



Unit 8

INSTRUMENTS IN MICROBIOLOGY

Structure

8.1 Introduction

Expected Learning Outcomes

8.2 Principle, Construction and Application of Laminar air Flow

8.3 Principle, Construction and Application of Bacteriological Incubator

8.4 Principle, Construction and Application of Shaker

8.5 Principle and Construction and Application of Centrifuge

8.6 Principle, Construction and Application of Spectrophotometer

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Bright Field and Dark Field Microscopy

Phase Contrast Microscopy

Fluorescence Microscopy

Electron Microscope

Comparison of Microscopes with their Magnification and Resolution.

8.8 Summary

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8.10 Answer

You are expected to be familiar with the use of common apparatus such as beaker, Petri dish, test tubes, culture tubes, watch glass, conical flask, distillation flask, Bunsen burner, measuring cylinder, jars, cover slides, glass slides, inoculation loops, Coplin jars and staining trough etc. in undergraduate microbiology lab.

8.1 INTRODUCTION

In the previous unit 6, you learned about staining techniques used for identifying bacteria. You will have used some kind of apparatus and equipment for performing the microbial techniques such as aseptic procedures, culturing techniques, counting of bacteria. This unit deals with the theoretical knowledge of common lab instruments used in microbiology. The study of microbes depends on the common instruments like microscope, centrifugation and spectrophotometer etc. When you work in a lab, you must follow laboratory safety rules and know the name, working principles and use of some common

lab apparatus and equipment. The common instruments such as laminar air flow, incubators, shakers, centrifuge, spectrophotometers and microscopes etc. are crucial for experimental work of microbiology. Laminar air flow device provides a sterilized environment for inoculation of microbes under control condition and incubators give ideal temperature for microbial growth in artificial medium. Shakers are used to mix microbial culture in a broth medium. Centrifuge is used for the separation of bacterial suspension from liquid culture; spectrophotometer is used for quantitative estimation of bacterial growth in culture while microscope is an essential tool for morphological observation and identification of different kinds of microbes and monitors the bacterial growth in culture media. Without the basic knowledge of lab equipment, you cannot perform the experimental work properly in the lab. Hence, understanding the use of these instruments is crucial in the Microbiology.

Therefore, in this unit you are going to learn the principle, instrumentation and application of laminar air flow, shakers, incubators, centrifuge, spectrophotometers and microscopes.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ describe the principle, basic parts, and application of lab instruments:
 - laminar Air flow
 - incubator
 - shaker
 - centrifuge
 - spectrophotometer
 - microscope
- ❖ compare the different types of microscope;
 - explain the magnification and resolving power of microscope
 - list the lab equipment used in microbiology lab.

8.2 PRINCIPLE, CONSTRUCTION AND APPLICATION OF LAMINAR AIRFLOW

Laminar airflow is one of the essential equipment in microbiology. It is utilised for inoculation of microbes in culture flask. Hence laminar airflow is also known as inoculation chamber. The laminar airflow provides the sterile environment for bacterial inoculation and it also protects the cell culture from pathogenic contaminants. However, sterile working environment is one of the essential requirement in microbial research laboratories.

Principle:

As its name, the laminar airflow (LAF) system works by generating a one-directional airflow to produce a contaminants free air drawn through the high-efficient filter known as HEPA filters which effectively eliminate airborne contaminants.

Construction: The basic components of LAF are given in Table 8.1.

Table 8.1: Basic parts of LAF

| Parts | Description | Use |
|-------------------------------|---|---|
| Cabinet | It is important part of LAF which have air filtration system. It is a glass shield which can either fully open or have hand openings for user access. | Insulates the inner sterile environment. |
| Working station | A flat, stainless steel surface where operations like culturing take place. | A platform to perform involution of microbes. |
| Filter Pad/Pre-filter: | Located at the top of the cabinet. | Prevent dust and some microbes from entering the working environment. |
| Fan/Blower: | Positioned below the filter pad. | Sucks in air, circulates it, and directs it towards the HEPA filter to trap remaining microbes. |
| UV Lamp: | UV germicidal lamp which should be turned on 15 minutes before operation to avoid user exposure to UV light. | to sterilize the interior (working station) before use |
| Lighting Lamp: | A LED to generate light | Provides proper lighting inside the cabinet during operations. |
| HEPA Filter: | After pre-filtration, the air passes through HEPA filter and making the interior conditions ideal for contamination-free environment. | Ensures a sterile environment by trapping fungi, bacteria, and dust particles. |

Types: Generally, there are two types of laminar airflow:

1. Vertical laminar airflow cabinet and 2. Horizontal airflow cabinet

Fig. 8.1 shows the laminar air flow cabinet. In a horizontal airflow cabinet, the position of the airflow is horizontal. Air enters the system from the backside of the HEPA filter, and the pure air (free from contaminants) is forced in a back-to-front direction across the workspace. Conversely, in a vertical airflow cabinet, the airflow is vertical. Air is directed downward from the top of the cabinet onto the working area. This configuration ensures that contaminants are filtered out before the air reaches the work surface, providing a sterile environment for handling sensitive biological materials.

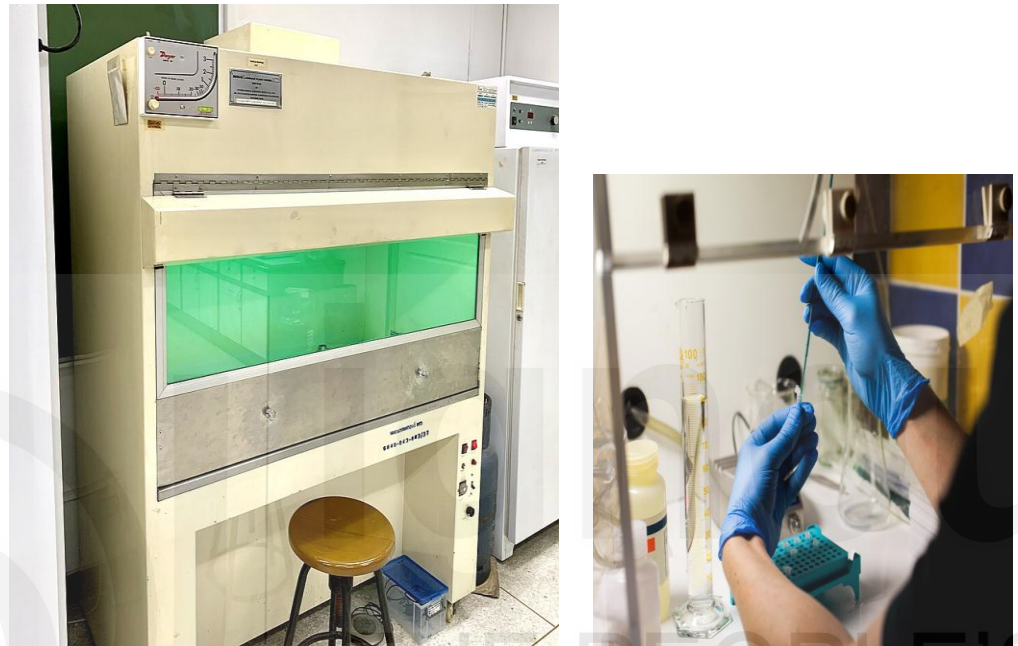


Fig. 8.1: A) Laminar air flow cabinet b) Working for inoculation of bacterial sample using the laminar flow.

