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1. Microscope – Bright field and Dark field.

Aim

To study and differentiate between Bright Field Microscopy and Dark Field Microscopy based on their principles, working mechanisms, and applications for observing biological specimens.

Principle of Bright field Microscope

For a specimen to be the focus and produce an image under the Brightfield Microscope, the specimen must pass through a uniform beam of the illuminating light. Through differential absorption and differential refraction, the microscope will produce a contrasting image. The specimens used are prepared initially by staining to introduce color for easy contracting characterization. The colored specimens will have a refractive index that will differentiate it from the surrounding, presenting a combination of absorption and refractive contrast. The functioning of the microscope is based on its ability to produce a high-resolution image from an adequately provided light source, focused on the image, producing a high-quality image. The specimen which is placed on a microscopic slide is viewed under oil immersion or/and covered with a coverslip.

Parts of the brightfield microscope

Eyepiece (Ocular lens) – it has two eyepiece lenses at the top of the microscope which focuses the image from the objective lenses. This is where you see the formed image from, with your eyes.

The objective lenses which are made up of six or more glass lenses, which make a clear image clear from the specimen or the object that is being focused.

Two focusing knobs i.e the fine adjustment knob and the coarse adjustment knob, found on the microscopes' arm, which can move the stage or the nosepiece to focus on the image. Their function is to ensure the production of a sharp image with clarity.

The stage is found just below the objectives and this is where the specimen is placed, allowing movement of the specimen around for better viewing with the flexible knobs and it is where the light is focused on.

The condenser: It is mounted below the stage which focuses a beam of light onto the specimen. It can be fixed or movable, to adjust the quality of light, but this entirely depends on the microscope.

The arm: This is a sturdy metallic backbone of the microscope, used to carry and move the microscope from one place to another. They also hold the microscope **base** which is the stand of the microscope. The arm and the base hold all the microscopic parts.

It has a **light illuminator** or a **mirror** found at the base or on the microscope's nosepiece.

The **nosepiece** has about two to five objective lenses with different magnifying power. It can move round to any position depending on the objective lens to focus on the image.

An aperture diaphragm (contrast): It controls the diameter of the beam of light that passes through the condenser. When the condenser is almost closed, the light comes through to the center of the condenser creating high contrast and when the condenser is widely open, the image is very bright with very low contrast.

Magnification by Brightfield Microscope

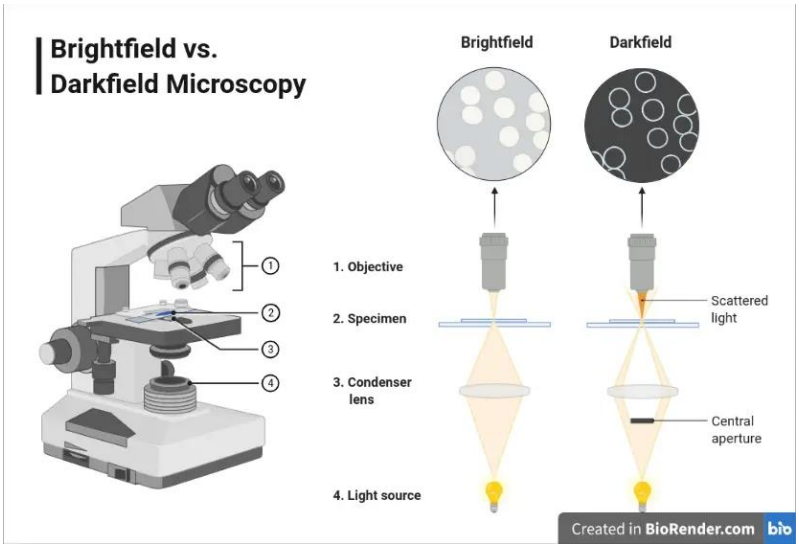
- The objective lenses are the main lenses used for focusing the image, on the condenser. This produces an enlarged clear image that is then magnified again by the eyepiece to form the primary image that is seen by the eyes.
- During imaging, the objective lenses remain parfocal in that, even when the objective lens has changed the image still remains focused. The image seen at the eyepiece is the enlarged clear image of the specimen, known as the virtual image.
- The magnification of the image is determined by the magnification of the objective against the magnification of the eyepiece lens. The objectives have a magnification power of 40x-1000x depending on the type of brightfield microscope while the eyepiece lens has a standard magnification power of 10x.

