Notes

# 31

# SOME COMMON TOOLS AND TECHNIQUES USED IN BIOLOGY

Long back, the biologists could learn about living things, only from what they could see with the naked eye. New tools and techniques were invented which helped in the study of finer structure of various kinds of organisms and their parts. Microscope not only revealed a world of minute organisms but also minute details of internal structure of organisms. In the course of history of biology, various new tools and techniques have developed, like microscopy, paper chromatography, etc. In this lesson you will learn about some of these.

## OBJECTIVES

After completing this lesson, you will be able to :

- *trace the development of microscopes and their working;*
- *list the parts of a compound microscope;*
- *compare the working principle of compound, electron and phase contrast microscope;*
- *Differentiate between transmission electron microscope and scanning electron microscope;*
- *describe the basic aspects of some other techniques like cytochemistry, autoradiography, paper chromatography, cell fractionation , ultracentrifugation and tissue culture.*

## 31.1 BRIEF HISTORY OF MICROSCOPES

Microscope as the name suggestes are instruments that help to enlarge minute (micro = very small) organisms or their parts. A microscope not only enlarges or magnifies the object but also 'resolves' it, that is makes it possible to differentiate between two points present close together in the objects being viewed.

The first microscope was constructed by Anton Van Leeuwenhoek (1632-1723). This microscope consisted of a **single biconvex lens** fitted in a small window of a "board" and the object was viewed through it. This was a simple microscope. Next stage was that of a very primitive compound microscope in which **two lenses** were used (Fig. 31.1). Improvements continued, newer and newer microscopes were designed and are still being improved.

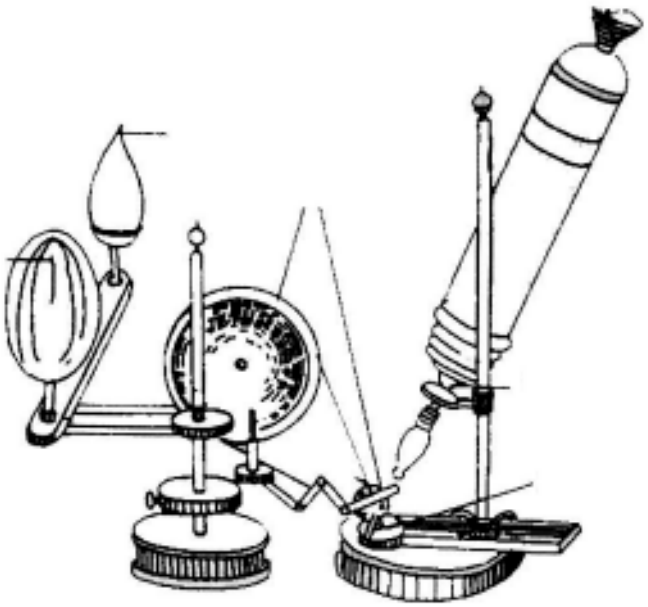


**Fig. 31.1** Crude microscope used by Robert Hooke.

## 31.2 VARIOUS TYPES OF MICROSCOPES

There are different types of microscopes which are used in studying the various structures and activities inside a cell. Some of these are as follows:

1. Simple microscope
2. Compound microscope
3. Phase-Contrast microscope
4. Transmission Electron microscope (TEM)
5. Scanning electron microscope (SEM)

| | | |
|---|---|---|
| **Resolving Power** | : | It is the ability of a microscope to show two closely lying points as two separate points. |
| **Magnification** | : | It is the ratio of the size of the image to that of the object. |

1. **Simple Microscopes :** These are of two types :

   (i) **Hand Lens** : It consists of a biconvex lens, mounted on a handle. The lens is of different sizes and different magnifying powers. It is commonly used to magnify an entire object.

**Fig. 31.2** A Hand Lens

   (ii) **Dissecting Microscope :** It consists of a biconvex lens which is moved up and down by an adjustment screw, to bring the object in sharp focus. The light is focussed with the help of a concave mirror fitted below. A magnified image of the full object can be seen through it.