[Image credit a)

https://commons.wikimedia.org/wiki/File:Laminar_flow_cabinet_Microbiology_Department.jpg , b) image credit [Karolina Fok](#) licensed under the Creative Commons Attribution 4.0 International license].

Application of LAF

- Performing aseptic techniques in microbiology and cell culture.
- Inoculation of bacterial sample in growth media
- Safeguarding biological samples against contamination during working with microbial sample.

SAQ 1

- Inoculation chamber is known as
- LAF is utilised for inoculation ofin culture media.
- The laminar airflow provides theenvironment and protects cell culture fromcontaminants.

- iv) The Laminar airflow (LAF) system works by generating a one-directional airflow to produce a contaminants-free air drawn through the high-efficient filter known as filters.
- v) In a horizontal airflow cabinet, the airflow undergoes in a back-to-front direction across the workspace while in a vertical airflow cabinet, airflow is directed the working area.

8.3 PRINCIPLE, CONSTRUCTION AND APPLICATION OF BACTERIOLOGICAL INCUBATOR

An incubator is a tool used to maintain and preserve cell or microbiological cultures by offering ideal temperature conditions in a controlled condition. The idea behind an incubator is to maintain the natural environments needed for the growth and development of microorganisms or cells by keeping the temperature, humidity, and gas levels constant. It is essential device for experimental work of *in vitro* laboratory work such as animal cell culture and bacterial cultures. The CO₂ Incubators are extensively utilized for cultivating cells and microorganisms that require CO₂.

Basic incubators are capable of maintaining temperatures between 60 to 65 °C (140 to 149 °F), with a few standard models can rise slightly higher temperatures (often not exceeding 100 °C). The most ideal temperature is about 37 °C employed for cultivating bacteria (i.e. E. Coli) as well as mammalian cells. These organisms can grow under this artificial conditions.

Construction of incubator:

The lab incubator comprises of the following components (Fig.8.2):

Cabinet:

- The incubator consists of insulated double walled cabinet and a secure door with a latch.
- The chamber is normally filled with water to help regulate the temperature, and there is an opening in the middle of the roof where a thermometer can be inserted to take the chamber's temperature.

Perforated shelves

- The interior of the incubator is equipped with one or many shelves designed to accommodate the samples and cultures during incubation.

Heating Unit and control knob:

- **The incubator's** base contains an electrically heated unit that supplies the required warmth.
- There is control knob located on one side of the base on which user can **on or off the** instrument and set the required temperature.

Thermostat

- Incubator is equipped with the thermostat at the back of the incubator controls to regulate the desired temperature.
- A status indicator bulb, located at the front near the control knob, shows whether the incubator is on or off.

Thermometer

- An external thermometer is positioned in L shaped on the upper section of the incubator's outside wall.
- The temperature reading is displayed on the outside of the incubator for convenient temperature reading in a control panel unit.



Fig. 8.2: a) Basic parts of incubator and b) inside view of incubator chamber.
(Image course: Public domain).

Application of incubator

- Cultivating bacterial and fungal cultures in microbiology.
- Sustaining mammalian cells for scientific investigation and manufacturing purposes.
Biochemical estimation of enzyme and proteins
- Egg Incubation: Creating ideal conditions for the hatching of eggs in the practice of chicken husbandry.
- Incubators are also useful to embed in paraffin wax (at 50°C -55°C) for making histological slides.
- CO₂ Incubators are used for cultivating of cells in growth medium.

SAQ 2

Fill in the Blanks:

- i) An incubator is a tool used to maintain and preserve or cultures by providing optimal temperature conditions in a controlled environment.
- ii) An incubator is designed to maintain the constant level of.....,, and to create a ideal condition for the growth and development of microorganisms or cells
- iii) Incubators are extensively utilized for cultivating cells and microorganisms that require
- iv) Basic incubators can maintain temperatures between to °C, with some models reaching slightly higher temperatures.
- v) The most ideal temperature for cultivating bacteria and mammalian cells is about °C.

8.4 PRINCIPLE, CONSTRUCTION AND APPLICATION OF SHAKERS

Laboratory shakers are small instrument in the field of microbiology, extensively utilized to consistent blending, mixing, and stirring of biological samples in a tube or flask. Their major purpose is to establish and uphold a regulated environment to facilitate the growth and cultivation of microorganisms by ensuring a uniform and continuous motion and blending.

A shaker contains an electric motor with the drive shaft oriented vertically and attached to a cupped rubber piece mounted slightly off-center.

Rotary shakers:

Rotary shakers are well-suited for shaking microbiological cultures (Fig. 8.3). The device rotates sample containers, such as flasks and tubes, in a circular motion at an ideal speed. a rotating oscillation. Cultivating microbes in beakers, test tubes, or Erlenmeyer flasks is quite beneficial.



Fig. 8.3: Rotary shakers

Image source

https://commons.wikimedia.org/wiki/File:Laboratory_microbiological_shaker_with_cultures-01.jpg

Application: Vortex mixer is ideal mixture device used in biochemical analysis. They are highly advantageous for the purpose of suspending cells in cell culture. Magnetic stirrers are common used in experimental work of molecular laboratories to blend chemicals for tests or to mix experimental samples with diluents.

SAQ3

- i) What is the primary use of laboratory shakers in microbiology?
 - ii) How do laboratory shakers contribute to the growth and cultivation of microorganisms?
-

8.5 PRINCIPLE, CONSTRUCTION AND APPLICATION OF CENTRIFUGE

Centrifuge is common lab equipment in biochemistry and microbiology labs (Fig. 8.4). You might see a bench top centrifuge in your biology/biochemistry lab. The sole purpose of a centrifuge is to generate the centrifugal force. It is primarily used for centrifugation techniques. Centrifugation is a separation technique employed to separate particles in liquid mixture based on their density, mass and shape in an applied centrifugal force. This action leads to the sedimentation of heavier particles at the lower part of the tubes, while lighter substances ascend to the upper part of test tube.

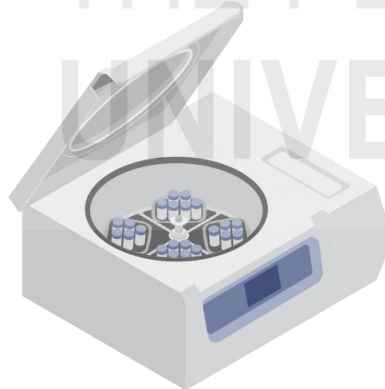


Fig. 8.4: The centrifuge.

[Image credit: https://commons.wikimedia.org/wiki/File:202301_Table-top_Centrifuge.svg]

Principle

Centrifugation is primarily based on the principle of sedimentation. Sedimentation is the phenomenon in which solid particles suspended in a liquid settle down over time to the lower section of a container under the influence of gravity. Nevertheless, gravity alone lacks the necessary strength to rapidly separate biological mixtures. Centrifugation employs centrifugal

force to accelerate sedimentation. Centrifugation involves subjecting particles to high-speed rotation, which generates an outward force that accelerates their settling process beyond what would occur under regular gravity.

