CHAPTER

1

The Study of Compound Microscope

I. THE STUDY OF COMPOUND MICROSCOPE

Learning Objectives

The student after completing the practical should be able to:

1. Identify and able to explain the uses of parts of a compound microscope.
2. Focus the given blood smear using low power, high power and oil immersion objective lenses.
3. Enlist precautions to be taken while using the microscope.
4. Discuss the care to be taken while transporting, handling, and cleaning and storage of the microscope.
5. Understand the importance of learning microscopy and its implication in clinical practice.

Aim of experiment: To study the compound microscope.

Instrument: Compound microscope.

Description of the instrument: Microscope is an instrument used to visualize objects which cannot be seen by the naked eye. Microscope may be simple or compound microscope. The compound microscope is commonly used by the students in haematology lab.

1. A simple microscope consists of a lens or set of lenses which produces an erect enlarged virtual image. Simple microscope does not produce high magnification.
2. The compound microscope is an instrument for magnifying small objects. It is consisting of an objective lens of short focal length for forming a real image of the object inside the microscope (Fig. 1.1) and is further magnified by a second lens (eyepiece) of longer focal length forming enlarged inverted virtual image of the object (Fig. 1.2). Refer to Fig. 1.1. The objective and eyepiece together allow much higher magnification and reduces chromatic aberration.

PRINCIPLE OF WORKING OF A COMPOUND MICROSCOPE

Principle: The objective lens focuses a real image (Fig. 1.1) of the object inside the microscope. That image is then magnified by a second lens or group of eyepiece lenses producing enlarged inverted virtual image (Fig. 1.2) of the object.

PARTS OF THE MICROSCOPE

Eyepiece lens: These are the lens at the top through which we make our observations. They are usually of 10X power.

Tube: It connects the eyepiece to the objective lenses.

Revolving nosepiece: It holds two or more objective lenses and can be rotated to easily change power.