Therefore to calculate:

Total Magnification power = Magnification of the objective lens x Magnification of the eyepiece

- For example: if the magnification of the objective is 45x and that of the eyepiece is 10x, the total magnification of the specimen will be 450x.
- The magnification is standard, i.e. not too high nor too low, and therefore depending on the magnification power of the lenses, it will range between 40X and 100X.

- The objective lens enlarges the image which can be viewed, a characteristic known as resolution. Resolution according to Prescott, is the ability of a lens to separate or distinguish between small objects closely linked together.
- Whereas the eyepiece magnifies the image at the end of the viewing, its magnification range is lower than that of the objective lens at 8X-12X (10X standard) and that of the objective lens at 40X-100X, magnification, and resolution of the microscope is highly dependent on the objective lens.



Dark field Microscope

Microbiology, the branch of science that has so vastly extended and expanded our knowledge of the living world, owes its existence to Antoni van Leeuwenhoek.In 1673, with the aid of a crude microscope consisting of a biconcave lens enclosed in two metal plates, Leeuwenhoek introduced the world to the existence of microbial forms of life.Over the years, microscopes have evolved from the simple, single-lens instrument of Leeuwenhoek, with a magnification of 300 X, to the present-day electron microscopes capable of magnifications greater than 250,000X.Microscopes are designated as either light microscopes or electron microscopes.

Light microscopes use visible light or ultraviolet rays to illuminate specimens. They include brightfield, darkfield, phase-contrast, and fluorescent instruments.This is similar to the ordinary light microscope; however, the condenser system is modified so that the specimen is not illuminated directly.The condenser directs the light obliquely so that the light is deflected or scattered from the specimen, which then appears bright against a dark background.Living specimens may be observed more readily with darkfield than with brightfield microscopy.

Principle of the Dark field Microscope

A dark field microscope is arranged so that the light source is blocked off, causing light to scatter as it hits the specimen. This is ideal for making objects with refractive values similar

to the background appear bright against a dark background. When light hits an object, rays are scattered in all azimuths or directions. The design of the dark field microscope is such that it removes the dispersed light, or zeroth order, so that only the scattered beams hit the sample. The introduction of a condenser and/or stop below the stage ensures that these light rays will hit the specimen at different angles, rather than as a direct light source above/below the object. The result is a "cone of light" where rays are diffracted, reflected and/or refracted off the object, ultimately, allowing the individual to view a specimen in dark field.

2. Structure observation of Prokaryotic cells

Aim

To observe and study the structure of prokaryotic cells under a microscope, including key features such as the cell wall, plasma membrane, cytoplasm, and nucleoid region.

Principle

Prokaryotic cells are unicellular organisms lacking membrane-bound organelles. They are simpler in structure compared to eukaryotic cells. Common examples include bacteria such as *Escherichia coli* and *Bacillus subtilis*. Using microscopy techniques like bright field microscopy, the basic structure of these cells can be observed. Staining techniques, such as Gram staining, enhance the contrast, allowing better visualization of the cells and their features.

Materials Required

Bacterial culture (e.g., *E. coli* or *Bacillus subtilis*), Glass slides and cover slips, Inoculation loop, Gram staining reagents (crystal violet, iodine, alcohol, safranin), Immersion oil, Microscope (bright field), Sterile water, Bunsen burner, Dropper.

Procedure

- A clean glass slide was taken, and a small amount of bacterial culture was collected using a sterile inoculation loop.

