



HISTOLOGY MANUAL



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CHAPTER ONE

MICROSCOPY

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The light microscope is a precision instrument that enables the user to see the order and organization inside a very small living structure. It plays an important role in understanding the compound picture of the structure(s) and function(s) of living material. For effective use of the microscope, it must be aligned correctly, handled carefully and kept clean at all times.

With regards to the microscope, the students should know and be able to do the following:

- Know the different components of the microscope;
- Understand the function of each component of the microscope;
- Insert and move a slide on the stage;
- Adjustment of the lighting mechanism of the microscope;
- Adjust the microscope to your eyes, including focus and inter-ocular distance;
- Recognise and understand different magnifications of an image;
- Understand and explain the pathway of light through the microscope; and
- Recognise and understand the different colours and shades on a slide.

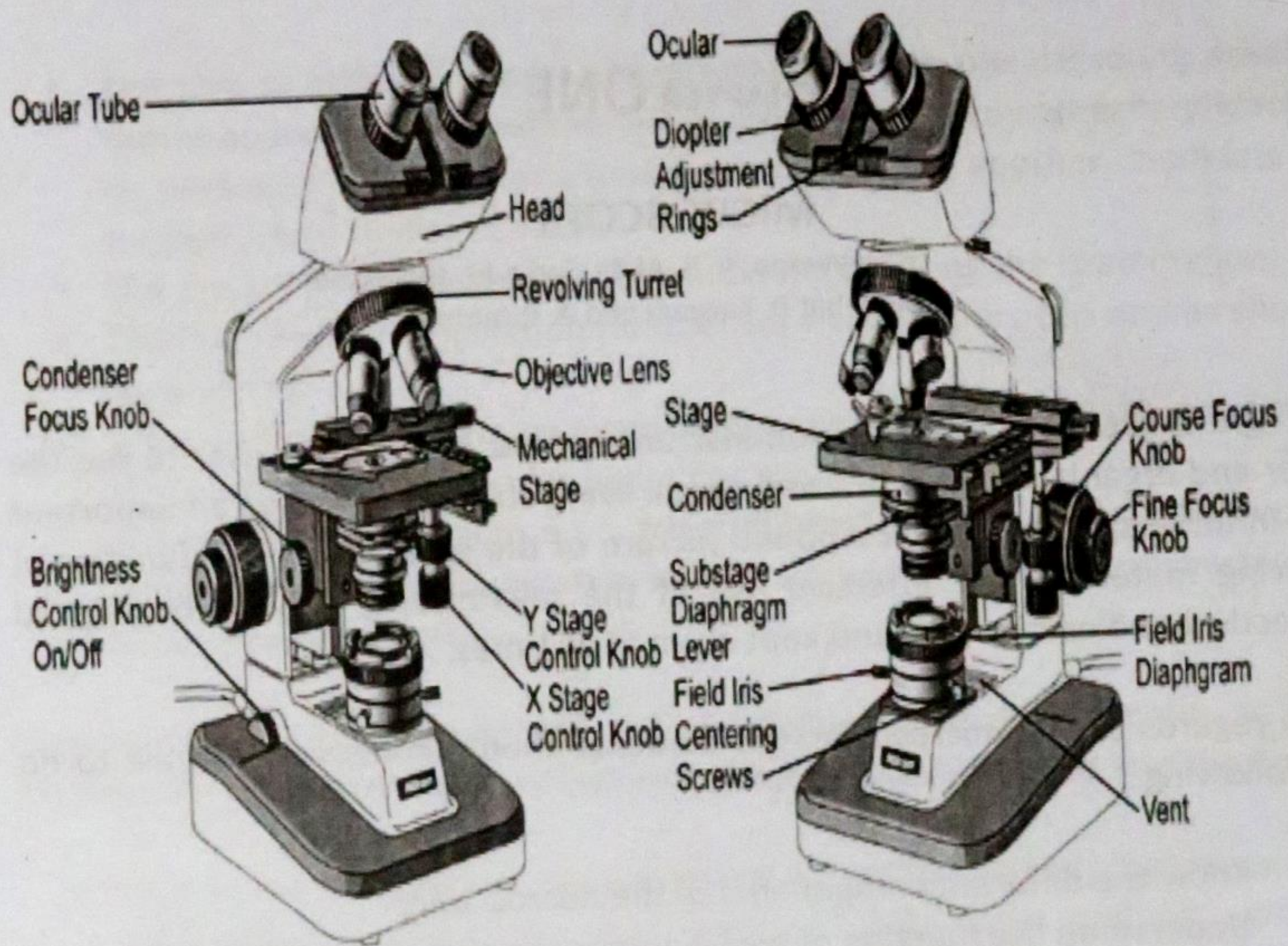


Figure 1: Anatomy of a Light Microscope.