The sedimentation rate of a particle depends on the

- Applied centrifugal force
- size, mass and density of particles
- Density and viscosity of medium and
- Frictional force of medium

The rate of centrifugation is expressed in revolutions per minute (RPM) and is specified by the angular velocity (ω). The relationship between RPM and angular velocity is given by:

$$\text{One revolution /minute (rpm)} = \frac{\omega \times 60}{2\pi}$$

$$\omega = \frac{2\pi \times \text{rpm}}{60}$$

Where, ω is the angular velocity in radians per second. One revolution of a particle is equal to 2π radians and the radial distance (r) of the particle from the axis of rotation (measured in centimetres)

Therefore, the centrifugal force (G) acting on particles during centrifugation can be derived as:

$$G = m \cdot \omega^2 \cdot r$$

Where:

- G is the centrifugal force,
- M is the mass of sedimenting particles,
- ω is the angular velocity in radians per second,
- r is the radius of rotation (the distance of migrating particles from the central axis of rotation).

The applied force on the sedimenting particle increases with the velocity of rotation and the r (distance from the particles from central axis of rotation).

In centrifugation, the more common measurement of centrifugal force G is expressed as RCF (relative centrifugal force)

$$\text{RCF} = (1.12 \times 10^{-5}) \cdot (\text{rpm})^2 \times r$$

Where 1.12×10^{-5} is an empirical factor,

r = radius of rotor from the center of rotation.

rpm= revolutions per minutes

Hence, RCF as defined in Eqn (vii), depends on the rpm and the radius of rotation and it is generally given in **units of gravity** or **× g**.

Construction- Parts of centrifuge

Centrifugation is carried out using a centrifuge. A centrifuge consists of three essential components (Fig. 8.5):

Rotor: It is the main rotating unit in centrifuge that holds and rotates the samples at high speeds. There are three types of rotors used in centrifuge: Vertical rotors, swing bucket rotor and fixed angle rotor. In vertical rotor, the sample tube is in upright position. In swing rotor, the sample tube moves in perpendicular direction during centrifugation. The sample tube is fixed at angle of 14°-40° to the axis of rotation in fixed angle rotor which is useful for differential pelleting.

Drive shaft: It connects the motor and rotor, allowing it to spin.

Motor: It gives necessary powers that forcing the rotor to spin and produce centrifugal force

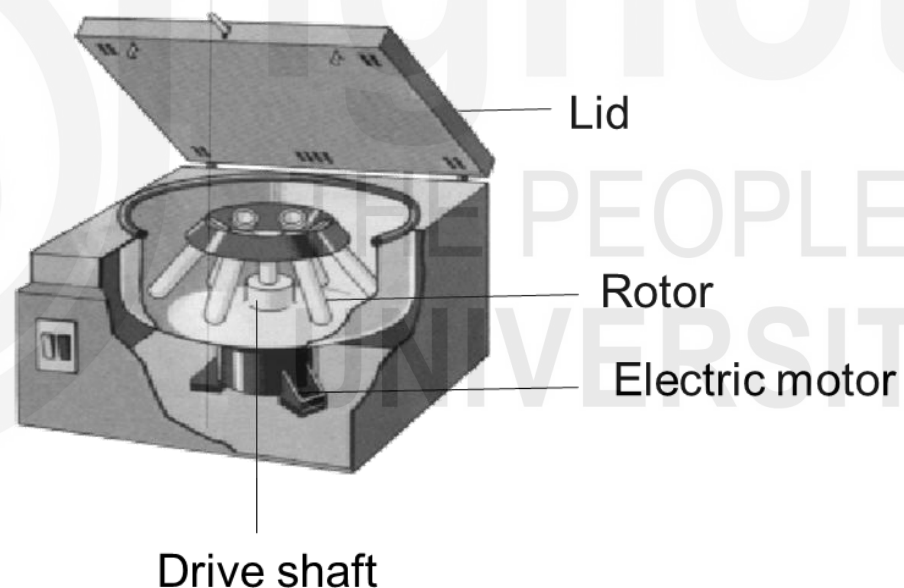


Fig. 8.5: Basic components of centrifuge.

Types of centrifuge

Based on the rotor speed, centrifuge is classified into three categories:

- Low speed or bench top centrifuge can produce maximum speed 4000-5000rpm
- High speed centrifuge can produce 5000-10,000 rpm
- Ultracentrifuge have maximum operating speed 1,50,000 rpm.

Applications of Centrifugation

The centrifuge is widely equipment in biochemistry, biotechnology and clinical laboratories. The main purpose of centrifuge is to isolate and separation microbial cell suspension from immiscible liquids in microbiology. However, there are many applications in biological science, including:

- Separation of subcellular organelles
- Isolation of protein, RNA, and DNA
- Separation of serum from blood sample
- Separation of bacterial suspension and viral particles
- Determination of sedimentation coefficient of cellular components:
- Separation of protein-bound or antibody-bound ligands from free ligands.
- Separation of lipid components like chylomicrons from other plasma components.
- Determines the purity and shape of biomolecules

SAQ 4

- i) The primary purpose of a centrifuge is
 - a) To generate heat
 - b) To create centrifugal force
 - c) To produce light
 - d) To measure pH
- ii) The principle is centrifugation based on
 - a) Evaporation
 - b) Sedimentation
 - c) Filtration
 - d) Absorption
- iii) The rate of centrifugation is expressed in:
 - a) Miles per hour (mph)
 - b) Revolutions per minute (RPM)
 - c) Meters per second (m/s)
 - d) Joules per second (J/s)

- iv) The centrifuge consists of
 - a) Rotor, Drive shaft, Motor
 - b) Filter, Heater, Pump
 - c) Light source, Lens, Slide
 - d) Beaker, Thermometer, Stirrer
- v) What is the maximum operating speed of an ultracentrifuge?
 - a) 5000 RPM
 - b) 10,000 RPM
 - c) 1,50,000 RPM
 - d) 50,000 RPM
- vi) Which type of rotor holds the sample tube at an angle of 14° - 40° to the axis of rotation?
 - a) Vertical rotor
 - b) Swing bucket rotor
 - c) Fixed angle rotor
 - d) Horizontal rotor
- vii) What does RCF stand for in the context of centrifugation?
 - a) Relative Centrifugal Force
 - b) Rotational Centrifugal Frequency
 - c) Rapid Centrifugation Factor
 - d) Rotational Centrifugal Field

8.6 PRINCIPLE, CONSTRUCTION AND APPLICATION OF SPECTROPHOTOMETER (UV/VIS)

Spectrophotometers are analytical devices utilized for the quantitative determination of biological molecules as well as the bacterial suspension. You are advised to recall the Unit 4 spectrophotometry in the course MBC-003 (Bioanalytical Techniques) to know more about the spectrophotometry techniques. The underlying principle of spectrophotometry is based on the Beer-Lambert Law, which establishes a relationship between the absorption of light and the concentration of the solute molecules in liquid solution.

Principle

Beer-Lambert Law states that the amount of light absorbed (A) is proportional to the concentration (c) of the absorbing substance and to the path-length, l of the sample.

