Microbiology Laboratory

Lab 2: The compound light microscope

History of microscope:

- In the 1st Century AD, the Romans invented the glass and used them to magnify objects.
- In the early 14th Century AD, eyeglasses were made by Italian spectacle makers.
- In 1590, two Dutch spectacle makers, Hans, and Zacharias Jansen created the first microscope. It was a simple tube with 2 lenses system and had 9X magnification.
- In 1903, Richard Zsigmondy invented the ultramicroscope. This could view objects smaller than the wavelength of light.
- In 1981, Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope. This allowed us to get the 3-D image of an object.

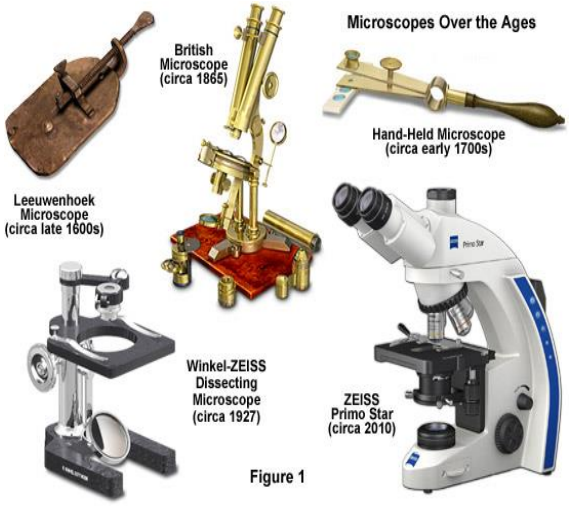


Figure 1

What is the Microscopy?

Microscopy can simply be understood as the 'use of microscope'. Microscopy can be defined as the scientific discipline of using microscopes for getting a magnified view of objects that can't be viewed by naked eyes.

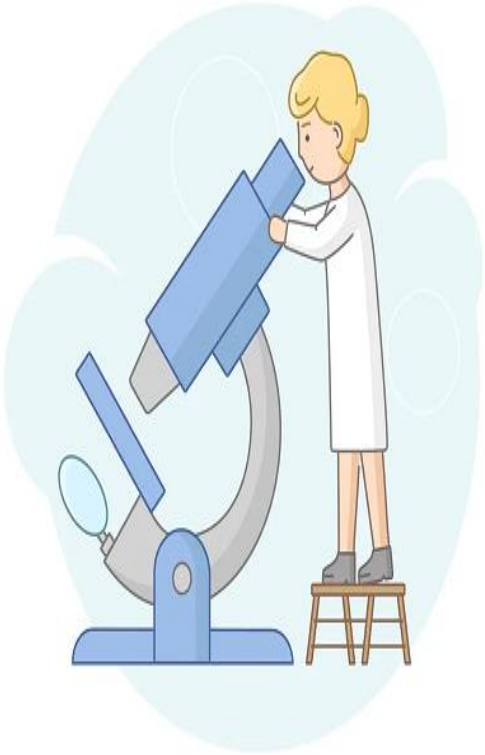
It is a very important tool in biology and nanotechnology. In microbiology, it is one of the most important tools used in observing microbial cells. Medical sectors, pathology, histology, molecular biology, and cytology are in great debt of microscopy.

Light Microscopy: is the microscopy technique that uses transmitted visible light, either natural or artificial, for developing the image of an object. It is the most common type of microscopy. Microscopes are instruments that are used in science laboratories to visualize very minute objects such as cells, and microorganisms, giving a contrasting image that is magnified. Microscopes are made up of lenses for magnification, each with its own magnification powers. Depending on the type of lens, it will magnify the specimen according to its focal strength.

Microscopes are generally made up of structural parts for holding and supporting the microscope and its components and the optical parts which are used for magnification and viewing of the specimen images. This description defines the parts of a microscope and the functions they perform to enable the visualization of specimens.

