



## 2

# LIGHT MICROSCOPY

## 2.1 INTRODUCTION

Microscopes are instruments designed to produce magnified visual or photographic images of objects too small to be seen with the naked eye. The microscope must accomplish three tasks: produce a magnified image of the specimen, separate the details in the image, and render the details visible to the human eye or camera. This group of instruments includes not only multiple-lens (compound microscopes) designs with objectives and condensers, but also very simple single lens instruments that are often hand-held, such as a loupe or magnifying glass.



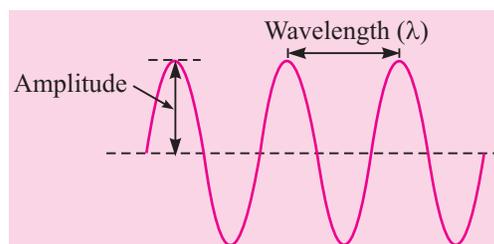
## OBJECTIVES

After reading this lesson, you will be able to:

- describe the principle of light microscope
- explain the parts of a light microscope
- learn how to use a microscope.

## 2.2 LIGHT AND ITS PROPERTIES

Light radiates in all directions, with each ray traveling straight till infinity, unless something interferes its path.



**Fig. 2.1:** Light represented by a wave showing amplitude and wavelength.



Notes

**Amplitude** refers to the strength of energy or brightness of light. When light passes through any medium, the amplitude decreases depending upon the medium.

**Wavelength:** The distance between the apex of one wave and the next is the wavelength and measured in nanometers, and determines the color.

**Retardation:** Media through which light is able to pass, will slow down the speed of light (proportionate to density of medium).

**Refraction:** If light enters a medium (eg glass) at an angle, a deviation of direction occurs

**Image Formation**

**Focal point:** Parallel rays entering a simple lens are brought together to a single point called focal point, where a clear image will be formed.

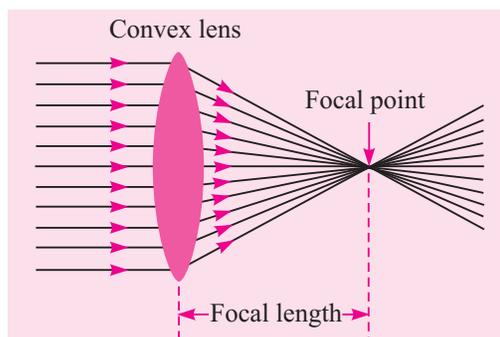


Fig. 2.2: Parallel rays entering a curved lens are brought to a common focus.

**Conjugate foci:** Object placed at one end of lens will form a clear image on a screen kept at other side of lens. Conjugate foci vary in position. If object is nearer the lens, the image will be formed further away, at a greater magnification and inverted. This “real” image is formed by objective lens of microscope. If the object is placed within focal point of lens, image is formed on same side as object, is enlarged, right way up and cannot be projected on a screen, this is the “virtual image”. The eye piece of microscope forms this image.

**Image Quality**

As white light is composed of all spectral colors, different wavelengths will be refracted to different extent. This lens defect is called **chromatic aberration**. **Spherical aberration** is caused when light rays entering at a periphery are refracted more than those entering the centre of lens. Both these faults can be corrected by using combination of lenses and lens elements.

## 2.3 COMPONENTS OF A MICROSCOPE

### Light source

Light source can be external or inbuilt. Dispersal of heat, collection of greatest amount of light, direction and distance are carefully calculated by the designers of microscope for greatest efficiency.