- The bacterial culture was spread on the slide to create a thin smear and left to air dry.
- The smear was then heat-fixed by passing the slide over a Bunsen burner flame.
- Crystal violet (primary stain) was added to the smear and left for 1 minute.
- The slide was rinsed with water to remove excess stain.
- Iodine solution (mordant) was applied to the smear and allowed to sit for 1 minute.
- The slide was again rinsed with water.
- The smear was decolorized with alcohol for 10-20 seconds and immediately rinsed with water.
- Safranin (counterstain) was applied to the smear and left for 1 minute before rinsing with water.
- The slide was blot-dried with a paper towel.
- The stained slide was placed on the microscope stage and observed under low (10x) and high (40x) magnifications.
- For more detailed observation, immersion oil was applied to the slide, and the oil immersion lens (100x) was used.
- The focus was adjusted to clearly view the bacterial cells.

Observation

The prokaryotic cells were observed as small, simple structures without internal organelles. When viewed under the microscope, Gram-positive bacteria appeared purple due to retaining the crystal violet stain, while Gram-negative bacteria appeared pink as they retained the safranin counterstain. The cells were observed in various shapes, such as rod-shaped (*Bacillus subtilis*) and spherical (*Staphylococcus*). No membrane-bound nucleus or other organelles were visible, consistent with the characteristics of prokaryotic cells.

Result

The prokaryotic cells displayed distinct shapes and were successfully stained using the Gram staining method. Gram-positive bacteria appeared purple, and Gram-negative bacteria appeared pink.

3. Structure observation of Eukaryotic cell

Aim

To observe and study the structure of eukaryotic cells under a microscope, identifying key organelles such as the nucleus, mitochondria, and other membrane-bound structures.

Principle

Eukaryotic cells are complex cells characterized by membrane-bound organelles, including the nucleus, which contains the cell's genetic material. They are found in plants, animals, fungi, and protists. Eukaryotic cells can be studied using microscopy techniques, such as bright field microscopy, and staining methods to highlight specific structures, like the nucleus and cytoplasmic organelles.

Materials Required:

Onion peel, glass slide, cover slip, forceps, iodine solution, dropper, blotting paper, microscope (bright field).

Procedure:

- A thin layer of onion peel was carefully removed using forceps.
- The onion peel was placed flat on a clean glass slide.
- A few drops of iodine solution were added to the onion peel using a dropper to stain the cells.
- A cover slip was gently placed over the onion peel to avoid air bubbles.
- The prepared slide was placed on the microscope stage.
- The slide was observed under low magnification (10x) and then under high magnification (40x) to view the eukaryotic cell structures.

Observation

Rectangular-shaped plant cells were observed with distinct cell walls. The nuclei, stained by iodine, appeared as dark, round structures within the cells. Large, central vacuoles occupied most of the cell space, with cytoplasm visible along the cell walls.

Result

The characteristic structures of eukaryotic plant cells were clearly observed, including the cell wall, nucleus, vacuole, and cytoplasm. The iodine stain helped enhance the visibility of the nucleus, confirming the presence of these key eukaryotic organelles.

4. Enumeration of Prokaryotic cells using Haemocytometer

Aim

To enumerate the number of prokaryotic cells (e.g., bacterial cells) using a hemocytometer.

Principle

A hemocytometer is a specialized counting chamber originally designed for counting blood cells. It is also used for counting prokaryotic cells such as bacteria. The hemocytometer has a grid etched into its surface, and a known volume of cell suspension is applied to this grid. By counting the number of cells within a specific area, the concentration of cells in the original sample can be calculated.

Materials Required:

Bacterial culture (*E. coli*), hemocytometer, microscope (bright field), sterile water, glass slides, cover slip, inoculation loop, Bunsen burner, pipette, staining solution (optional, such as methylene blue), dropper.

Procedure

- A fresh bacterial culture was obtained using a sterile inoculation loop.
- The bacterial culture was diluted with sterile water to an appropriate concentration for counting.
- The hemocytometer was cleaned and a clean cover slip was placed on it.