Therefore,

$$A = \epsilon \cdot c \cdot l$$

Where,

A = absorbance,

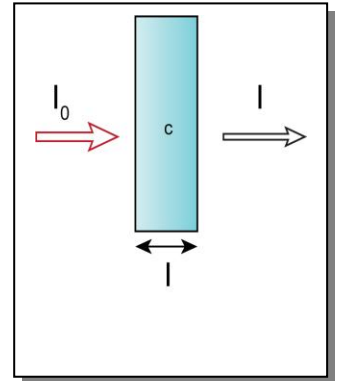
ϵ = Molar extinction coefficient (L/mol·cm)

c = molar concentration (M),

l = optical path length of the sample (cm)

Therefore, the spectrophotometer determines the absorbance (A) by calculating the ratio of the transmitted light (I) to the original light intensity (I_0). The equation $A = \log(I_0/I)$ represents the logarithm of the ratio between I_0 and I .

$$A = \log \left[\frac{I_0}{I} \right] = \epsilon \cdot c \cdot l$$



Construction of spectrophotometer

Spectrophotometers are fitted with deuterium or hydrogen lamps to emit ultraviolet (UV) light in the range of 200-400 nm, and tungsten lamp is used for producing the visible light in spectrophotometer. Visible spectrometer is generally called colorimeter which is used for qualitative determination of substance. The modern spectrophotometers are equipped with both UV-Vis light sources (Fig.8.6). This enables them to operate in both the ultraviolet and visible spectrums. A monochromator is used to convert polychromatic radiation into a single wavelength with a very narrow bandwidth before it reaches to the sample. A monochromator is a prism that associated with the rotating metal grid known as a grating which selectively determine specific wavelength of light. It is situated between the source of light and the sample corvette.



Fig. 8.6: UV-Vis spectrophotometer.

The basic part of spectrophotometer is given in Table 8.2.

Table 8.2: Components of Uv-Vis Spectrometers

| Components | Ultraviolet Spectrometer | Visible Spectrometer |
|---------------------------------|---|---------------------------------------|
| Light Source | Hydrogen or deuterium lamp | Tungsten filament lamp |
| Electromagnetic spectrum | 200-400nm (UV light) | 400-700nm (Visible light) |
| Optical System | Prism or different grating serves monochromator | Glass filters or interference filters |
| Cuvette (Sample holder) | Quartz or fused silica | Glass |
| Detectors | Photomultiplier | Photovoltaic cell |

Applications:

- Quantitative estimation of substance in solution, such as proteins, nucleic acids, and other biomolecules and phytochemicals etc.
- Study of enzymatic reactions.
- Assessment of purity of pharmaceutical products.
- Widely used in clinical biochemistry and microbiology.
- Analyzing and examining the characteristics of various substances and materials.

SAQ5**Tick the true or false:**

- Spectrophotometers quantify light absorption by specimens at various wavelengths. []
- The Beer-Lambert Law relates absorption of light to solute concentration and path length. []
- Spectrophotometers calculate absorbance (A) as the logarithm of the ratio of transmitted light (I) to initial intensity (I_0). []
- Ultraviolet spectrometers typically operate in the range of 200-700 nm, not just up to 400 nm. []

8.7 PRINCIPLE, CONSTRUCTION AND APPLICATION OF MICROSCOPE

The study of microorganism is only possible with the use of microscope. Hence, microscope is an indispensable tool in the study of cellular structure and microorganisms. There are various kind microscope is utilized in the study of microorganisms. However, there are two types on microscope based on the utilization of source illumination.

1. Optical microscope or Light microscope. It used light to view the microscopic things.
2. Electron microscope. It uses a beam of electron to study of microorganisms and cell structure at finer detail.

In this section we will first study the different types of light microscope and then the study of electron microscope

Light microscope

Light microscope uses the series of lens to magnify the final details of a object with good resolution. It can produce 1500X magnification.

The advanced light microscope can also produce 2000X magnification.

Principle

The primary principle underlying in a microscope is the use of lenses to provide better magnification and resolution, allowing for finer examination of small structures of an object. When light interacts with the specimen, it can be absorbed, transmitted or refracted by a specimen. Refraction-bending of light is significant feature of light which key factor to produce clear and magnified image in microscopy.

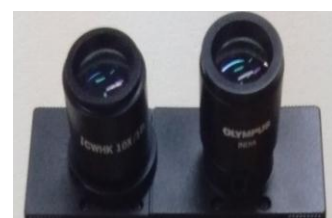
The magnification and resolving power are important to define the working principle of a microscope.

Magnification is a unique feature of optical lens that shows how well the microscope can enlarge and magnify an object, giving the user a clearer picture of the object. Basically, it's the ratio of how big an object looks in a microscope picture to how big it really is. Compounds microscope has two or three objective lens of different magnification with single eye piece (10X).

It is generally denoted by English alphabet "M". You can easily calculate magnification (X) of a microscope by multiplying the objective lens and eyepiece lens magnification. For instance, when you combine a 40X objective lens with a 10X ocular lens, the resulting magnification is calculated as $(40 \times 10) X = 100X$. But higher magnification doesn't mean the higher resolution.

Apart from the magnification, the resolving power is more important in microscopy.

Note: To know more about the microscopy, study the **unit 6 Optical Microscopy** and **unit 7 Electron Microscopy** of the course Bioanalytical Techniques (MBC-003), MSCBCH



Eyeiece (10X)

Resolving power is the resolution ability of an objective lens to clearly distinguish between two closely positioned objects that being seen through a microscope. The Fig. 8.6 shows three images of two closely points in it. Only the b image labeled “b” displays clearly resolved points compared to (a) and (c) images. The resolving power determines the degree of clarity in discerning between two closely spaced spots at a given magnification level. Hence, the resolving power of an optical system determines the level of clarity in distinguishing between two closely spaced spots at a specific magnification level.

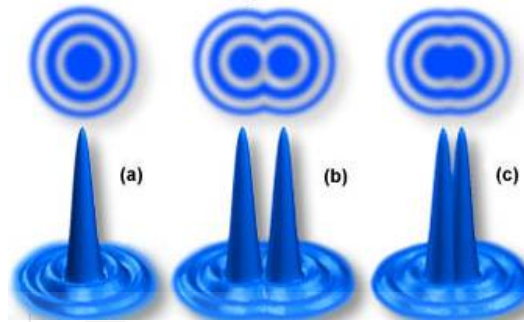


Fig. 8.6: a) No clear resolution between two points b) clearly resolved two points C) little resolution under the optical microscope. Resolution is to see clearly and separate two point within the specimen.

[Credit: Adapted from <http://microscopy.fsu.edu/primer/anatomy/numaperture.html>]

The resolving power of a microscope is based on the wavelength of the light and the numerical aperture of lens system. Shorter wavelength gives higher resolution than the higher wavelength used in a microscope. The numerical aperture indicates the lens's light-gathering and detail-resolving capabilities. The limit of the resolving power is denoted by “d”

Therefore, the **resolving power** of a microscope can be calculated by the given.

$$d = \frac{0.61\lambda}{NA}$$

Where, d is the minimum distance between two points that can be resolved, λ is the wavelength of light (0.61 constant λ), and NA is the numerical aperture ($n \sin \theta$). The limit of resolving power of the optical microscope is $0.2\mu\text{m}$ (200nm).