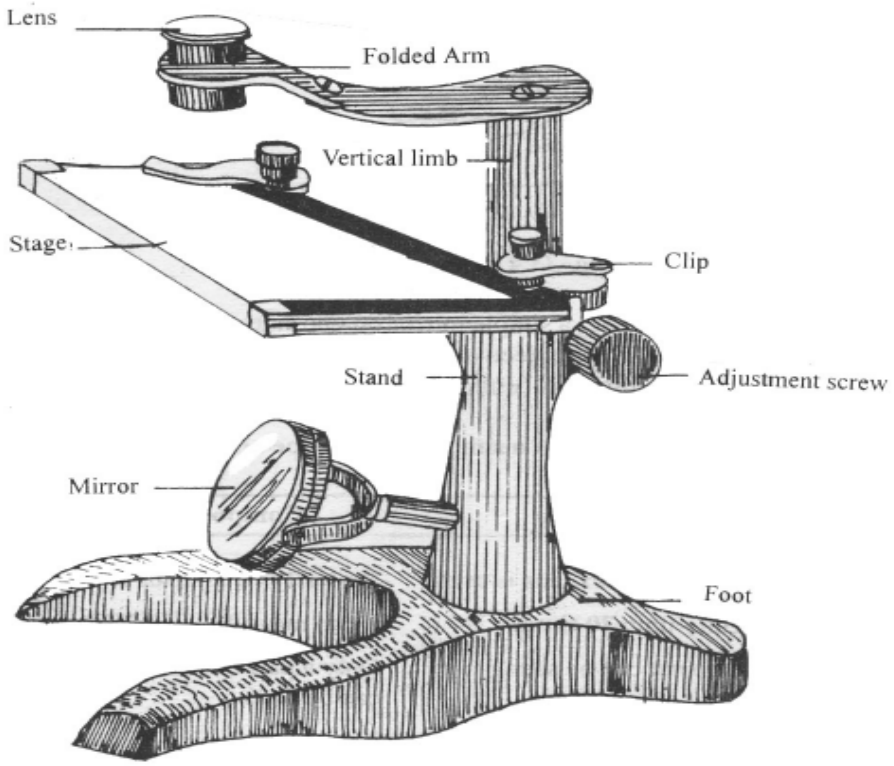



**Fig. 31.3** Dissecting microscope.

2. **Compound Microscopes** : It is commonly used in the laboratories to view extremely minute organisms and parts and sections of larger organisms. Fig. 31.4 shows various parts of a compound microscope and table 31.1 gives the differences between simple and compound microscope.

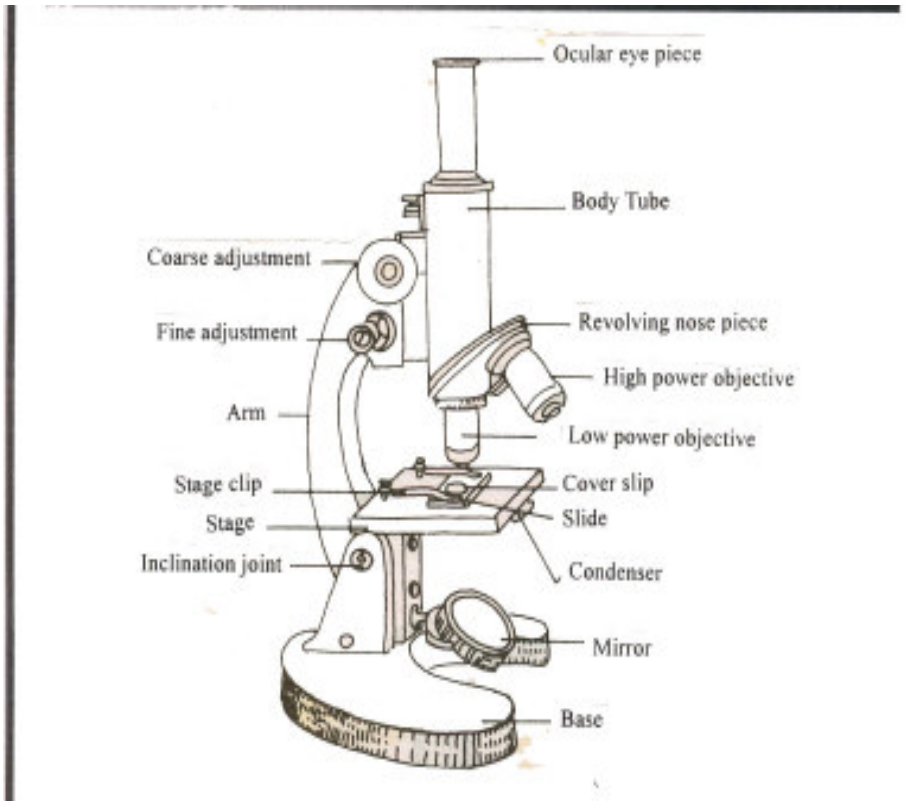



**Fig. 31.4** A compound Microscope

Apart from the lenses, it also has a condenser, having a simple mirror on one side and concave mirror on the other. The object is placed first below the objective lens over the stage. The objective lens forms an image of the object. This image is further magnified by the eye piece.