- Using a pipette, a small amount of the diluted bacterial suspension was drawn and carefully applied to the edge of the cover slip, allowing the suspension to be drawn under the slip by capillary action, filling the chamber.
- The hemocytometer was placed on the microscope stage.
- The bacterial cells in the grid were observed under low magnification and adjusted to focus on the cells.
- The number of bacterial cells within the grid was counted.
- If the cells were stained with a solution like methylene blue, the count was easier, as the stain helped to distinguish live and dead cells.
- The total number of cells per milliliter of the original bacterial suspension was calculated based on the known volume of the hemocytometer grid.

Observation

The bacterial cells were evenly distributed across the grid of the hemocytometer. Each small square of the grid contained several bacterial cells, which were counted under the microscope.

Result

The concentration of bacterial cells in the original suspension was found to be -------- cells/mL.

5. Enumeration of Eukaryotic cells using Haemocytometer

Aim

To enumerate the number of human blood cells (eukaryotic cells) using a hemocytometer.

Principle

A hemocytometer is a specialized counting chamber with a grid pattern used for counting cells. By placing a known volume of diluted blood onto the hemocytometer, the number of cells within specific grid areas can be counted under a microscope. This allows for the calculation of cell concentration in the original blood sample.

Materials Required

Human blood sample, hemocytometer, microscope (bright field), glass slide, cover slip, pipette, staining solution (optional, such as Wright's stain or Giemsa stain for differentiating cell types), dropper, sterile water.

Procedure

- A human blood sample was collected using standard venipuncture techniques and mixed with an anticoagulant to prevent clotting.
- The blood sample was diluted with sterile water or a suitable buffer to achieve an appropriate concentration for counting, typically 1:10 or 1:20 dilution.
- The hemocytometer was cleaned thoroughly.
- A cover slip was placed over the hemocytometer.

- Using a pipette, 10 µL of the diluted blood sample was drawn and carefully applied to the edge of the cover slip, allowing the suspension to be drawn into the hemocytometer chamber by capillary action.
- The hemocytometer was placed on the microscope stage.
- The cells were observed under low magnification (10x) to locate the grid areas and then under high magnification (40x) to count the cells clearly.
- The number of blood cells in the grid area of the hemocytometer was counted.
- Cells were counted in multiple squares or fields for accuracy.
- The total number of cells per milliliter of the original blood sample was calculated based on the known volume of the hemocytometer grid.

Observation

The blood cells were observed within the grid of the hemocytometer. Depending on the staining method used, red blood cells (RBCs) appeared as small, circular, and pale, while white blood cells (WBCs) were larger with visible nuclei. The cells were distributed across the grid, and their counts were recorded.

Result

 The concentration of red blood cells (RBCs) in the original blood sample was determined to be 1.2 × 10^6 RBCs/mL.

 The concentration of white blood cells (WBCs) in the original blood sample was determined to be 3.0 × 10^5 WBCs/mL.

(Diagram side use pencil)

2. Calculation:

- The volume of the central square of the hemocytometer is 0.1 mm^3 (or 0.1 µL).
- Assuming no dilution factor and the volume of the grid as 0.1 µL:

$$\text{Cell concentration (cells/mL)} = \frac{\text{Number of cells counted} \times \text{Dilution factor} \times 10^4}{\text{Volume of grid in µL}}$$

- For RBCs:

$$\text{RBC concentration (cells/mL)} = \frac{120 \times 10^4}{0.1} = 1.2 \times 10^6 \text{ RBCs/mL}$$

- For WBCs:

$$\text{WBC concentration (cells/mL)} = \frac{30 \times 10^4}{0.1} = 3.0 \times 10^5 \text{ WBCs/mL}$$

6. Observation – Different types of cells – parenchyma, collenchyma, sclerenchyma,epithelium

Aim

To observe and identify different types of plant and animal cells, specifically parenchyma, collenchyma, sclerenchyma, and epithelium cells, using a light microscope.

Principle

Different cell types have distinct structural features that can be observed under a light microscope. By preparing and staining tissue samples, the unique characteristics of each cell type can be visualized and identified.