Let us know the basic parts of the optical or light microscope.

Construction of Light Microscope

The main components of a light microscope are the light source, condenser lens, optical lens and eye piece (ocular lens). The image of biological specimen is formed by the optical lens and ocular lens. Look at Fig 8.7 which

Numerical Aperture is a measure of the resolving power of a microscope objective. It is equal to the product of the refractive index of the medium (n) in front of the objective and the sine of the angle ($\sin \theta$) between the outermost ray entering the objective and the optical axis. Therefore, NA is an estimate of how much light from the sample is collected by the objective lens.

the basic parts of the optical microscope. The description of microscopic components is given in Table 8.3.

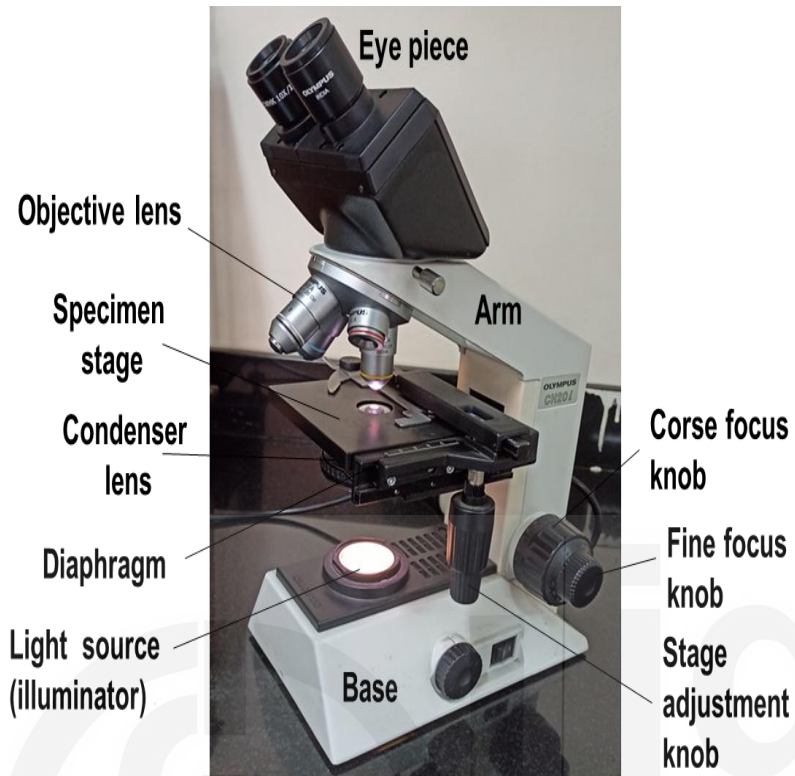


Fig. 8.7: Basic parts of light microscope.

Table 8.3: Components of light microscope

| Parts of Microscope | Description |
|--------------------------------|--|
| Light source | It can be sunlight or external source (bulb) to provide visible light |
| Condenser lens | Located just below the specimen stage and focus the light on the specimen from the light source. |
| Specimen stage | A plate form where a sample slides is placed. A pair of clips attached to the center stage holds the slide on the stage and can be moved horizontally and vertically using the adjustment knob |
| Objective Lenses: | Primary source of magnification and resolution. There are multiple objective lenses (usually 4x, 10x, 40x, and 100x) are fixed on a rotating nosepiece. |
| Ocular Lens (Eyepiece): | Located upper side of microscope and near to viewer. The eyepiece, often with a magnification of 10x and enhances the magnification of the image produced by the objective lens. |

| | |
|-----------------------|--|
| Focusing knobs | The coarse and fine adjustment knobs are utilized to achieve focus on the specimen by displacing either the stage or the objective lenses. |
|-----------------------|--|

Now let us learn the types of microscopes used in microbiology.

8.7.1 Bright Field Microscopy

Bright field microscopy is often suitable for observing stained biological samples. The important feature of this microscope is that forms a dark image against a brighter background. When transmitted light enters the objective lens and is magnified for observation and then an image is formed by diffracted light interfering with diffracted light from the specimen (Fig. 8.8).

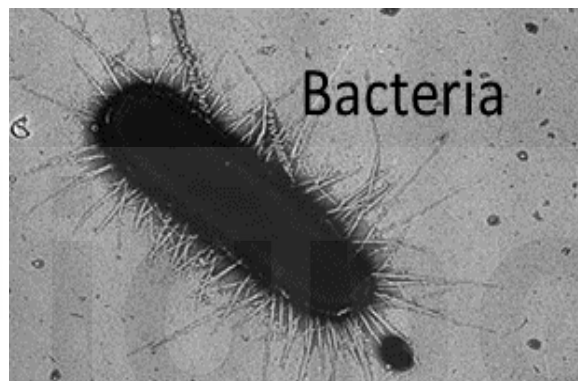


Fig. 8.8: Bright-field image of bacteria.

8.7.2 Dark-Field Microscopy

Darkfield microscopy is popular technique used to observe transparent or translucent specimens that are difficult to visualize by brightfield microscope. Therefore, it is used in viewing unstained specimens.

In this microscope, a condenser lens (patch stop) is equipped with a dark field stop to block the light. The scattered light from the specimen enters the objective lens, producing a bright image of the specimen against a dark background. It is widely used microscopic observation of various microorganisms and other animal cells. It is valuable microscope for studying the movement and structure of living bacteria and other microorganisms (Fig.8.9)

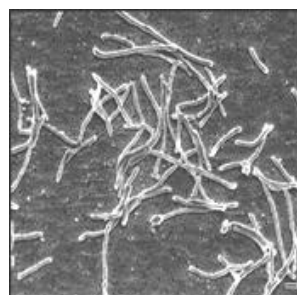


Fig. 8.9: A bacterial micrograph in the dark field microscope.

8.7.3 Phase contrast Microscopy

Phase Contrast Microscopy (PCM) is a specific type of light microscopy. Basically, it uses to generate contrast images of transparent samples. This technique is also known as a contrast-enhancing optical microscopy. It is used to view those microorganisms that cannot stain.

Unlike simple compound microscope, the phase contrast microscope (PCM) has a specialised condenser equipped with a circular-shaped annular ring and the phase plate. The annular ring facilitates the passage of a circular beam of light via the condenser, directing the light onto the specimen and a circular phase plate is located in the focal plan of the objective lens.

The working principle of Phase Contrast Microscopy (PCM) is based on the detection of slight changes in the refractive index of light. The object in case of bacterial specimen have different kind medium such as cytoplasm and chromosomes, bio molecules etc. When light rays pass through the different these medium within specimen, they will be slightly bent in their pathway which is referred to the deviated or the diffracted light rays. This causes the minor changes in refractive index of light. Those light rays that pass through only background medium of the specimen are called undiffracted light or direct light. Therefore, we can say that phase contrast microscopy divides light rays into two parts, one is diffracted light and another is undiffracted light. The difference between diffracted and undiffracted light is detected by the objective phase plate and is referred to the phase shift. The light rays that diffracted from the biological specimen and the undiffracted light rays recombine or sync with each other at image plane, resulting in the formation of the contrast and clear image of specimen. The image is viewed as contrasts in grey background (Fig. 8.10).