**Table 31.1 Differences between a Simple Microscope and a Compound Microscope**

| Simple Microscope | Compound Microscope |
|---|---|
| 1. Basically **one** biconvex lens is used. | 1. Basically **two** lenses are used |
| 2. It can magnify upto **20 times**. | 2. It can magnify upto **1500 times** |
| 3. The whole object may be seen. | 3. Only a part of the object or a thin section can be seen. |
| 4. It uses light which is reflected by the mirror and passes through the object or simply which is reflected by the object. | 4. It uses light which is transmitted through the object. |

3. **The Electron Microscope :** The organelles of the cell became known after the electron microscope was invented. As is seen in table 31.2 the magnification and resolution of the electron microscope are much higher than that of the compound microscope. Table 31.3 gives the comparison between transmission electron microscope and scanning electron microscope.

**Table 31.2 Comparison between the working of a Compound Microscope and an Electron Microscope**

| Compound Microscope | Electron Microscope |
|---|---|
| 1. It is operated in **open** condition. | 1. It is operated only in **vacuum** condition. |
| 2. The objective lens is simply **a glass** lens. | 2. The objective lens is **electromagnetic** lens. |
| 3. The source of illumination is **light**. | 3. The source of illumination is an **electron beam** |
| 4. The final image of an object is observed through an eye-piece. | 4. The final image of an object is projected on a flourescent screen. |
| 5. It magnifies the object upto **1500 times**. | 5. It magnifies the object upto **200,000 times**. |
| 6. Resolution power is upto **2500Å**. | 6. Resolution power is upto **2.5Å**. |
| 7. It can be used to see both **living and dead cells**. | 7. It can be used to see only **dead cells**. |

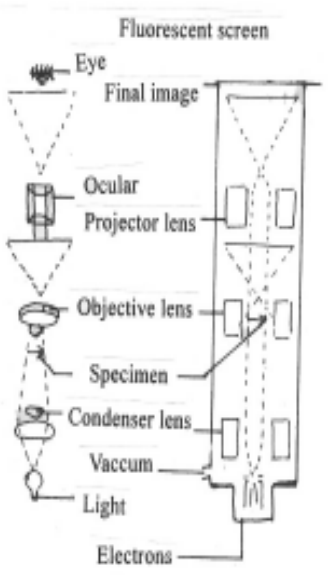



**Fig. 31.5** Showing similarities and differences between the light (compound) and electron microscope

**Table 31.3 Comparison between Transmission Electron Microscope and Scanning Electron Microscope**

| Transmission Electron Microscope | Scanning Electron Microscope |
|---|---|
| 1. Beam of electrons is passed through section of material to produce the image. | 1. Whole specimen is scanned by a beam of electrons. |
| 2. Only ultra thin sections or very small objects can be examined. | 2. Larger specimens can be viewed |
| 3. Resolution very high. | 3. Resolution inferior than that in case of Transmission electron microscope. |

4. **Phase-Contrast microscope :** It has an annular diaphragm located below the condenser an objective having a phase plate. When light is transmitted through lenses, some of its rays pass directly while others are diffracted laterally. The diffracted light rays are thus separated from the direct light and an image of strong contrast is produced. Mainly it is used to :

   (i) examine living cells.

   (ii) observe the nuclear and cytoplasmic changes taking place during mitosis.

   (iii) study phagocytosis and pinocytosis.

   (iv) observe the effect of different chemicals inside the living cells.

5. **Scanning Electron Microscope (SEM)**

In this type of microscope, three dimensional images are developed. It gives more detailed and clear structure of **surface of cells**. It is particularly used for the study of the surface of an object.

## INTEXT QUESTIONS 31.1

1. Name the type of lens used in a simple microscope.

   .....

2. How many times can the image of an object be magnified in a compound microscope?

   .....

3. Mention any two differences between a compound microscope and a simple dissection microscope.

   l. ..... 2. .....

4. What is the source of illumination in an electron microscope?

   .....

## 31.3 SOME OTHER TECHNIQUES

There are other types of tools and techniques that have been developed which helped in the progress of biology as a subject. Some of them are given below :

1. **Cytochemical Methods :** These methods are used to locate specific chemical constituents within the cells by differentiating a particular part from other parts by colouring them with a specific stain or dye. It is done either by the use of certain dyes or by using the substrates of enzymes e.g. Schiff's reagent used in Feulgen staining, is used to localize the presence of DNA in a cell.
2. **Autoradiography :** This technique is used for study the steps and location of synthesis of molecules and to trace metabolic events in the cells. The radio-labelled compounds are injected into the organism. Then various tissues are investigated to find out where the radioactivity is located. This is done by using

photosensitive film of silver bromide. Whenever in the cell or tissue or the organism, the radio labelled substance is present, silver gets reduced by radiation and is seen as black patches in the **autoradiographs**.

