

Experiment 1

Study of Compound Microscope

Aim

To study the compound microscope.

Relevance in Practice

Compound microscope is the scientific tool that allow us to see tiny objects in detail, such as animal or plant cells, bacteria, algae, and protozoa. They use multiple lenses to magnify those specimens which are not visible to the naked eye. This magnification is essential for various applications, including academic research, forensic analysis, medical diagnosis and the study of microorganisms. This experiment will allow the students to handle the microscope and to focus the tissues and cells.

IMPORTANT THEORETICAL CONTEXT

Types of Microscopes

- **Simple microscope:** Single lens magnification
- **Compound microscope:** Multiple lenses system
- **Electron microscope:** Uses electron beam
- **Fluorescence microscope:** Uses fluorescence or phosphorescence
- **Digital microscope:** Displays image digitally
- **Dark field microscope:** Uses scattered light
- **Dissecting microscope (stereo microscope):** Views 3D objects

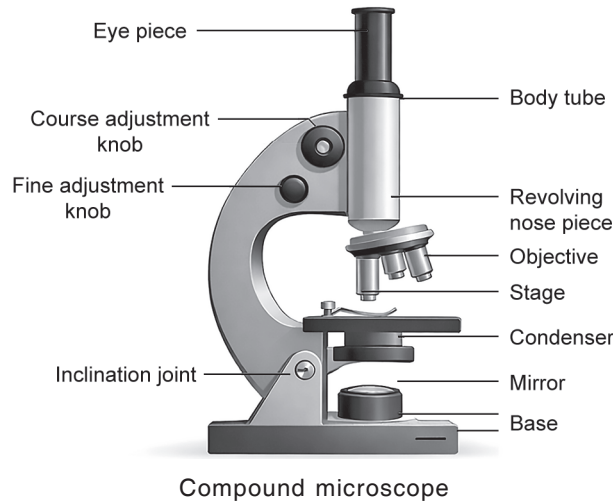
Terminology

'Micro' and 'scope' are two distinct words that can be used interchangeably. "Micro" denotes small or tiny, while "scope" means to watch or observe. Consequently, it is described as an optical device that is used to observe and magnify minuscule objects that are invisible to the human eye. Cell morphology, histology, histopathology, and microbiology are all studied with it.

Resolution: Distinguishing adjacent items as distinct entities.

Magnification: Makes smaller objects larger.

1. **Foot or base:** This U-shaped component bears the compound microscope's full weight. The base is a hefty, metallic, "horseshoe" or "U" shaped construction that gives the microscope the most stability while holding it in place on the platform.
2. **Pillars:** It is the vertical projection. This stands by resting on the base and supports the stage.
3. **Arm:** The solid C-shaped arm is used to hold the microscope when it is being moved around the lab. It also serves to attach the tube to the microscope's base. A fine and coarse adjustment knob is included. A robust, curving structure called the arm, controls the entire microscope.
4. **Stage:** This is a platform where slides of sample specimens are set up for viewing. This has an aperture in the middle and can be round, square, or rectangular in shape. The stage may be mechanical or fixed. The slide is secured in place by two clips on the fixed stage. The right side of the mechanical stage is equipped with a metal frame that has been calibrated. The slide is held in place by a spring-mounted



clip, and it can be moved forward, backward, and side to side using two screw heads. To show the degree of movement, a Vernier scale is also included.

5. **Inclination joint:** A tilting joint makes it easier to view the microscope more comfortably. There is a junction where the arm and pillar are joined. This joint connects the arm to the pillar of the compound microscope. Using the inclination joints, the microscope can be inclined.
6. **Clips:** The upper parts of the stages are connected to two clips, the slide can be held in its position with the help of the clips.
7. **Diaphragm:** The diaphragm is fastened below the stage. It controls and adjusts intensity of light that passes into the microscope. The diaphragm can be two types:
 - Disc diaphragm
 - Iris diaphragm
8. **Nosepiece:** The rotating turret, sometimes referred to as the nose piece, is a circular rotating metal component with three to five holes where the objective lenses are attached. It is connected to the bottom of the body tube. The round nosepiece is a rotating metal component that is attached to the bottom end and body tube. The objective lenses are inserted into the nosepiece's three holes.
9. **Body tube:** To visualize the image, light travels *via* a cylindrical tube that is 16–17 cm long. There is an adjustable knob to move the body tube up and down. The microscope's body tube is a hollow, tubular structure that makes up the top portion of the arm. Through the use of the adjustment knobs, the body tube can be moved up and down.
10. **Coarse adjustment knob:** It is a large knob, used to move the body tube up and down for proper focusing of slide. It brings the object to be examined under exact focus.
11. **Fine adjustment knob:** It is a smaller knob, used for sharp and fine focusing of the object. It is employed for accurate focusing.
12. **Optical parts of compound microscope:** Eyepiece, lens or ocular: The detachable lens that is positioned at the top of the body tube is known as the eyepiece, lens, or ocular. The sizes are 5X, 6X, 8X, 10X, or 15X. There are two lenses in each eyepiece: A field lens at the bottom and an eye lens at the top. The image is further magnified by the field lens, which gathers divergent rays and sends them through the eye lens. A lens known as an eyepiece is positioned at the top of the body tube, allowing for the observation of the magnified picture.
13. **Objectives:** There are three objectives fitted into a revolving nosepiece. Different types of objective lenses are: low power objective of 10X, high power objective of 45X, and oil immersion objectives of 100X. Low power objective $10X = 10 \times 10 = 100$ times magnifies the image

High power objective $45X = 45 \times 10 = 450$ times magnifies the image, used for viewing blood cells, blood smear and histological studies.

