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((Theoretical Histology))

Stage (-3-)

LEC- ((1))

Microscope

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Microscope: study the microscope parts of types and lines, how can be used.

A microscope is an optical instrument consisting of one or more lenses that magnifies an image and allows visualization of greater detail than is possible with the unaided eye.

Historians credit the invention of the compound microscope to the Dutch spectacle maker, Zacharias Janssen, around the year 1590.

Human can see about 0.5 mm sized object whereas the light microscopes can be used to visualize up to 1 μ m and EM (electron microscopes) can be used to view 0.5 nm objects.

Types of microscopes

1/ Optical microscopes :that utilizes light for image formation and are classified as follows:

- A. Simple microscope: It consists of a single convex lens or a single set of lenses for magnifying objects.
- B. Compound light microscope: It consists of two sets of lenses, one near the sample (objective lens) and the second lens near the eye (eyepiece). It is the most commonly used microscope.

2/ Electron Microscope: A system of electromagnetic lenses and a short beam of electrons are used to obtain magnification. It is of two types: (I) Transmission electron microscope (TEM) (ii) Scanning electron microscope (SEM).

Compound light microscopes are of the following types:

1. **Bright-field microscope:** In this microscope, the specimen is placed between the light source and the optical system.
2. **Dark-field microscope:** It is useful for observing unstained samples.
3. **Phase-contrast microscope:** It is useful for visualization of live, unstained organism and living cells.
4. **Fluorescence microscope:** It uses UV light. Tissue sections are usually irradiated with ultra violet (UV) light and the emission is in the visible portion of the spectrum.



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The fluorescent substances appear bright on a dark background.(DNA and RNA)

This microscopy is useful for immuno istochemistry (identification of specific antigen/ protein), and in situ hybridization.

Parts of microscope :

The compound microscope is a bright field microscope consists of:

STRUCTURAL COMPONENTS

- **Head/Body** :houses the optical parts in the upper part of the microscope
- **Base**: of the microscope supports the microscope and houses the illuminator.
- **Arm** :connects to the base and supports the microscope head. It is also used to carry the microscope.
- **Stage** : The platform that is used for placing the slides under observation .
- **Stage clip**: hold the slides in proper place .
- **Adjustment Knobs** Each microscope is filled with coarse and fine adjustment knobs. These knobs are useful for moving the sample upward and downward to bring it into focus..
- **Eyepiece Tube** holds the eyepieces in place above the objective lens.
- **Nosepiece (Turret)**: A rotating turret that houses the objective lenses. The viewer spins the nosepiece to select different objective lenses.

OPTICAL COMPONENTS

- **Eyepiece or Ocular** :is what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 5x, 10x, , 20x.



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- **Objective Lenses** :are the primary optical lenses on a microscope. Standard objectives include 4x, 10x, 40x and 100x , although different power objectives are available.
- The 100x lens is immersed in a drop of oil placed on the slide in order to eliminate any air gaps and loss of light due to refraction (bending of the light) as the light passes from glass (slide) → air → glass (objective lens). Immersion oil has the same refractive index of glass.
- **Illuminator:** is the light source for a microscope, typically located in the base of the microscope. Most light microscopes use low voltage, halogen bulbs .
- **Aperture** :is the hole in the stage through which the transmitted light reaches the stage.
- **Condenser:** is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.
- **Iris Diaphragm** : controls the amount of light reaching the specimen. It is located above the condenser and below the stage.
- **Condenser Focus Knob** :moves the condenser up or down to control the lighting focus on the specimen.
- **Power switch:** This switch on the base of the microscope turns the illuminator off and on.

Magnification :A lens magnifies the image. For a microscope, magnification is the multiplication of eyepiece magnification and objective magnification. For example, while using a microscope of 40X objective and 10X eyepiece, a sample is magnified 400 times (40×10).

Resolution: is the ability of microscope to distinguish between closest two points as a separate entity.



