrical rod. Its one end is fixed at the foot or base.
- It provides support to the mirror, adjustment screw and other parts.

Vertical Limb:

- It is short and movable rod that fits into the hollow tube of the stand.
- With the help of the adjustment screw, this limb can be moved up and down.

Folder Arm:

- It is a horizontal arm. Its one end is attached with the vertical limb and on it’s another end is attached lens.
- Folded arm is movable. It can be moved up and down as well as left and right.

Stage:

- It is rectangular glass plate attached to the upper end of the stand or limb.
- Slide or the object, to be observed, is kept on the stage.

Clips:

- Two clips are fitted on the stage.
- They are used to hold the slide in the desired position.

Adjustment Screw:

- This is a screw used to adjust or move the vertical limb up and down.

Optical parts:

- These parts are involved in passing the light through the object (specimen) and magnifying its size.

Lens:

- It is a simple convex lens of either 2X, 3X, 5X, 10X or 20X magnification.

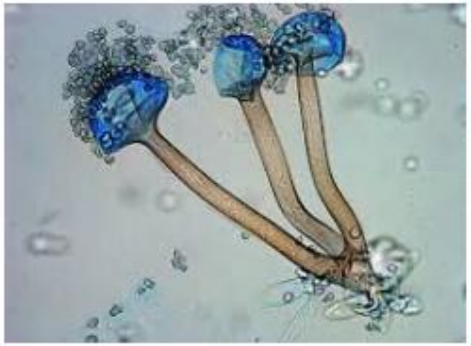
Mirror:

- It is concave reflecting mirror attached to the lower inner side of the stand.
- Light rays are reflected or focused on the stage by the mirror.

Applications:

- It is usually used for the study of microscopic algae, fungi, and biological specimens.
- It is used to see the magnified view of different particles of different types of soils.
- It is used by skin specialists to find out various diseases of the skin.

Algae

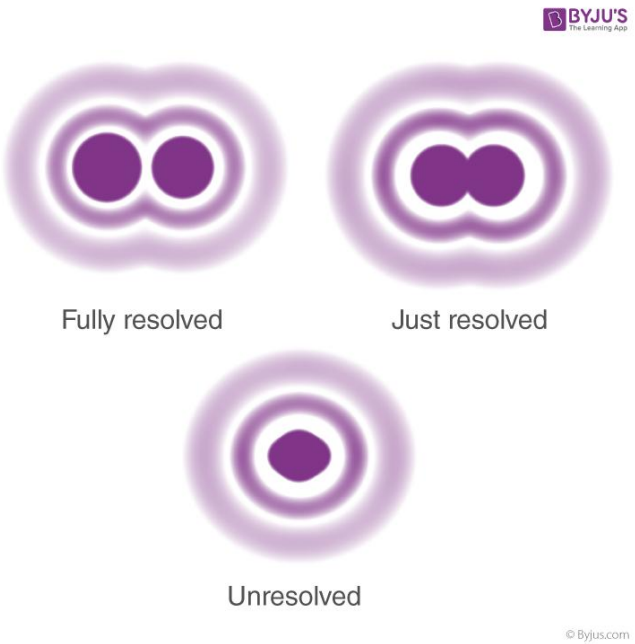


Fungi

insect

Compound microscope

- **Definition:** compound microscopes refers to the microscope having more than one lens.
- It consists of **two optical parts**, namely the **objective lens** and the **ocular lens**.



Principle:

Compound microscopes have a **combination of lenses** that enhances both **magnifying** powers as well as the **resolving** power.