Oil immersion objective ($100X$) = $100 \times 10 = 1000$ times magnifies the image, as the name indicates it requires oil (cedar wood oil) to focus the image.

14. **Condenser:** A condenser is a group of lenses that gather light from the source and direct it toward the specimen. To raise or lower the condenser, turn a condenser knob. Below the stage, it is installed. Beneath the stage is a condenser with a standard diameter of 39.5 mm that collects and concentrates light that travels through the sample or brings light beams to a shared focus on the object under inspection. There could be two or more lenses.
15. **Iris diaphragm:** To regulate the amount of light that enters the condenser, a knob on the inside is utilized to open or close the opening. Usually, this fixed lever on the condenser is used to adjust the aperture. The diaphragm is used to either raise or reduce the light's intensity by closing and opening it.
16. **Mirror:** A double-sided mirror with one flat side and other concave is located below condenser and can be rotated in all directions. The flat side is used for oil-immersion and the concave side is used for low and high-power objectives. It can be rotated in any direction and is situated below the condenser.
17. **Light source:** Both internal (electric built-in light source) and external (natural daylight, electric lamp, or tube light) are possible. Its components include a light source that can be either electric or natural light. Because it is easier to operate, electric lighting is more popular. For everyday use, an external light source of 60 watts can be placed 18 inches away from the microscope. These days, many microscopes have built-in understage lighting. If not, daylight might be utilized. Both the eyes and the microscope are at risk from exposure to direct sunlight. For best results, utilize reflected sunlight on a white background.

Requirements

Apparatus: Compound microscope, and permanent slides to examine.

Procedure

1. Examine the specimen, blood film, or permanent slide with your unaided eyes first.
2. Set the body tube up about 7–8 cm above the stage and place the microscope upright on the worktable. The eyepiece, objectives, substage, mirror, and stage window should all have their glass surfaces cleaned using a silk cloth and cleaning solution.
3. Position the slide on the stage, then raise the specimen over the centre aperture using the mechanical stage assistance.
4. Pick an objective with a low magnification ($10X$).
5. Choose and align the concave or plane mirror so that the specimen is illuminated.
6. Reduce surplus light by adjusting the condenser well down and partially closing the diaphragm.
7. With the body tube lowered using the coarse adjustment, the low power lens should be around 1 cm above the slide when viewed from the side. Lift the tube slowly until the slide is in focus while looking into the eyepiece.
8. After that, decide the area of interest to view at a higher-magnification.
9. To focus at high magnification, just turn the nosepiece until the $45X$ high magnification objective "clicks" into place. Open the diaphragm and raise the condenser to the middle position to allow for adequate light. Make precise adjustments as needed. For focusing under oil immersion objective ($100X$), raise the body tube ~ 8–10 cm above the slide.
10. Place a drop of cedar wood oil, paraffin or glycerine on the slide. Looking from the side, bring down the objective till it just enters the oil drop. Use other adjustments as required.

Precautions

1. Maintain the microscope in an upright position at all times.
2. Do not use direct sunlight.
3. Do not let the microscope touch the edge of the table.
4. The item to be examined should be covered with a coverslip and placed in mounting media before the observation.
5. Apply just enough mounting material.
6. Always make sure the microscope is clean, dust-free, and secure.
7. Concave mirrors are utilized with low power lenses, while plane mirrors are employed with high power or oil immersion lenses. Appropriate mirror placement is necessary for optimal and uniform illumination.
8. Reposition the condenser and temporarily remove the eyepiece for better results. Adjusting the iris diaphragm is necessary.
9. After the iris and condenser adjustment, target is clearly visible, replace the eyepiece and reobserve.
10. Using an oil immersion lens, a drop of cedarwood oil is smeared on the slide. Cedarwood oil is better than other oils since its refractive index is more like that of glass.
11. Wipe the oil off the target with a dry, soft cloth, and then apply a small amount of xylol to finish the job. Avoid using too much xylol.
12. Never take out any of the screws on the microscope. Alcohol destroys the cementing compound used to clean microscope lenses; hence it should not be used to fix lenses.

Result

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Viva Voce

1. Name the optical parts of compound microscope?
2. Explain role of diaphragm?
3. Define condenser.
4. What is compound microscope.
5. Describe the illumination system.