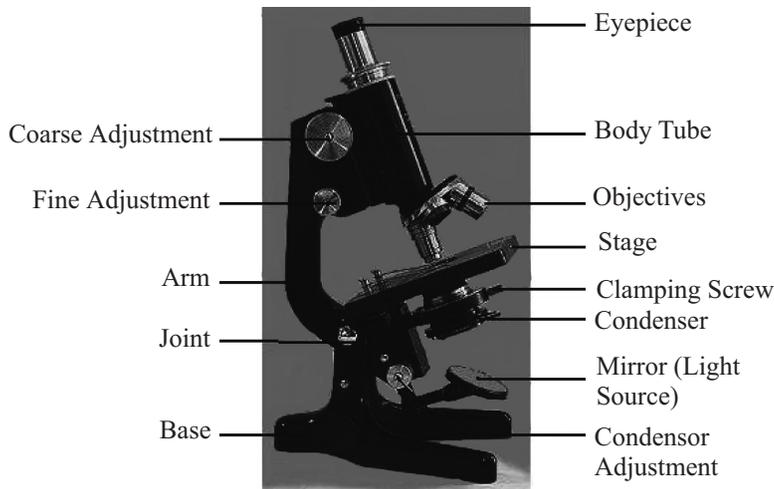


Fig. 2.3: Microscope

### Condensers

The purpose of condenser is to concentrate the light into the plane of the object. The more the light at the specimen, better is its resolution. All condensers have aperture diaphragm with which the diameter of the light beam can be controlled.

### Object stage

It is a rigid platform with an aperture through which the light can pass. It supports the glass slide. It allows controlled movement in two directions.

### Objectives

They are the most important parts of microscope. The main task of objective is to collect the maximum amount of light from the object, unite it and form a high quality magnified real image. Magnifying powers of objectives are from 1:1 to 100:1.

### Body tube

Body tube can be monocular, binocular and the combine photo-binocular (also called trinocular). Binocular tubes have provision for inter-pupillary distance adjustment, enabling each observer to adjust for his eyes.



Notes



**Notes**

**Eyepiece**

The final stage in optical path, the eyepiece’s function is to magnify the image formed by the objective within the body tube, and present the eye with a virtual image.

**Use of the Microscope**

- Illumination should be centered.
- The condenser should be centered and in proper position.
- Objectives should be properly screwed.
- Optical parts should be clean and free from dust.
- Use oil only for oil immersion objective. After use, clean the oil objective with lens tissue. Avoid use of xylene, alcohol or acetone. Eyepieces get dirty by grease from eyelashes, clean them with lens paper.
- When changing slide, always lower the stage before removing the slide or change objective lens to scanner view.
- Make sure the slide is the right way up.



**INTEXT QUESTIONS 2.1**

1. Parallel rays entering a simple lens are brought together to a single point called
 

(a) Focal point	(b) Optical point
(c) Conjugate	(d) All of the above
2. Speed of light on entering a medium
 

(a) Increases	(b) Decreases
(c) Remains constant	(d) Variable
3. Choose the false statement
 

(a) Illumination of microscope should be centered
(b) Objectives should be properly screwed
(c) Use oil only for oil immersion objective
(d) When changing slide, always raise the stage
4. Instruments designed to produce magnified visual images of objects are .....
5. Strength of energy or brightness of light is referred as .....
6. Distance between apex of one wave and the next is .....



### WHAT HAVE YOU LEARNT

- Microscopes are instruments designed to produce magnified visual or photographic images of objects too small to be seen by naked eye
- Light radiates in all directions, with each ray travelling straight till infinity, unless something interferes its path
- Amplitude refers to the strength of energy or brightness of light
- Wavelength is the distance between the apex of the wave to the next is the wavelength and is measured in nanometers
- Media through which light is able to pass, will slow down the speed of light is described as retardation
- Parallel rays entering a simple lens are brought together to a single point called focal point
- Object placed at one end of lens will form a clear image on a screen kept at other side of lens is described as conjugate lens
- Light source, Condensers, Object stage, Objectives, Body tubes and Eyepieces are the components of microscope



### TERMINAL QUESTIONS

1. Define amplitude and wavelength of light with diagram.
2. Define conjugate foci
3. Differentiate between chromatic and spherical aberration
4. Enumerate the different components of light microscope



### ANSWERS TO INTEXT QUESTIONS

#### 2.1

1. (a)
2. (b)
3. (d)
4. Microscope
5. Amplitude
6. Wavelength