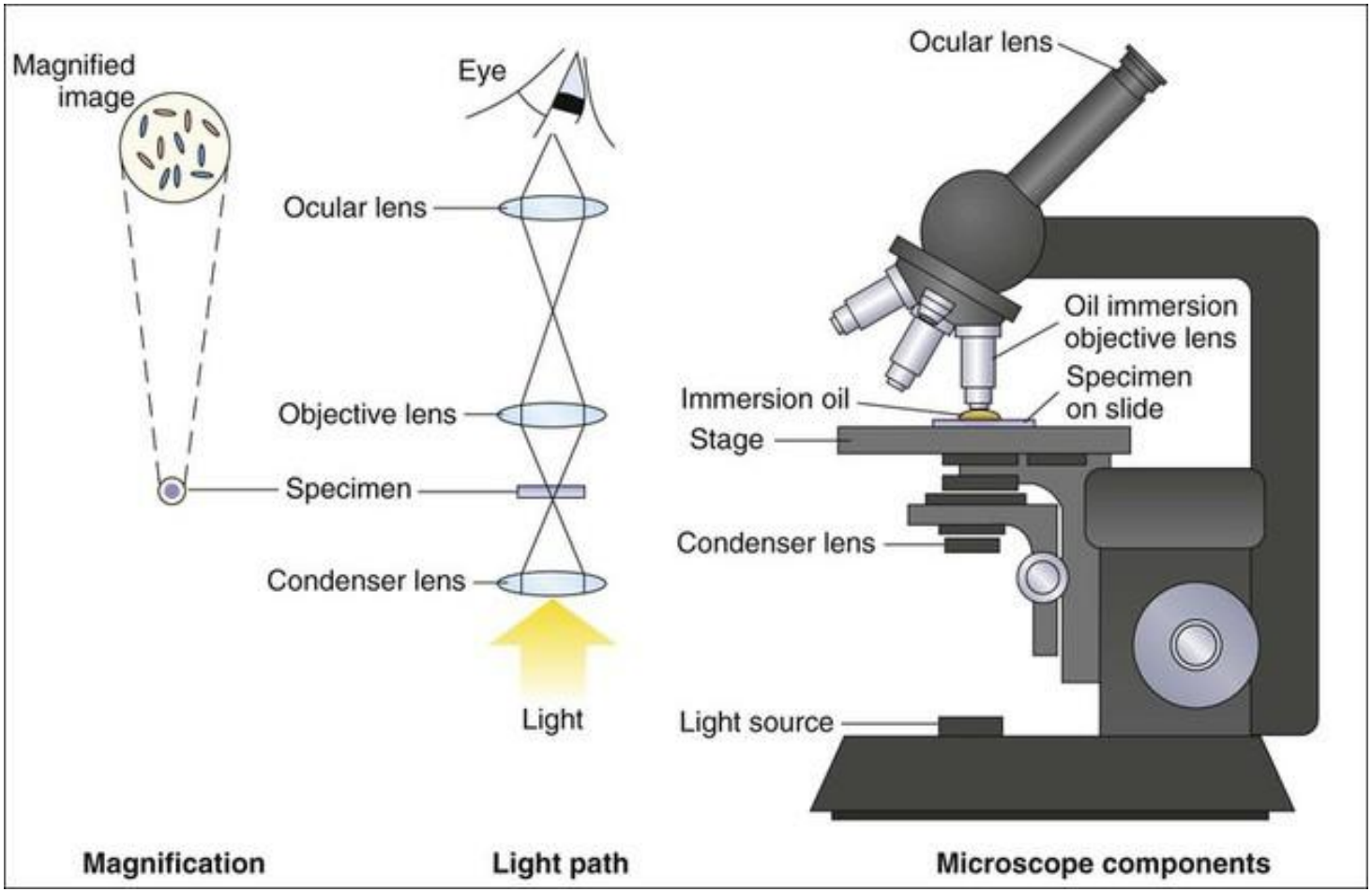
- The specimen or object, to be examined is usually mounted on a transparent **glass slide** and positioned on the specimen stage between the **condenser lens** and **objective lens.**

- A beam of visible light from the base is focused by a condenser lens onto the specimen.

- The **objective lens** picks up the light transmitted by the specimen and creates a **magnified** image of the specimen called the primary image inside the body tube.

- This image is again magnified by the **ocular** lens or eyepiece.

Image magnification in compound microscope



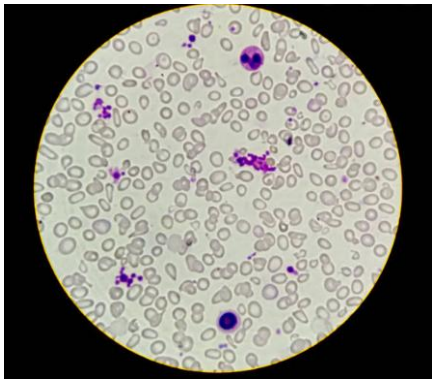
- When higher magnification is required, the nose piece is rotated after low power **(10x)** focusing to bring the objective of a higher power **(45X)** in line with the illuminated part of the slide.

- For very high magnification an **oil immersion** objective lens (**100X**) is used. e.g. for observing bacterial cell.

- The common light microscope is also called a **bright-field** microscope because the image is produced amidst a brightly illuminated field.

- The **image appears darker** because the specimen or object is denser and somewhat opaque than the surroundings.

- Part of the light passing through or object is absorbed.