Types of Microscopes: there are 16 types of microscopes

1. Light simple Microscope
2. Compound Microscope
3. Phase Contrast Microscope
4. Fluorescence Microscope
5. Electron Microscope (SEM & TEM)
6. Dark Field Microscope
7. Dissecting Microscope (Stereo Microscope)
8. Digital Microscope
9. Scanning Probe Microscope (SPM)
10. Inverted Microscope
11. Acoustic Microscope
12. X-Ray Microscope
13. Polarizing Microscope
14. Metallurgical Microscope
15. Pocket Microscope
16. USB Microscope



What is the light microscope? (The light microscope is also known as an optical microscope)

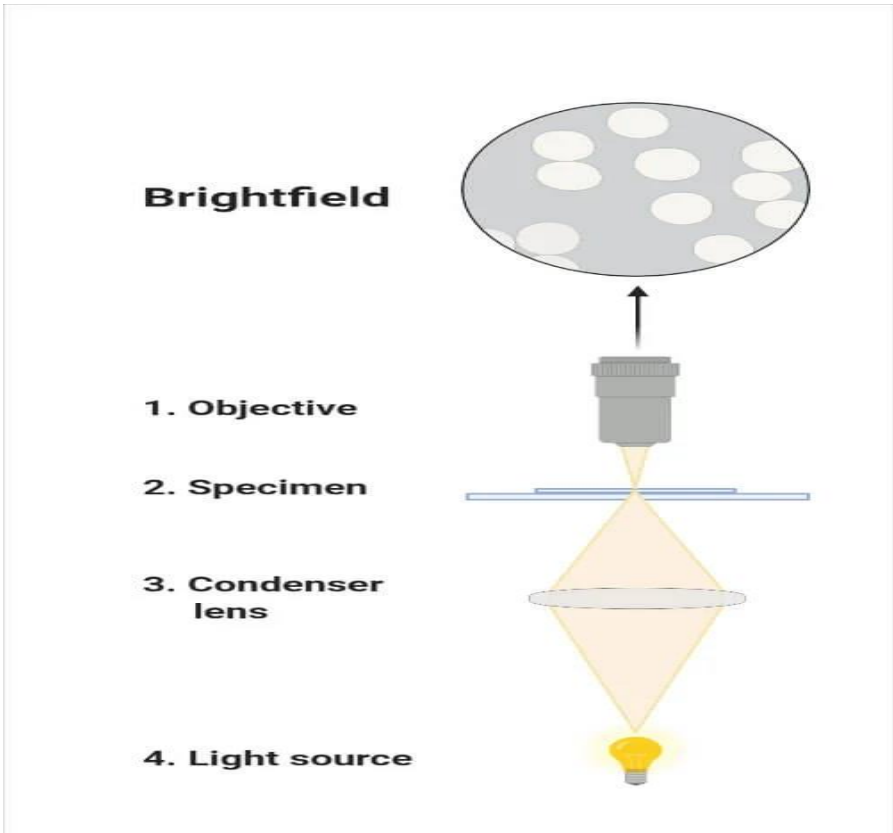
- A light microscope is a biology laboratory instrument or tool, that uses visible light to detect and magnify very small objects and enlarge them.
- They use lenses to focus light on the specimen, magnifying it thus producing an image. The specimen is normally placed close to the microscopic lens.
- Microscopic magnification varies greatly depending on the types and number of lenses that make up the microscope. Depending on the number of lenses, there are two types of microscopes i. e Simple light microscope (it has low magnification because it uses a single lens) and the Compound light microscope (it has a higher magnification compared to the simple microscope because it uses at least two sets of lenses, an objective lens, and an eyepiece). The lenses are aligned in that, they can be able to bend light for efficient magnification of the image.
- 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. Specimens can vary from bacterial to cells and other microbial particles.

Principle of a light microscope (Optical microscope):

A light microscope visualizes an image by using a glass lens, and magnification is determined by, the lens's ability to bend light and focus it on the specimen, which forms an image. When a ray of light passes through one medium into another, the ray bends at the interface causing **refraction.** The bending of light is determined by the **refractive index**, which is a measure of how great a substance slows the speed of light. The direction and magnitude of the bending of the light are determined by the refractive indexes of the two mediums that form the interface.

Microscopy works strictly with a factor of resolution whereby resolution is the ability of a lens to be able to differentiate small objects that are closely packed together. The resolution of a light microscope is determined by a **numerical aperture** of its lens system and by the wavelength of the light it employs; a numerical aperture is a definition of the light wavelengths produced when the specimen is illuminated.