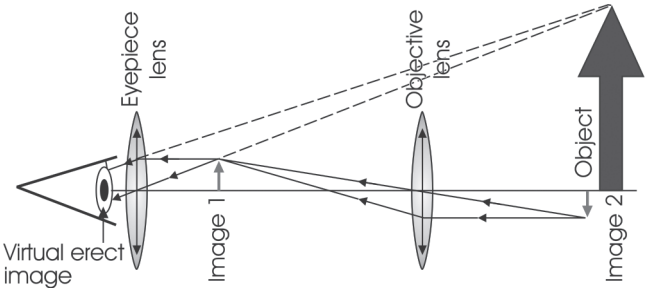

Fig. 1.1: Image formation under compound microscope

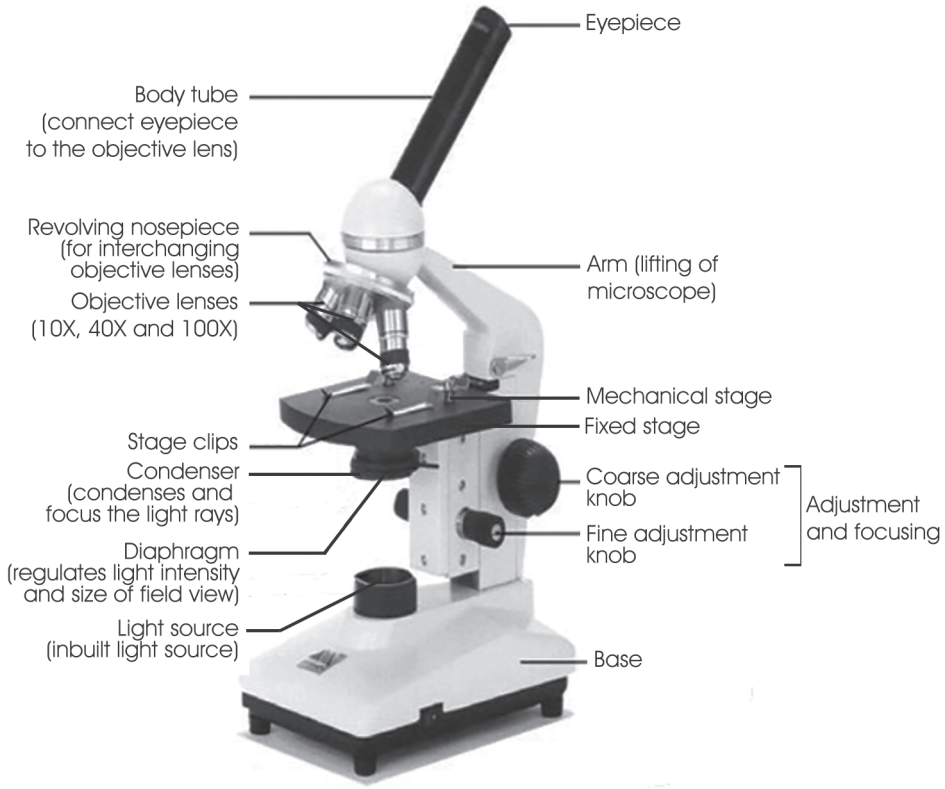

Fig. 1.2: Compound microscope

Objective lenses: The three objective lenses commonly present on a microscope are having 10X, 40X and 100X powers (Fig. 1.3). Together with a 10X eyepiece lens the total magnifications produced will be of 100X (10X times 10X), 400X and 1000X.

Arm: The handle supports the optical system of lenses and can be used for lifting the microscope.

Key Notes

The oil immersion lens, the highest magnification and least working length. It is so called oil immersion lens because it is to be used with a drop of oil having the same refractive index as that of the glass slide so that the loss of light rays coming from the object can be minimized. Cedar-wood oil is commonly used to minimize the loss of light rays coming from the object as its refractive index is 1.515 which is the same as that of glass.

Fig. 1.3: Objective lenses of microscope

Illumination: Light from an electric bulb or sunlight acts as the source of illumination. The light rays are reflected by a plane concave mirror provided at the base. Modern microscope has inbuilt light source of 110 volts in place of a mirror.

Stage: It consists of a horizontal platform on which the slide is mounted. The stage clips hold the slides in place and keep it stable. It has a central aperture which allows the reflected light rays to fall on the object. A mechanical stage is fitted to the fixed stage so that the object can be moved from side to side or from front to back.

Substage: This is located below the stage and it consists of a condenser and an iris diaphragm. The condenser consists of lenses which condense and focus the light rays from the mirror onto the object. The condenser can be lowered or raised by moving of the knob which is located at the side. The iris **diaphragm** is used to control the amount of light reaching the object.

Diaphragm: The diaphragm consists of varied sized holes due to which various intensity of light can be projected upward into the slide. The setting of diaphragm is carried out by the observer depending on transparency of the specimen, the degree of contrast expected and the type of objective lens which is in use.

Focus adjustment knobs: There are two focus adjustment knobs present at the side of the microscope. The bigger knob is utilized for coarse adjustment and focusing while the smaller knob is utilized for fine focusing. The distance between the objective lens and object can be adjusted using these knobs so that the object lies at the focal length of the objective.

Base: The microscope has a solid base which provides stability.

II. MAGNIFICATION

The product of power of eyepiece and that of power of objective determines the total magnifying power of the microscope. The total magnification achieved when viewing an image under a compound microscope, under the low-power lens (10X), the high-power lens (40X), and the oil-immersion lens (100X) shall be the power of the eyepiece which is 10X multiplied with power of lenses, that is, 10X, 40X and 100X respectively. Thus, magnification achieved is 100X with the low-power lens, 400X with the high-power lens, and 1000X with the oil-immersion lens.

III. RESOLUTION

The **resolution** of the microscope refers to the ability to see two items as separate objects and

with clarity under the microscope. The resolving power depends on the numerical aperture of the lens and the wavelength of the light used for visualization. Shorter the wavelength of light, better will be the resolution. The **numerical aperture (NA)** refers to the widest cone of light entering the lens.

IV. WORKING DISTANCE

At low magnification your working distance is more and so vice versa when magnification is increased. This objective has the least working distance when observed under oil immersion lenses.

V. FOCUSING PROCEDURE

Under Low Power (10X)

a. Keep the slide to be focused on the mechanical stage and ensure that the stage clips are approximated so that it does not get displaced.
b. Focus the light on the object by adjusting the concave mirror.
c. Lower the low power objective (10X).
d. Lower the condenser from the higher position till the object is properly illuminated.
e. Keep the iris diaphragm partially opened and adjust the focus with the fine adjustment for a sharper image.

For High Power Magnification (40X)

a. Place the slide to be focused on the mechanical stage and ensure that the stage clips are approximated so that it does not get displaced.
b. First focus under low power and carefully observe and select the required area.
c. Rotate the revolving nosepiece and focus the high-power objective lens into visualization position.
d. Keep the iris diaphragm half opened.
e. Use plane mirror to condense the light on the object.
f. Raise the condenser and keep it in high position.
g. Use the fine focusing knob to focus the object and visualize and note your observations carefully.