3. **Paper Chromatography :** In this method the chemical substances present in a mixture can be separated. A drop of the mixture is put on one end of a long strip of the Whatman filter paper. The filter paper is hung in a manner that the end with the drop of the mixture dips into the solvent mixture kept in the tray/jar. As the liquid is drawn up on the paper, different substances in the mixture begin to separate aceording to their molecular weight, size and solubility in the solvent and rise up to different heights on the paper. It is then analysed by using certain chemicals for further investigation.

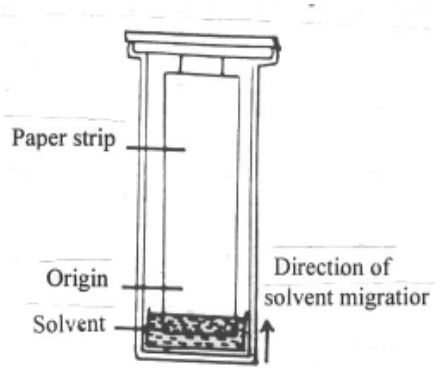



**Fig. 31.6** Paper Chromatography

4. **Cell fractionation :** By this method different organelles of cells such as nucleus, mitochondria, ribosomes etc. having different particle size and weight are separated by rotating them in a centrifuge at different speeds.

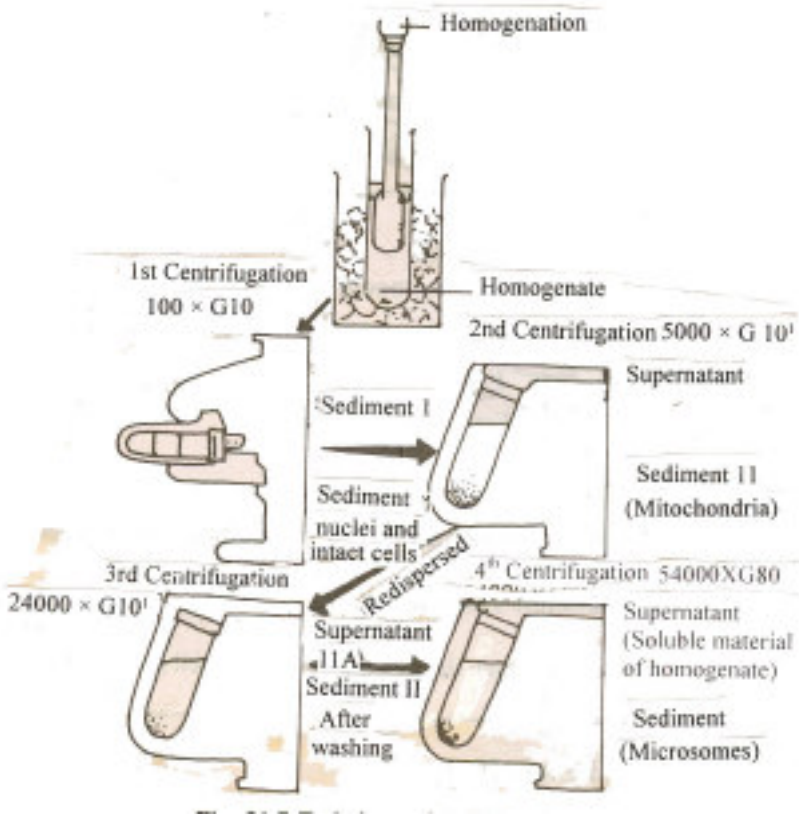



**Fig. 31.7** Technique of Cell Fractionation

The cells are first homogenised or broken down by a special method. The homogenate (crushed cells) is then put into tubes and tubes are placed in a centrifuge. The centrifuge is rotated at a high speed. By doing so under the influence of centrifugal force, organelles separate according to their particle density and sizes. The lighter particles settle at the top and the heaviest particles settle at the bottom. The layers are then studied separately and the structure in details gets to be known.

5. **Ultracentrifugation :** By rotation at a high speed, particles/organelles of different sizes and shape separate, according to their density. Since the rotation is at very high speed, friction with air produces heat, so has to run under refrigeration and vacuum. Nucleus, mitochondria etc. separate out at different speeds.

6. **Tissue Culture :** This technique involves growing living cells outside the organism by providing all necessary conditions for their survival and growth. The cells from an organism are grown in the laboratory on a nutritive medium at a suitable temperature. Using this technique it has been possible to develop a whole organism from a single cell. Some new fully grown plants have been developed in this way. (Fig. 31.8).