Principle of a light microscope

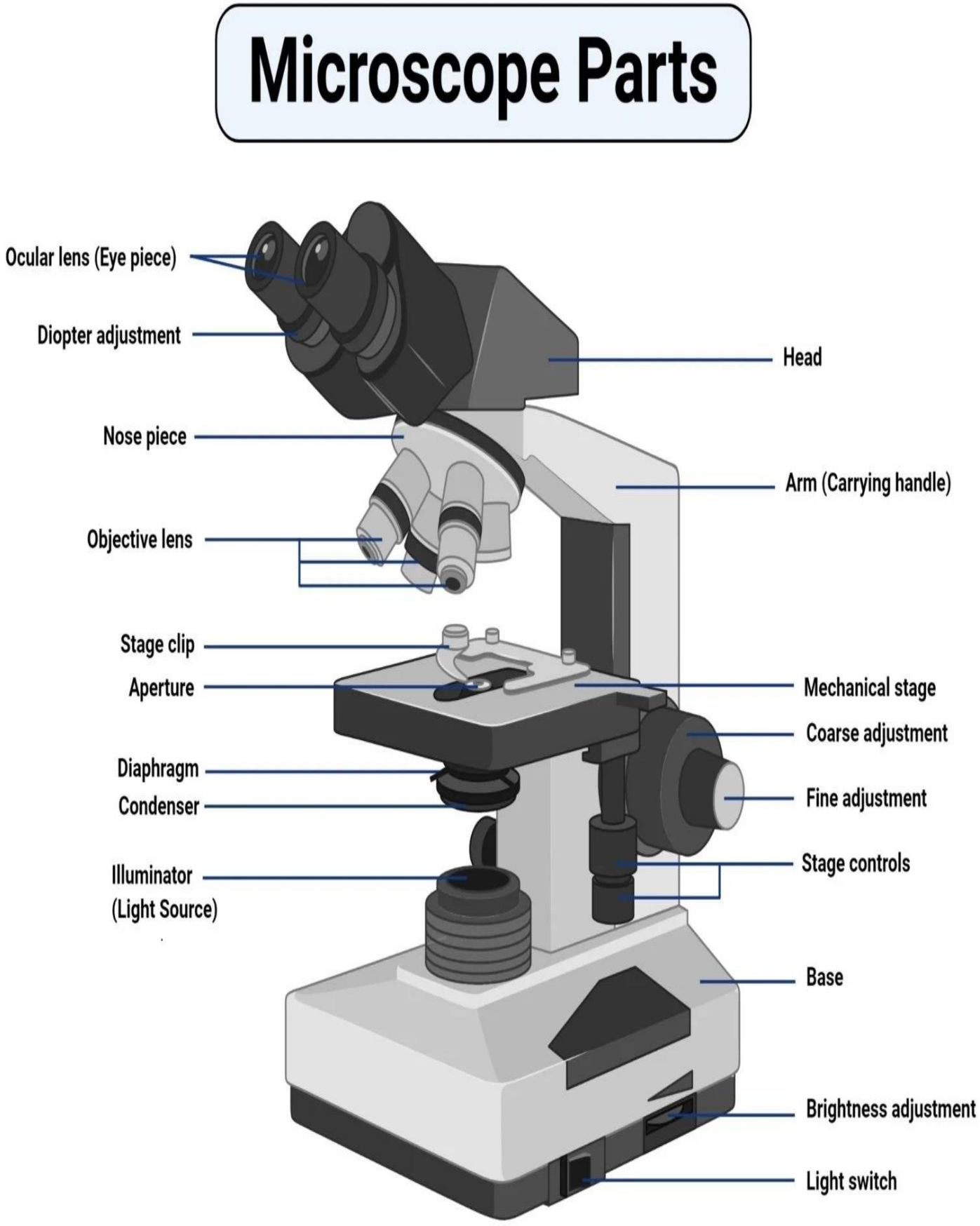


Diagram of parts of a microscope

Structural parts of a microscope and their function:

There are three structural parts of the microscope i.e. head, base, and arm.

1. **Head** – This is also known as the body. It carries the optical parts in the upper part of the microscope.
2. **Base** – It acts as microscopes support. It also carries microscopic illuminators.
3. **Arms** – This is the part connecting the base and to the head and the eyepiece tube to the base of the microscope. It gives support to the head of the microscope and it is also used when carrying the microscope. Some high-quality microscopes have an articulated arm with more than one joint allowing more movement of the microscopic head for better viewing.