1.1. Identification and Functions of the Component Parts of a Microscope (Figure 1).

The microscope comprises the following:

(a) Microscope Stand, which is made up of:

- (i) Frame
- (ii) Coarse and fine focus knobs
- (iii) Light source
- (iv) On/Off power switch
- (v) Variable light control

(b) Integrated Binocular Tube, which consists of:

- (i) Eyepiece
- (ii) Revolving Nosepiece carrying the objectives of various magnifications:
 - (a) Very low magnification 4X
 - (b) Low magnification 10X
 - (c) High magnification 40X
 - (d) Oil immersion 100X

- (c) **Mechanical Stage**, which is made up of:
 - (i) Specimen holder with spring-loaded clamps and Control Knobs for movement of the specimen.
 - (ii) Condenser.

Each part of the microscope has the following functions:

Frame

The various parts of the microscope are mounted on the frame.

Coarse and Fine Focus Knobs

Adjustment wheels move either the stage or the microscope tube to focus the image seen at the ocular lens. At higher magnifications, only the fine focus knob is used.

Light Source

This provides illumination of the specimen.

On/Off Power Switch

Turns power to the light source on or off.

Variable Light Control

The variable light control adjusts the intensity of the illumination.

The Mechanical Stage

The stage below the objective lenses with a spring loaded clamp arrangement to support and move the slide on the stage.

Condenser

The condenser focuses light from the source(s) in a cone onto the tissue. It usually incorporates diaphragms and filters to manage both quality and intensity of illumination.

Revolving Nose Piece

The revolving nose piece or objective turret carries the objective lens and enables the user to easily change to a higher or lower magnification.

Objective Lenses

Usually, but not restricted to 4X, 10X, 40X and 100X (oil immersion) magnification. The objective lenses receive light from the tissue on the slide and project an enlarged image in the direction of the ocular lens. Some types of objective lenses use oil or water placed on top the specimen or coverslip to obtain greater resolution at higher magnifications.

Ocular or Eyepiece Lens

The eyepiece or ocular lenses further magnified and enhance the image from the objective lens and brings the image into focus for the eye. The final optical magnification of the specimen is the product of the magnification of the ocular and objective lenses. The eyepiece often contains a diopter adjustment ring which can be used to adjust the focus for the user's own eyes.

Lens Structure and Focal Point

Objective lenses of microscopes are made of special optical glasses with higher quality than ordinary glasses. The lens is shaped like a circular disk with two outward curving convex faces. When parallel rays of light strike one face of the objective lens, they are focused while passing through the lens to meet at a single spot called the Focal Point.

Focal Length of an Objective Lens

The focal length of an objective lens is the distance from the centre of the lens to the focal point. It is the distance from the lens to the point of convergence of parallel rays passing through the lens. The larger created image is, therefore, essentially the object viewed by the ocular- or eyepiece- lens as created by an objective lens with a smaller focal length. The Focal Length of convex lenses usually has a positive sign because the image occurs on the other side of the lens from where the object is positioned. However, Focal Length of concave lenses (where the faces of lens curve inward) is usually negative.

Lens Strength

The strength of a lens is determined by its focal length. The Lens Strength is calculated by taking the inverse of the Focal Length. A lens with a shorter focal length will have a higher lens strength with capacity to

enlarge the image more. Hence, microscopes have shorter focal length so that they can greatly enlarge images.

Calculation of Total Magnification and Focal Length

Total Magnification = Power of the Objective Lens X Power of the Ocular Lens

Magnification (Objective) = Focal Length of Ocular Lens / Focal Length of the Objective Lens

1.2. How to use the Light Microscope

This is a very condensed version of the correct way to use a light microscope. Consult a manual or textbook if you plan to do serious work using a microscope.