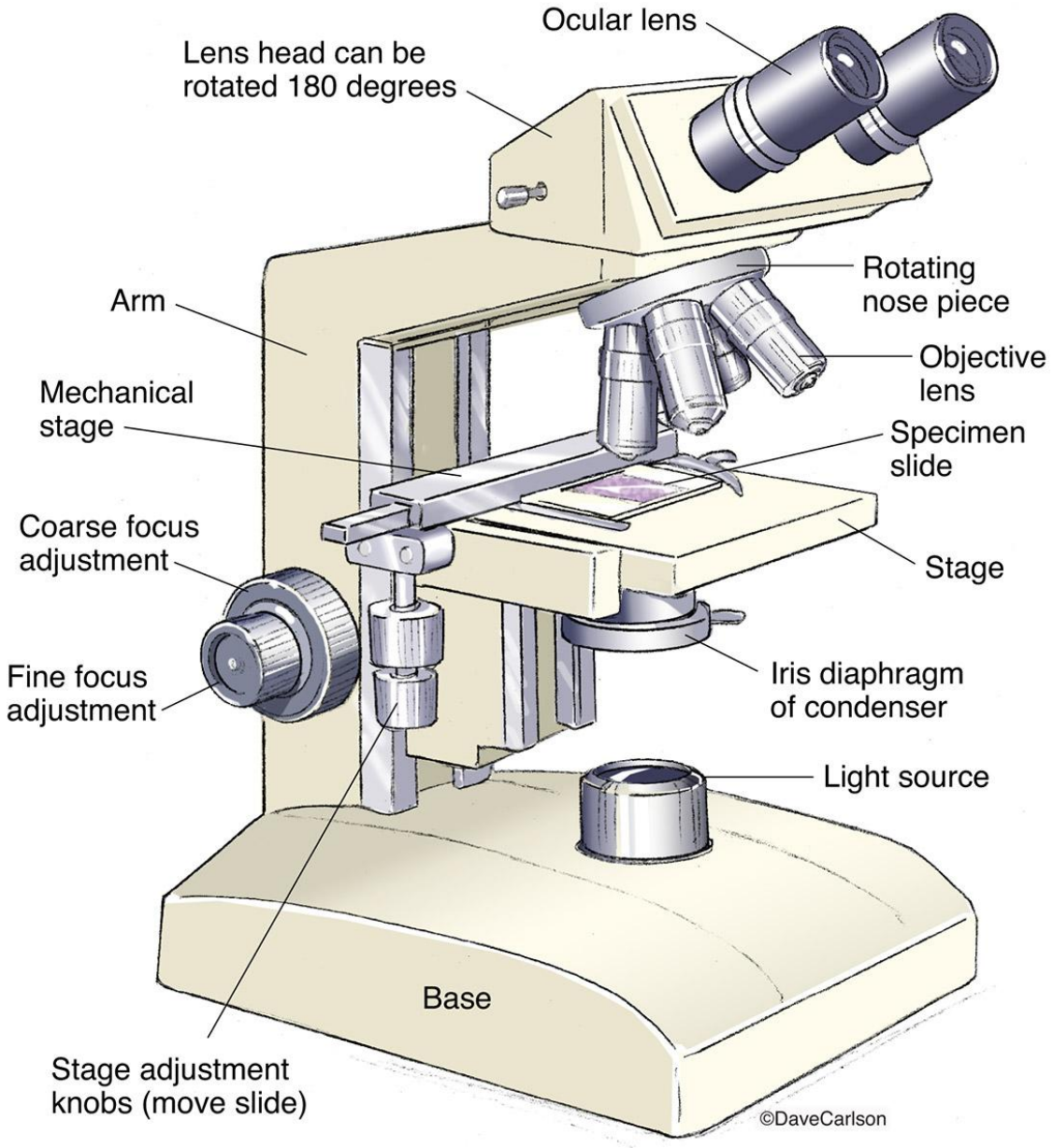


Magnification:

- To calculate **total magnification** of an image take the power of the objective lens which is at 4x, 10x or 40x and multiply it by the power of the eyepiece which is typically **10x**.
- Therefore, a **10x** eyepiece used with a **40X** objective lens will produce a magnification of **400X**.

- Alternatively, the magnification of the compound microscope is given by:
- $\mathbf{m = D/ f_o * L/f_e}$
- where, D = Least distance of distinct vision (25 cm)
  L = Length of the microscope tube
  fo = Focal length of the objective lens
  fe = Focal length of the eye-piece lens

Parts of Compound Microscope



Eyepiece And Body Tube.

- The eyepiece is the lens through which the viewer looks to see the specimen.
- It usually contains a 10X or 15X power lens.
- The body tube connects the eyepiece to the objective lenses.

Objectives and Stage Clips

- three to four objective lenses which range from 4X, 10X, 40X to 100X.
- Stage Clips are metal clips that hold the slide in a place.

Arm and Base

- The Arm connects the Body Tube to the base of the Microscope.
- The Base supports the Microscope and its Illuminator.

Illuminator and Stage

- The illuminator is the light source for a microscope.
- Has a low voltage bulb as an illuminator.
- The stage is the flat platform where the slide is placed.

Nosepiece and Aperture

- Nosepiece is a rotating turret that holds the objective lenses.
- The aperture is the middle of the stage that allows light from the illuminator to reach the specimen.

Condenser, **Iris diaphragm, and Diaphragm**

- A **condense**r gathers and focuses light from the illuminator onto the specimen being viewed.

- **Iris diaphr**agm adjusts the amount of light that reaches the specimen.

- The **diaphragm** is a five holed disk placed under the stage.

- Each hole is of a different diameter. By turning it, you can vary the amount of light passing through the stage opening.

Applications

- It helps to see and understand the microbial world of **bacteria** and **viruse**s, which is otherwise invisible to the naked eye.

- A compound microscope is of great use in **pathology labs** so as to identify diseases.

- To examine human cells under the microscope in forensic laboratories.
- Plant cells are examined and the microorganisms thriving on it.
- It is crucial to biologists.

- The presence or absence of minerals and the presence of metals can be identified.

- Students in schools and colleges are benefited by the use of a microscope for conducting their academic experiments.

Advantages

- Simplicity and its convenience.
- A compound light microscope is relatively small, therefore it’s easy to use and simple to store, and it comes with its own light source.
- Because of their multiple lenses, compound light microscopes are able to reveal a great amount of detail in samples.