Optical parts of a microscope and their function:

The optical parts of the microscope are used to view, magnify, and produce an image from a specimen placed on a slide. These parts include:

1. **Eyepiece** – also known as the ocular. This is the part used to look through the microscope. Its found at the top of the microscope. Its standard magnification is 10x with an optional eyepiece having magnifications from 5X to 30X.
2. **Eyepiece tube** – it's the eyepiece holder. It carries the eyepiece just above the objective lens. In some microscopes such as the binoculars, the eyepiece tube is flexible and can be rotated for maximum visualization, for variance in distance. For monocular microscopes, they are none flexible.
3. **Objective lenses** – These are the major lenses used for specimen visualization. They have a magnification power of 40x-100X. There are about 1- 4 objective lenses placed on one microscope, in that some are rare facing and others face forward. Each lens has its own magnification power.
4. **Nose piece** – also known as the revolving turret. It holds the objective lenses. It is movable hence it cal revolve the objective lenses depending on the magnification power of the lens.
5. **The Adjustment knobs** – These are knobs that are used to focus the microscope. There are two types of adjustment knobs i.e fine adjustment knobs and coarse adjustment knobs.
6. **Stage** – This is the section in which the specimen is placed for viewing. They have stage clips that hold the specimen slides in place. The most common stage is the mechanical stage, which allows the control of the slides by moving the slides using the mechanical knobs on the stage instead of moving them manually.
7. **Aperture** – This is a hole on the microscope stage, through which the transmitted light from the source reaches the stage.

8. Microscopic illuminator – This is the microscopes light source, located at the base. It is used instead of a mirror. It captures light from an external source of a low voltage of about 100v.
9. **Condenser** – These are lenses that are used to collect and focus light from the illuminator into the specimen. They are found under the stage next to the diaphragm of the microscope. They play a major role in ensuring clear sharp images are produced with a high magnification of 400X and above. The higher the magnification of the condenser, the more the image clarity. More sophisticated microscopes come with an Abbe condenser that has a high magnification of about 1000X.
10. **Diaphragm** – it's also known as the iris. Its found under the stage of the microscope and its primary role is to control the amount of light that reaches the specimen. It's an adjustable apparatus, hence controlling the light intensity and the size of the beam of light that gets to the specimen. For high-quality microscopes, the diaphragm comes attached with an Abbe condenser and combined they are able to control the light focus and light intensity that reaches the specimen.
11. **Condenser focus knob** – this is a knob that moves the condenser up or down thus controlling the focus of light on the specimen.
12. **Abbe Condenser** – this is a condenser specially designed for high-quality microscopes, which makes the condenser to be movable and allows very high magnification of above 400X. High-quality microscopes normally have a high numerical aperture than objective lenses.
13. **The rack stop** – It controls how far the stages should go preventing the objective lens from getting too close to the specimen slide which may damage the specimen. It is responsible for preventing the specimen slide from coming too far up and hitting the objective lens.

Features of Light Microscope

No.	Character	Light Microscope
1.	Alternatively known as	Optical microscope
2.	Invented by	It is believed that Dutch spectacles makers Zacharius Jansen and his father Hans were the first to invent the compound microscope in the 16th century.
3.	Illuminating source	Uses light (approx. wavelength 400-700 nm) to illuminate the objects under view.
4.	Principle	The image is formed by the absorption of light waves.
5.	Structure	Light microscopes are smaller and lighter.
6.	Lenses used	Lenses are made of glass.
7.	Vacuum	Not used under a vacuum
8.	Specimen type	Fixed or unfixed, stained or unstained, living or non-living.
9.	Specimen observed	Both live and dead specimens can be observed.
0.	Specimen preparation	Less tedious and simple.
11.	Preparation time	Specimen preparation takes usually a few minutes to hours.
12.	Thickness of specimen	5 micrometer or thicker
13.	Dehydration of Specimen	Specimens need not be dehydrated before viewing.
14.	Coating of specimen	Stained by colored dyes for proper visualization.
15.	Mounting of specimen	Mounted on the glass slide.
16.	Focusing	Done by adjusting the lens position mechanically.
18.	Resolving power	Low resolving power, usually below 0.30μm.
19.	Viewing of the image formed	Light microscope images can be viewed directly. Images are viewed by the eyes through the eyepiece.
20.	Nature of Image formed	Poor surface view
21.	Image Color	Colored images.

23.	Living processes	Visualization of living processes such as microscopic pond life in action and even cell division is possible.
24.	Room settings	No special settings are required.
26.	Electric Current	No need for high voltage electricity.
29.	Radiation leakage	No radiation risk.
30.	Complexity	Less complex
31.	Expense	Cheap to buy and has low maintenance costs.
32.	Suitability / Practicality	Suitable for most basic functions, and is very common in schools and other learning institutions.
33.	Advantages	Easy to use, Cheap True color but sometimes require staining Live specimens
34.	Disadvantages	Low resolution due to shorter wavelength of light (0.2nm) Low magnification The specimen used is thin.
36.	Application	It is used for the study of detailed gross internal structure.