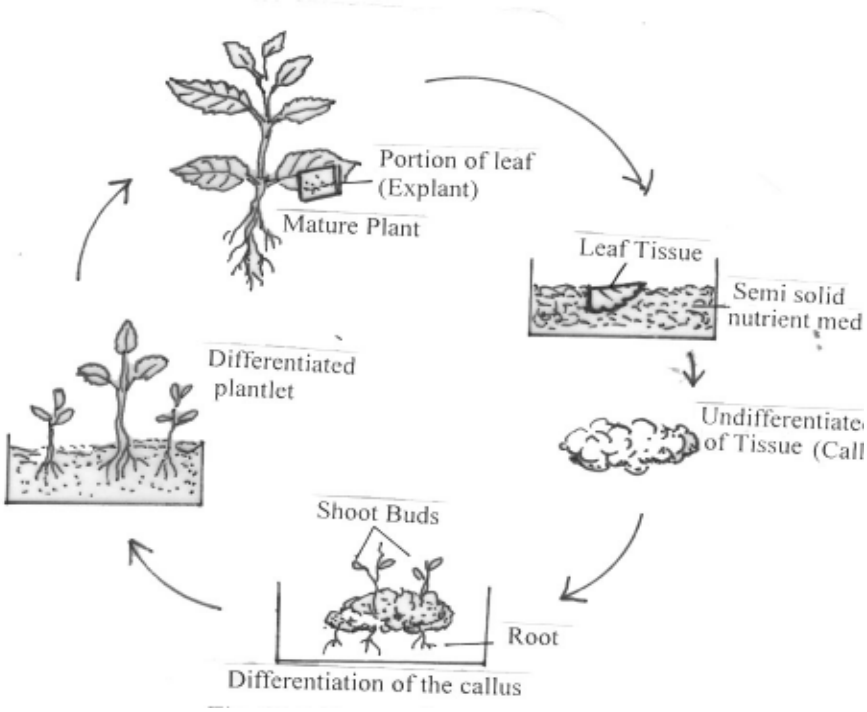
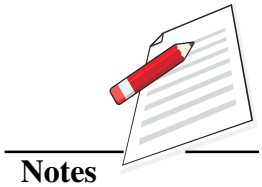



**Fig. 31.8** Tissue culture.

The steps in tissue culture are given in Fig. 31.8. Tissue is removed from the plant body and grown in a nutrient medium. The cells divide to form an undifferentiated mass of cells called **callus** which then differentiates into a plant. In the diagram leaf tissue culture has been shown but tissue from any





part of the plant has the ability to follow the similar path as shown in the Fig. 31.8, and produce an entire plant. The tissue taken from the plant is called an explants. It is now possible to culture a single cell into a whole plant.

## INTEXT QUESTIONS 31.2

1. What special type of substances are injected in an organism for autoradiography?

   .....

2. In which technique is Schiff's reagent used?

   .....

3. Name the technique by which the organelles from a cell can be separated.

   .....

## WHAT YOU HAVE LEARNT

- Biologists depend heavily on a number of tools and techniques for studying organisms.
- Microscopes, such as the simple (dissection) microscope, compound microscope and the electron microscope are used to study organisms.
- Compound microscope uses light and can give magnification up to about 1500 times whereas the electron microscope uses electron beam and magnifies the image upto 2,00,000 times.
- Phase contrast microscope is chiefly used for observing activities inside the living cells.
- Scanning electron microscope is used for introducing three-dimensional images chiefly of the surfaces.
- Cytochemical methods, autoradiography, centrifugation are helpful in studying cell chemistry, synthesis of substances inside the living organism and isolation of cell organelles respectively.
- Paper chromatography is used for separating chemical substances in a mixture.
- Tissue culture involves growing of cells and tissues outside the body of the organism.

Notes

## TERMINAL QUESTIONS

1. Name the scientist who constructed the first microscope?
2. Mention three differences between a compound microscope and an electron microscope.
3. Define the term ultracentrifugation.
4. Name the microscope used in the study of a living cell and instrument used in separating cell organelles.
5. List the main points of the technique of autoradiography.
6. Give uses of cytochemical methods and centrifugation.
7. Mention the importance of tissue culture.

## ANSWER TO INTEXT QUESTIONS

**31.1** 1. Biconvex lens
2. upto 1500 times
3. any two points given in the table 38.1
4. Electrons

**31.2** 1. Radiolabelled
2. Cytochemical methods
3. Ultracentrifugation