However, you are expected to be able to:

- Focus and view slides effectively
 - Know what objective lens to be use at a particular time when viewing the slide
 - Know how and when to use the fine and coarse adjustment knob
 - Know how to calculate the magnification of the specimen.
- (a) Microscope quick checklist:
1. Condenser at the top
 2. Iris should be opened
 3. Focus with both eyes or one eye depending on the number of eye piece available
 4. Rheostat supply enough light
- (b) Using the microscope:
1. Handle the microscope by the stand only
 2. Remove the microscope from its box beneath the table and place it on the desk
 3. Ensure that there are no wires, books or pens under the base.
 4. Turn the rheostat to 5
 5. Open the iris on the condenser all the way
 6. Turn the condenser as low as it can go
 7. Open the spring-loaded finger of the specimen holder and insert the slide. The specimen should be above the condenser in the path of the light. Ensure that the cover slip faces up

8. Switch on the microscope
9. Turn the condenser directly beneath the slide
10. Adjust your chair until the level of your eyes is slightly higher than the level of the oculars
11. Look through any of the two oculars and adjust the light (using the rheostat) to a comfortable level (5-8)
12. Move the oculars apart
13. Position your eyes approximately 2cm from the oculars
14. Slowly move the oculars closer to each other until you see two circles or two half circles
15. Move the oculars slowly until you see only one circle
16. Close your right eye. Your left eye must see an image. If the right ocular has a pointer, it should not be visible
17. Open both eyes. You should see one image with both eyes. If the ocular has a pointer, it should be visible with both eyes
18. If you see one image with both eyes, you can start focusing the image.
19. Ensure that the 4x objective is in position above the slide. This can be done by turning the nosepiece until the desired objective lens is in position
20. Close your left eye
21. Turn the coarse focus adjustment knob and focus the image in the right ocular
22. Adjust the right image by turning the fine focus knob
23. Close your right eye
24. Focus the right image by turning the diopter adjustment ring
25. Open both eyes and focus the image by turning the fine focus knob After this it is not necessary to turn the diopter adjustment ring
26. Turn the nosepiece until the 10x objective clicks into place above the condenser
27. Focus the image only with fine focus knob
28. Turn the nosepiece until the 40x objective clicks into place above the condenser
29. Focus the image only with fine focus knob
30. Turn the nosepiece until the 10x objective clicks into place above the condenser

31. Focus the image only with fine focus knob
32. Turn the nosepiece until the 40x objective clicks into place above the condenser
33. Focus the image with the fine focus knob

Questions:

1. Calculate the highest total magnification for a microscope with an ocular lens of 15X and objective lenses of 5X, 10X, and 30X.

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2. If linear magnification of the objective lens of a microscope is 30, angular magnification of the ocular lens is 55 and the focal length of the ocular lens is 15cm. Calculate the focal length of the objective lens.

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1.3. *Identification of the Structural Components of the Cell*

Slide 1: This specimen (Amphibian Blood) serves as an example of an animal cell and is used for orientation regarding the size, shape, colour and shading of a cell stained with haematoxylin and eosin. Red blood cells (erythrocytes), are anucleated biconcave discs, which are packed with the O₂-carrying protein hemoglobin. The biconcave shape provides erythrocytes with a large surface-to-volume ratio to ease gas exchange. Human erythrocytes are 7.5µm in diameter, 2.6µm thick at the rim, and 0.8µm thick in the center when suspended in an isotonic medium. The normal concentration of erythrocytes in blood is approximately 3.9–5.5 million per microliter in women and 4.1–6 million per microliter in men.

At the end of this section, you should be able to identify:

- The red blood cell
- The cell membrane
- The shape of the cell and its nucleus
- The location of the nucleus in the cell i.e. central or eccentric

- The ratio of the size of the nucleus to the size of the cell
- The colour of the nucleus
- The colour of the cytoplasm
- Any other organelle within the cytoplasm of the cell
- Any granule within the cell

Drawing:

Draw a red blood cell: Draw the shape and size of the cell and nucleus in the correct ratio to one another.

Questions:

1. What is understood by the terms acidophilic and basophilic?

.....

2. Complete the table:

	Cytoplasm	Nucleus	Cell membrane
Colour			
Stain			
Visible			