Under Oil Immersion (100X)

a. Place the slide to be focused on the mechanical stage and ensure that the stage clips are approximated so that it does not get displaced.
b. Place a drop of cedarwood oil over the area which is to be visualized.
c. Keep the condenser at the highest position.
d. Keep the iris diaphragm fully opened.
e. Adjust the plane mirror to focus the light on the object.
f. Adjust the objective lens as it touches the slide, ensure that the slide is not broken.
g. Visualize the object and note your observations.

> **Note:** In microscope having inbuilt light source; there is no mirror and hence no such mirror adjustment is required.

BENEFITS OF USING A COMPOUND MICROSCOPE

The compound microscope is simple and convenient to use; it has also inbuilt light source; they have multiple lenses with varied magnification power (for example, 10X, 40X and 100X) that reveals a greater amount of details; and being small in size it is convenient to store and handle easily.

Precautions

1. **Transporting the microscope:** Handle the microscope with care. Support the base of the microscope and hold it uprightly while lifting it. Do not swing the microscope.
2. **Handling and cleaning the microscope:** Clean the eyepiece and objective lens

gently with xylene before using the microscope. Do not touch the lens with hand as this will leave a fingerprint marks on lens; which will smudge the images. Use sterile gauze piece for cleaning the lenses and the glass slide.

3. **Care while using:** Do not lower the optical tube when you are looking through the eyepiece. After using the oil immersion lens, it should be cleaned with cotton soaked in xylol. Prefer minimal use of fine adjustment while viewing through low power, high power or oil immersion lens.
4. **Cleaning:** The students should clean all slides, place materials in rack or assigned appropriate place, and finally clean the work area. Discard the cover slips in the bin.

Types of Microscopes

1. **Simple microscope:** Simple microscope has a single lens for magnification. It has lower magnification as compared to compound microscope.
2. **Compound microscope:** The common magnifications of a compound microscope are 10X, 40X and 100X. The compound microscopes have high magnification but a low resolution.
3. **Dissection microscope or stereoscope:** This microscope is binocular (two eyes) and provides a three-dimensional image of the specimen and is especially used for studies related to anatomical dissection.
4. **Confocal microscope:** This microscope uses laser light which scan across the specimen and image is focused on a digital computer screen for further studies and evaluation.
5. **Scanning electron microscope:** This equipment uses electrons (negatively charged electrical particles) to magnify objects up to two million times. The 3-dimensional image is formed. The magnification and resolution of scanning electron microscope is high.
6. **Transmission electron microscope:** This equipment also uses electrons for illumination, but instead of scanning the surface (as with scanning electron microscope) electrons are made to pass through very thin specimens. This forms a 2-dimensional view. The magnification and resolution of transmission electron microscope is high.
7. **Fluorescence microscope:** This type of microscope utilizes fluorescence to create an image. The sample specimens are stained with a fluorescent dye which gets bounded to specific component of the specimen. The light of a specific wavelength (or wavelengths) is absorbed by the fluorophores. This causes them to emit light of longer wavelengths producing auto-fluorescence image based on their chemical constitutional makeup.
8. **Bright field microscope:** The bright field microscopes use transmitted light to observe targets at high magnification. They are used to observe live or stained cells.
9. **Phase contrast microscope:** The phase contrast microscopes are used for viewing transparent, unstained, live cells. Phase-contrast microscopy converts phase shifts in light passing via the transparent specimen to bright outlook of the image, thereby making them visible and identifiable so that their characteristics can further be studied.

Snap box 1

Clinical Perspective

Microscope has been a diagnostic tool in clinical practice. Microscope has been in use for manual blood cell count of red blood cell, white blood cell, platelet, reticulocyte, Arneth, absolute eosinophil count, sperm count, etc. It is immensely helpful in histological and histopathological studies. Its application in diagnosis of anemia, malaria, filaria, leukemia, diagnosis of cancerous conditions, parasitic disorders, cell culture studies, etc. has been boon to medical sciences.