Microscope Parts

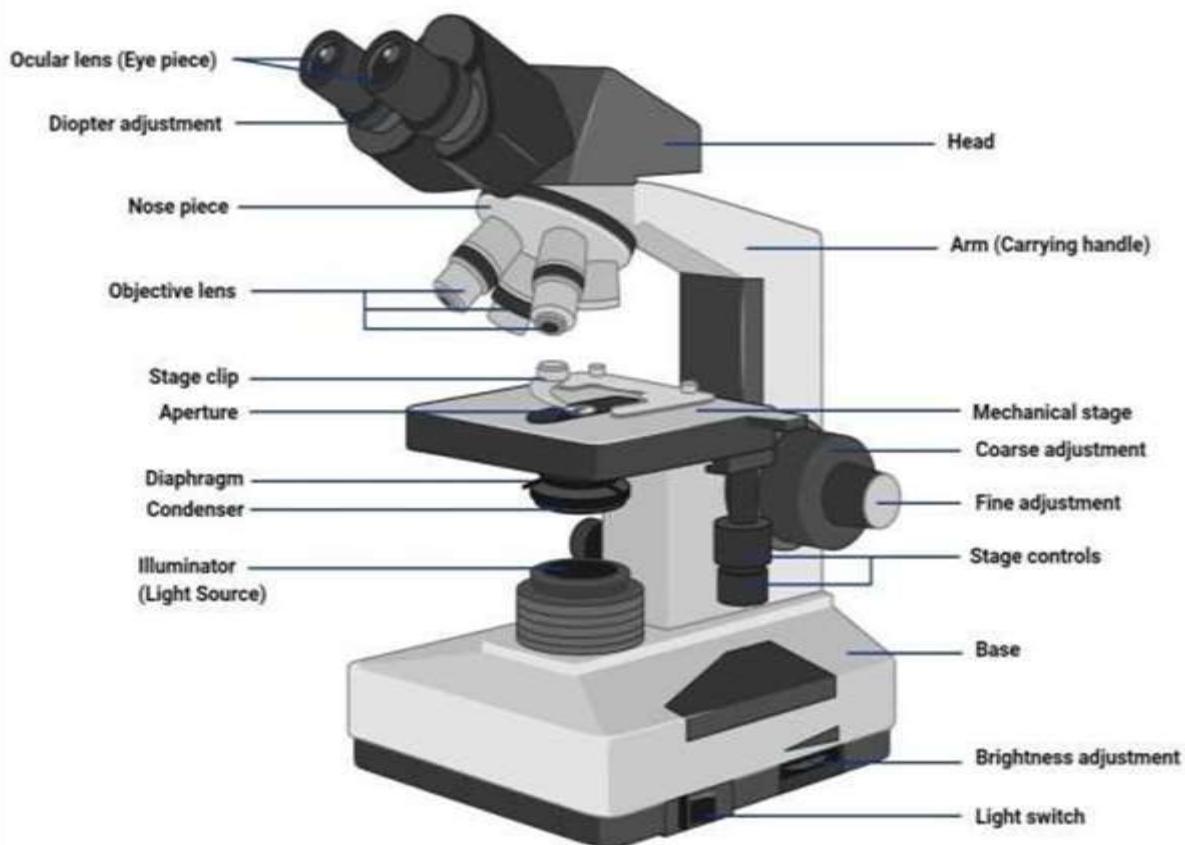


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Magnifying object/ Focusing image:

1. When viewing a slide through the microscope make sure that the stage is all the way down and the 4X scanning objective is locked into place.
2. Place the slide that you want to view over the aperture and gently move the stage clips over top of the slide to hold it into place.
3. Beginning with the 4X objective, looking through the eyepiece making sure to keep both eyes open, slowly move the stage upward using the coarse adjustment knob until the image becomes clear.



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4. To magnify the image to the next level rotate the nosepiece to the 10X objective. While looking through the eyepiece focus the image into view using only the fine adjustment knob
5. To magnify the image to the next level rotate the nosepiece to the 40X objective. While looking through the eyepiece focus the image into view using only the fine adjustment knob.
6. High power – oil immersion ($\times 100$). Place a small drop of immersion oil on the part to be examined. Lower the objective until it is in contact with the oil. Look through the eyepiece and turn the fine adjustment very slowly until the image is in focus.

Routine maintenance of the microscope

The microscope is a delicate instrument that must be handled gently.

- It must be installed in a clean environment away from chemicals, direct sunlight, heating sources or moisture.
- Because storage in a closed compartment encourages fungal growth, keep microscope standing on the bench protected by a light plastic cover.
- After use of the microscope, wipe the oil-immersion objective with lens tissue, absorbent paper, soft cloth or medical cotton wool. If other lenses are smeared with oil, wipe them with a small amount of toluene or a solution of 40% petroleum ether, 40% ethanol and 20% ether.
- Clean non optical parts with mild detergent and remove grease or oil
- Remove dust from the inside and outside of the eyepieces with a blower or soft camel-hair brush. Clean the condenser in the same way as the lenses with a soft cloth or tissue moistened with toluene

Never force the controls. If movement of the focusing screws or mechanical stage becomes difficult, lubricate them with a small drop of machine oil.