This phase difference is viewed as a contrast image under the phase contrast microscope. Therefore, PCM enables the examination of live cells or transparent specimens without the need for staining, thereby enhancing the contrast and visibility of microorganism.

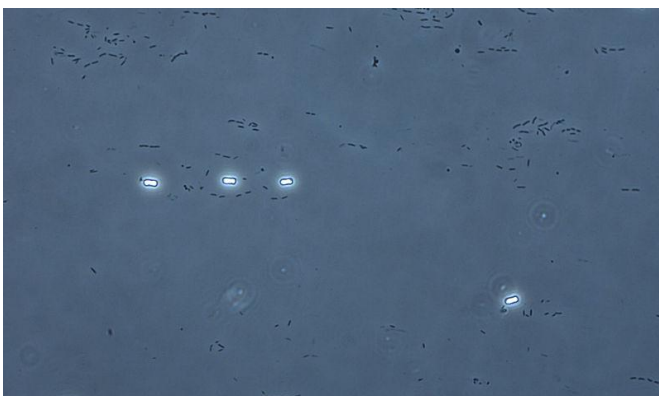
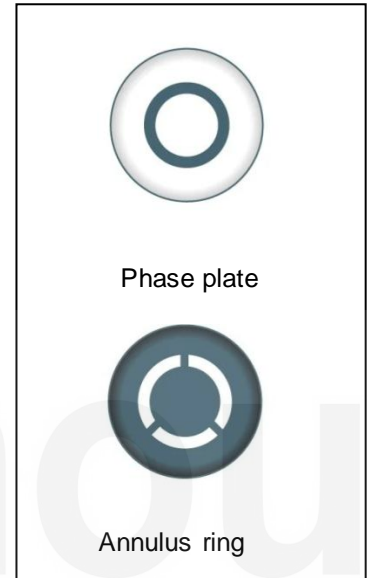


Fig. 8.10: Phase contrast image of bacteria.

[Image credit https://en.wikipedia.org/wiki/File:Bacteria_-_Phase_Contrast.jpg]

Let us discuss the basic components of Phase contrast microscope.

The common components of phase contrast microscope are shown in Fig. 8.11:

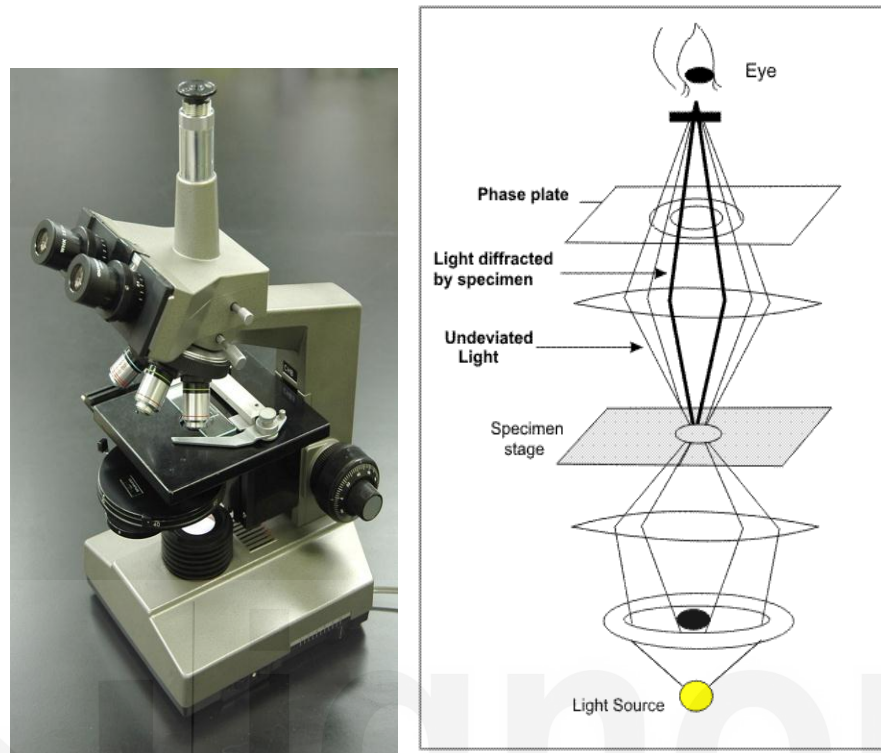


Fig. 8.11: a) Phase contrast microscope b) Basic part of PCM.

- **Light source:** A simple light source is used.
- **Annular Diaphragm or annular ring:** It is placed beneath the condenser in a microscope. It is a circular disk that contains a **ring-shaped** groove that allows light to pass through the trinocular head of a phase-contrast microscope. When the light beams reach the annular groove of the annular diaphragm, they all converge onto the biological object. The objective aperture at the backplane forms the image of a biological sample. The annulus selectively permits just a hollow cone of light to reach the specimen.
- **Phase Plate:** The phase plate within the objective lens distinguishes between the light that is not deviated and the light that is diffracted. A black annulus present in the phase plate causes a delay of $\frac{1}{4}$ wavelengths in the diffracted waves.
- **Phase condenser and phase objective lenses** are employed to measure the refractive index of the sample medium. All light rays that have undergone diffraction reach the condensers where from they subsequently reach the objective lens. The wave fronts originating from the light beams that have undergone diffraction converge at a single point, resulting in the formation of distinct and contrasting specimens.
- The combined interference of an unaltered and diffracted light resulting in formation of contrast and clear resolved image of live transparent sample.

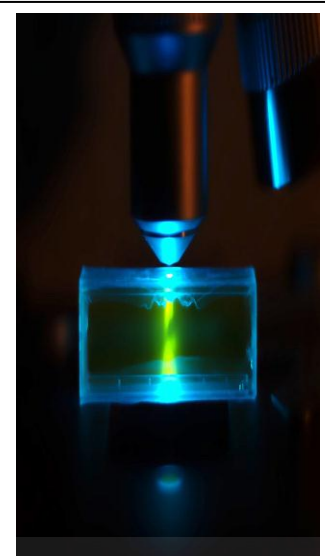
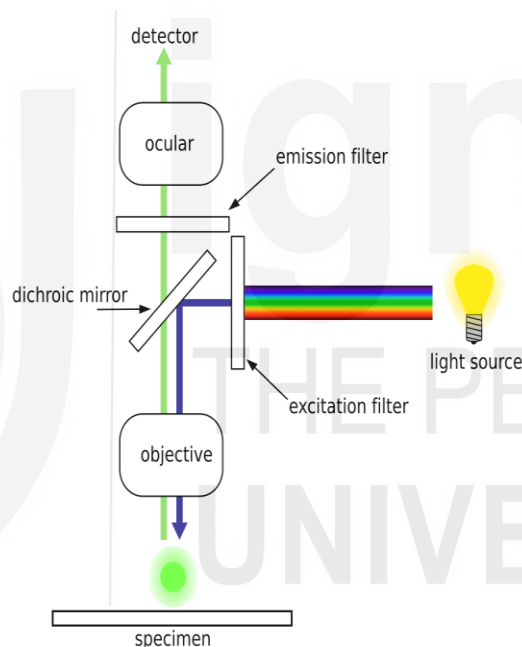
Application

PCM helps in investigating the final details of intracellular structure of organisms and studying the live transparent sample. It is widely used in cell culture techniques to view live cell growth. Specifically, this technique improves the contrast by magnifying variations in refractive indices, which makes it well-suited for examining vital cellular components such as nuclei, vacuoles, and organelles as well as the internal and external structure of bacteria which are not stained.