EXERCISE FOR STUDENTS

OSPE Non-skilled

1. Label the parts of microscope as given in Fig. 1.4.

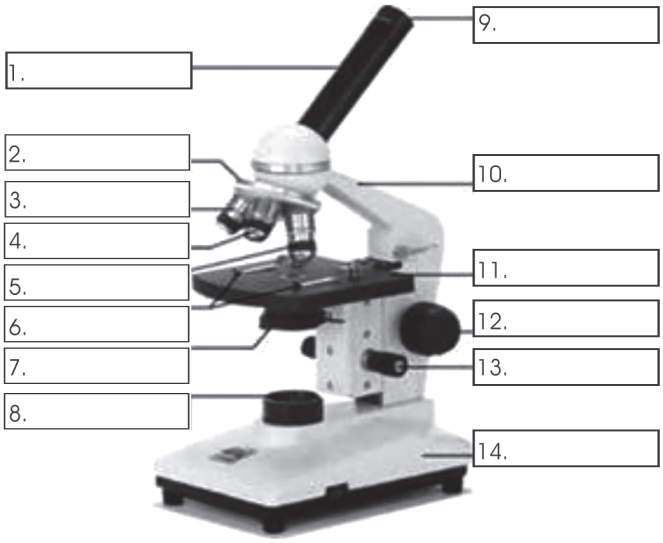

Fig. 1.4

Snap box 2

Nobel Prize in Chemistry for the year 2014 was awarded to Eric Betzig, William Moerner and Stefan Hell 2014, for the development of super-resolved fluorescence microscopy. The newly developed technique brought optical microscopy into the Nano dimension.

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Viva Voce Questions

Q1. What is the power of eyepiece lens?

Ans. The power of eyepiece lens usually 10X or 15X power.

Q2. What is the function of tube in compound microscope?

Ans. The tube connects the eyepiece to the objective lenses.

Q3. What is the function of arm in compound microscope?

Ans. The arm supports the tube and connects it to the base.

Q4. What is the function of base in compound microscope?

Ans. The base is the bottom of the microscope, used for support.

Q5. What is the source of illumination in your compound microscope?

Ans. The microscope has a mirror which reflects light from an external light source up via the bottom of the stage. In modern compound microscope a light source of 100 volts is inbuilt instead of mirror.

Q6. What is the function of stage in compound microscope?

Ans. The stage is flat platform where slides are placed. The stage clips keep the slide approximated in place. A mechanical stage is fitted to the fixed stage so that the object can be moved from side to side or from front to back.

Q7. What is the function of nosepiece in compound microscope?

Ans. The nosepiece is the part that holds two or more objective lenses which can be rotated to change power.

Q8. What are the power of objective lenses of compound microscope?

Ans. The power of objective lenses of compound microscope commonly used is of power 10X, 40X and 100X.

Q9. What are the types of commonly used microscopes in clinical research lab?

Ans. The commonly used microscopes in clinical research lab are simple microscope, compound microscope, dissection microscope or stereoscope, confacial microscope, fluorescence microscope, bright field microscope, phase contrast microscope, electron microscope (includes transmission electron microscope and the scanning electron microscope), etc.

Q10. Which are the desired positions of condenser, for seeing object in low power, high power and oil immersion?

Ans. The desired position of the condenser is as follows:

Objective	Lens power	Condenser position
i. Low power	10X	Lowest
ii. High power	40X	Midway
iii. Oil immersion	100X	Highest

Q11. How are the illumination achieved for observation of sample under microscope?

Ans. The normal daylight is natural illuminating source which is directed via a mirror onto the sample. Modern microscopes have halogen lamp, LEDs and lasers as an adjustable and controllable light source.

Q12. What is the role of condenser in the microscope?

Ans. The condenser is a lens designed to focus light from the illumination source onto the sample. The condenser consists of a diaphragm to control the quality and intensity of the illumination.

Q13. What do you understand by magnification of a compound optical microscope?

Ans. The magnification of a compound optical microscope is the product of the powers of the ocular (eyepiece) lens and the objective lens.

Q14. How much is the total magnification achieved when viewing an image under a compound microscope, under the low-power lens (10X), the high-power lens (40X), or the oil-immersion lens (100X)?

Ans. The total magnification achieved when viewing an image under a compound microscope, under the low-power lens (10X), the high-power lens (40X), or the oil-immersion lens (100X) is obtained by multiplying the power of the eyepiece which is 10X with power of the lens, thus the total magnifications of the low-power lens will be 100X, 400X of the high-power lens, and 1000X of the oil-immersion lens.

Q15. What is the principal advantage of an oil immersion objective?

Ans. The oil-immersion objectives offer greater resolution at high magnification.

Q16. Which oil is used for magnification under oil immersion objective? What is its advantage?

Ans. Cedarwood oil is used as index-matching material. The refractive index of the cedarwood oil is higher than air; due to which the objective lens achieve a larger numerical aperture (greater than 1). The larger numerical aperture allows minimal refraction enabling more light to be transmitted and object can be observed with clarity.