8.7.4 Fluorescence Microscopy

Fluorescence microscope employs fluorescent dye to study specific microorganisms and cellular components. Fluorescent dye is used to produce fluorescence image of particular cellular structures. Hence, it is called Fluorescence microscope.

Key components of fluorescence microscope (Fig.8.12)



A view fluorescent light (SYBR green dye)

[Image course
https://commons.wikimedia.org/wiki/File:FluorescenceMicroscopeSample_HerringSpermSYBRGreen.jpg]

Fig. 8.12: a) Fluorescence microscope, b) A schematic view of fluorescence microscope.

- **Light source:** Mercury or xenon arc lights and lasers.
- **Filter:** There three types of filters are used:
 - i) **The excitation filter** selectively allows only the desired wavelength of excitation light to pass through.
 - ii) **Dichroic mirrors, also known as dichromatic beam splitters,** are optical devices that reflect the excitation light from the objective to the sample. They are positioned at an angle in the light path.

- iii) **The barrier (emission) filter** eliminates any excitation light that may diminish the contrast of the image. Filters are integrated inside filter cubes, allowing for convenient filter rotation.

Detection: The fluorescent image can be observed or captured with a camera equipped with sensors against a dark backdrop.

Application: It is widely useful for detecting specific biomolecules, live cell imaging and study of antigen and antibody.

8.7.5 Electron Microscope

Electron microscopy is a highly effective but expensive microscope employed to examine and analyse intracellular structures at significantly greater resolution compared to the conventional light microscopy. This technique uses electrons to view image of the biological specimen to give greater magnifications as well as resolving power. The tissue samples to be examined undergo a series of steps like fixation, sectioning and staining and grid preparation. It is important to note that an electron has a thousand times shorter wavelength than the visible light, resulting in a higher resolving power which is about 0.1 nm. Electron microscopy has wide range of application specially in the scientific fields like molecular cell biology, microbiology, Biotechnology, toxicology and medical sciences, materials science, nanotechnology, and physics etc.

The principle of electron microscopy underlies on the interactions between electron and specimen material. However, the magnification and resolving power are the two important features in electron microscope as same as in light microscopy. This microscope uses the electromagnetic lens system and vacuum chamber in the electron microscope because electron can move only in vacuum condition.

The electron gun, equipped with a tungsten filament cathode, serves as the electron source and provides the illumination in an electron microscope. It generates a focused stream of high-velocity electrons moving in a certain direction. The electromagnetic lenses are employed to focus electrons beam a specimen. The electrons that pass through the specimen can be either scattered or unscattered, based on their interaction. The emergent electron beam contains data regarding the specimen's structure and is subsequently amplified by additional lenses to produce a high-resolution image at the atomic level. The image is captured by either projecting it onto a fluorescent screen or photographing it with a charged coupled device (CCD) camera.

Basic parts of transmission electron microscope

The basic instrumentation of the TEM comprises of an electron emission source, electromagnetic lenses, fluorescent screen and image recording system (Fig. 8.13) which is summarized in the Table 8.4 along with their role(s).

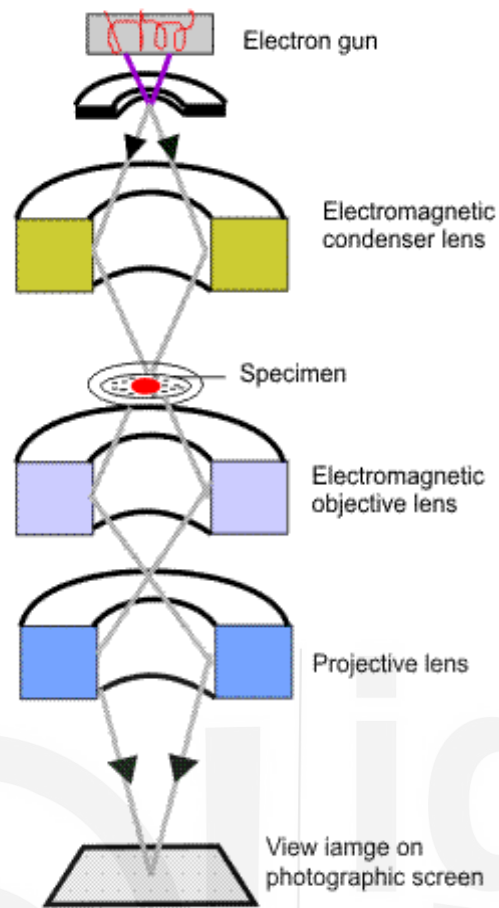


Fig. 8.13: A Schematic diagram of transmission electron microscope

Table 8.4: Basic parts of TEM

| Parts of TEM | Role(s) |
|--|---|
| 1) Electron source: An electron gun (voltage 5-100 KV) | produces an electron beam consists of a cathode and an anode |
| 2) A large cylindrical vacuum column | Maintain vacuum state within in microscope and prevents the collision of moving electron beam with gas molecules. |
| 3) Electromagnetic condenser lens | focuses the electron beam onto the specimen |
| 4) Electromagnetic objective and projector lenses | receive the transmitted electrons from the specimen and focuses onto projector lens that projects the final magnified image onto the viewing screen or camera |
| 5) Fluorescent screen | Collection and analysis of the image |
| 6) The image-recording system | Image transmitted to the digital camera for recording. |

Application

Transmission electron microscopy (TEM) is extensively utilized in the field of Cell and Molecular biology research for the purpose of histological inspection of cells and tissue, drug exploration, illness diagnostics, and the study of subcellular organelles such as the nucleus, mitochondria, endoplasmic reticulum, etc., as well as biomolecules.

Scanning Electron Microscope (SEM)

A scanning electron microscope offers a detailed visual representation of the surface of a biological specimen. The fundamental components of a scanning electron microscope (SEM) are similar to those of a transmission electron microscope (TEM) except secondary electron detector. In scanning electron microscopy (SEM), a focused electron beam interacts with the material, causing the emission of secondary electrons. These secondary electrons are then detected and recorded by a detector, as illustrated in Fig. 8.14. The detector forms an image of surface view. Therefore, SEM is based on the detection of secondary electrons that are reflected from the surface of the object as a result of being excited by the original electron beam.

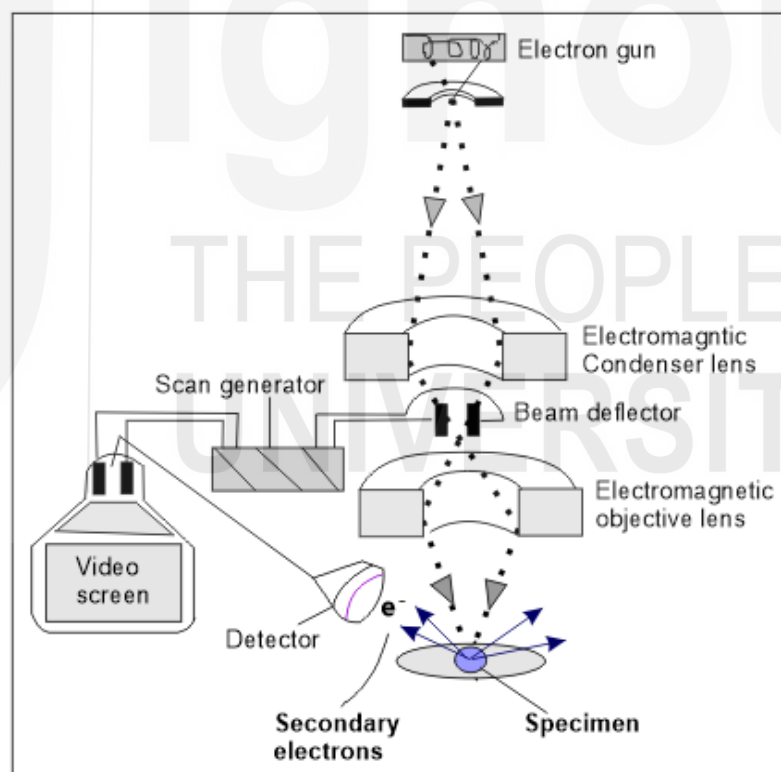


Fig. 8.14: Basic setup of Scanning electron microscope (SEM).

The scanning electron microscope (SEM) has a resolution limit ranging from 3 to 15 nm, whereas the transmission electron microscope (TEM) has a resolution of 0.2 nm. However, SEM offers a higher depth of view and produces images that closely depict the three-dimensional surface structure of the sample.

Application: The SEM is primarily used to examine of topography, composition and morphology of surface of biological specimens.

8.7.6 Comparison of Microscope with their Magnification and Resolution

Table 8.5: Overview of Microscopy

| Types of Microscope | Magnification | Resolution | Application |
|---------------------|---------------------|------------|---|
| Bright field | 1500X | 200nm | Visualization of microorganism and viewing of stained specimen |
| Dark field | 1500X | 200nm | Visualization of microscopic cells |
| Phase contrast | 1500X | 200nm | Examination of unstained live specimen with contrast image, staining not required |
| Fluorescence | 1500X | 200nm | Examine the specific cellular structure, |
| TEM | 500,000X-10,00,000X | 1nm | Used to examine ultrastructure of cells with finer details |
| SEM | 500,000X-10,00,000X | 1nm | Used to view detailed surface of biological specimen |

SAQ 6

Identify whether the given sentence are True (T) or False (F).

- i) Spectrophotometers are indispensable tools for studying cellular structure and microorganisms. []
- ii) Electron microscopes use photons of light to achieve high-resolution imaging of microorganisms. []
- iii) Phase Contrast Microscopy enhances contrast in transparent specimens without the need for staining. []
- iv) Dark-field microscopy is effective for observing stained biological samples due to its bright image formation. []
- v) Fluorescence microscopy uses fluorescent dyes to label specific cellular components, aiding in their visualization. []
- vi) Light microscopes can achieve magnifications up to 1500X, and some advanced models can reach 2000X magnification. []

- vii) The resolving power of a microscope depends on the wavelength of light and the numerical aperture of its lens system. []
- viii) Electron microscopes operate in a vacuum to allow the movement of electrons without interference from gas molecules. []
-

8.8 SUMMARY

- Laminar airflow cabinets are essential in microbiology for providing sterile environments during microbial inoculation. They utilize HEPA filters to eliminate airborne contaminants, ensuring a contamination-free workspace.
- An incubator provides ideal temperature, humidity, and gas levels to support cell and microbiological cultures, crucial for *in vitro* experiments. It is used to maintain microbial culture and animal cell culture.
- Laboratory shakers are important lab devices for performing blending, mixing, and stirring of biological samples. They facilitate growth of microorganisms by ensuring uniform motion.
- Centrifuge is an important instrument for centrifugation techniques. Centrifugation is a separation technique that separates particles that differ in their size, shape, and density. Centrifugal force accelerates the rate of sedimentation of particles and separates them based on their size, mass, and density. As a result, heavy dense particles separate first at low rpm than light dense particles. By employing centrifugation, it becomes convenient to isolate cellular and subcellular components of cells for research studies.
- UV-Vis spectrophotometers are common instruments used to measure light absorption across various wavelengths. Based on the Beer-Lambert Law, which links absorbance to solute concentration and path length. They are used to measure the concentration of a substance in a solution.
- Microscopes are essential tools in microbiology, enabling the study of cellular structures and microorganisms. The optical (or light) microscopes and electron microscopes are two popular microscopes. Optical microscopes use lenses to magnify objects, achieving up to 2000X magnification, while electron microscopes utilize electrons for getting much higher resolutions.
- Techniques like phase contrast microscopy enhance the visibility of transparent specimens without staining, while fluorescence microscopy uses fluorescent dyes to label specific cellular components. These microscopes play crucial roles in various scientific fields by providing detailed insights into biological structures at different scales.

8.9 TERMINAL QUESTIONS

1. Explain the importance and basic components of laminar airflow.
2. Write a short note on incubator and laboratory shaker.
3. Discuss the construction and application of centrifuge.
4. Explain the basic components and principle of spectrophotometer.
5. Compare and contrast the operating principles and applications of optical microscopes (light microscopes) and electron microscopes. Highlight their differences in resolution and magnification.
6. Describe the working principle of a phase contrast microscope. Include how it enhances contrast in transparent specimens and its applications in biological research.
7. Write the working principle and application of TEM and SEM

8.10 ANSWER

Self-Assessment Questions

1.
 - i) Laminar airflow
 - ii) microbes in culture media.
 - iii) Sterile, contaminants.
 - iv) HEPA filters.
 - v) in a back-to-front direction across, the downward from the top of the cabinet onto the working area.
2.
 - i) cell or microbiological cultures
 - ii) Temperature, humidity, and gas levels constant.
 - iii) CO₂.
 - iv) 60 to 65 °C
 - v) about 37 °C.
3.
 - i) Laboratory shakers are primarily used for consistent blending, mixing, and stirring of biological samples in a tube or flask.
 - ii) They establish and uphold a regulated environment by ensuring uniform and continuous motion and blending, which is essential for the growth and cultivation of microorganisms.
4.
 - i) B. To create centrifugal force
 - ii) B. Sedimentation

- iii) B. Revolutions per minute (RPM)
 - iv) A. Rotor, Drive shaft, Motor
 - v) C. 1,50,000 RPM
 - vi) C. Fixed angle rotor
 - vii) A. Relative Centrifugal Force
- 5.
- i) True
 - ii) True
 - iii) True
 - iv) False
- 6.
- i) False
 - ii) True
 - iii) True
 - iv) True
 - v) False
 - vi) True
 - vii) True
 - viii) True

Terminal Questions

1. Refer to Table 8.1
2. Refer to section 8.2 and 8.3
3. Refer to section 8.4
4. Refer to section 8.5
5. Refer to section 8.7.6
6. Refer to section 8.7.3
7. Refer to section 8.7.5