Q17. Define numerical aperture, working distance and resolving power of the lens.

Ans. The numerical aperture is a measure of the amount of light entering the objective lens. The working distance is approximately equal to the focal length. The resolving power of an objective lens is measured by its ability to differentiate two lines or points in an object:

Minimum separable difference

$$= \frac{0.61 \times \text{wavelength of light}}{\text{Numerical aperture}}$$

Q18. What will happen to image under focus, if the aperture of oil immersion objective lens is more than a pin-hole aperture?

Ans. In case the aperture of oil immersion lens is more than a pin-hole; spherical and chromatic aberration would distort the image.

Q19. What is phase contrast microscopy?

Ans. The phase contrast microscopy is an optical microscopy illumination technique; in which small phase shifts in the light passing through a transparent specimen are converted into amplitude for contrast changes in the image. This microscope technique made it possible to study the cell cycle in live cells.

Q20. Name the variants of the electron microscopes.

Ans. The major variants of electron microscopes are scanning electron microscope (SEM) and the transmission electron microscope (TEM).

Q21. What is fluorescence microscopy?

Ans. The sample is illuminated through the objective lens by narrow set of wavelengths of light. The light interacts with fluorophores in the sample eventually emitting light of a longer wavelength. The emitted light which forms up the image. This illuminating technique with the aid of fluorophores is called fluorescence microscopy.

Q22. Enlist the applications of optical microscopy.

Ans. Optical microscopy is used in histopathological studies for medical diagnosis and so also in nanotechnology, biotechnology, pharmaceutical research, microbiology, microelectronics and mineralogy.

Q23. How does digital microscope work?

Ans. The digital microscope uses optics and a digital camera to output an image to a monitor. A digital microscope often has its own in-built LED light source. The image is focused on the monitor.

Q24. What are the monocular, binocular and trinocular microscopes?

Ans. A. Monocular microscope has provision for one eyepiece for viewing the specimen.

B. Binocular microscope has provision for two eyepieces; and is comfortable and easy to use.

C. Trinocular microscope has provision for third eyepiece tube that can be used by the third individual, for example, a learner student simultaneously or by a CCD camera.

Q25. What is the basic precaution to be taken in use of a microscope after its use?

Ans. The basic precaution to be taken in use of a microscope after its use is: Switch off the microscope when not in use. Place it in rack. Support the base of the microscope and holds it uprightly while lifting it. Do not swing the microscope.

Q26. Who received the Nobel Prize in Physics in 1986 for his work in scanning tunneling microscopy?

Ans. Gerd Binnig along with his colleague Heinrich Rohrer was awarded the Nobel Prize in Physics in 1986 for his work in scanning tunneling microscopy. Binnig and Rohrer developed the powerful microscopy technique that could form an image of individual atoms on a semiconductor surface or metal by scanning the tip of a needle over the surface at a height of only a few atomic diameters.

Q27. What are the advantages of binocular microscope?

Ans. The advantages of binocular microscope are the true depth perception of an image and moreover the use of dual eyepieces reduces strain on eyes especially when work on microscope is to be carried for long hours.

Snap box 3

HISTORICAL ASPECTS—INVENTION OF MICROSCOPE

Zacharias Jansen (1580–1638): He was a Dutch spectacle-maker from Middelburg and credited with inventing the first microscope. Although Zacharias Jansen (often written as Zacharias Janssen or Zacharias Jansen) is generally believed to be the first creator of a compound microscope, the accomplishment is dated around the 1590s, and many scholars believe that his father Hans must have played an important role in the creation of the instrument.

Anton van Leeuwenhoek (1632–1723): The father of microscopy Anton van Leeuwenhoek of Holland made several biological discoveries. He was the first to see and describe bacteria, yeast plants, the teeming life in a drop of water and the circulation of blood corpuscles in capillaries.

Robert Hooke (1635–1703): He was the English father of microscopy and he re-confirmed Anton van Leeuwenhoek's discoveries of the existence of tiny living organisms in a drop of water. Hooke made a copy of Leeuwenhoek's light microscope and then improved upon his design.

James Hillier (1915–January 15, 2007): Physicist James Hillier is recognized for his contributions to the development of the electron microscope. Hillier's work on the electron microscope began in college. He and a fellow graduate student built a model that magnified 7,000 times in the year 1937.

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OBSERVATIONS: Exercise for Students

1. Draw a well-labelled diagram of microscope. Describe its parts and their functions.

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