Materials Required

Microscope, glass slides, cover slips, pipette, staining solutions (e.g., iodine solution for plant cells, hematoxylin and eosin for animal cells), forceps, razor blade, sterile water, and tissue samples (e.g., plant tissues and animal tissues).

Procedure

Parenchyma, Collenchyma, and Sclerenchyma

- Fresh plant tissues (e.g., leaf or stem) were collected.
- Thin sections of the plant tissues were prepared using a razor blade.
- The sections were placed on glass slides, and a few drops of iodine solution were added to stain the tissues.

- A cover slip was gently placed over the tissue.
- Observations were made under low magnification.

Epithelium

- A small piece of epithelium tissue (e.g., from the cheek or skin) was collected.
- Thin sections or smears of the tissue were prepared and placed on glass slides.
- The sections were stained using hematoxylin and eosin or other suitable stains.
- A cover slip was placed over the stained tissue.
- The prepared slides were examined under the microscope.
- Observations were made under low magnification (10x) to locate the cells and then under high magnification (40x or 100x) to observe detailed cell structures.

Observation and Results

Parenchyma Cells:

Large, thin-walled cells with central vacuoles, often rectangular or spherical. Iodine staining revealed thin walls and starch granules. Thin walls and large vacuoles indicate roles in storage and metabolism.

Collenchyma Cells:

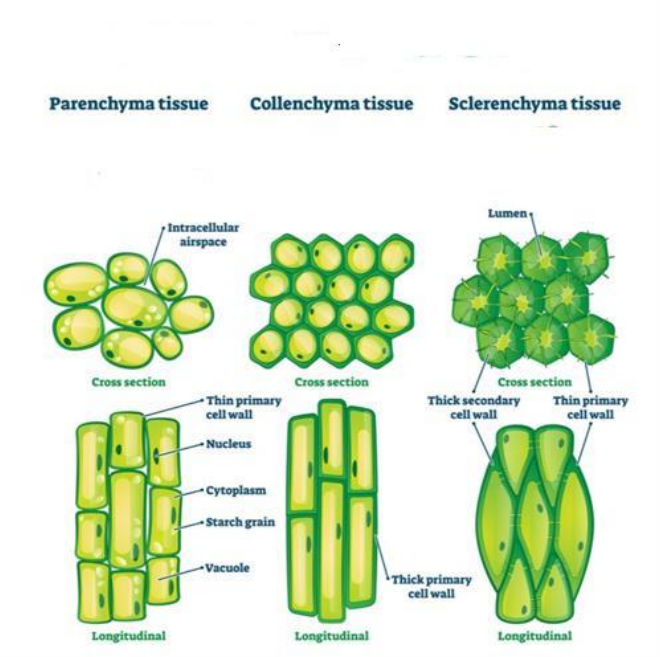
Elongated cells with thickened primary walls at corners, found in strands or layers. Iodine staining showed uneven wall thickness. Thickened walls provide flexible support in growing plant parts.

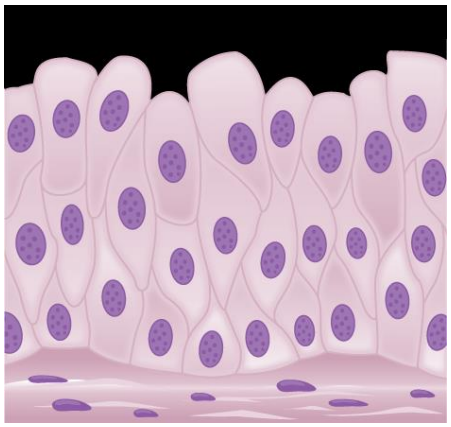
Sclerenchyma Cells:

Smaller cells with very thick, lignified secondary walls, often angular or irregular. Iodine staining darkened the lignified walls.Thick, lignified walls offer structural support and strength.

Epithelium Cells:

Tightly packed cells with uniform shapes (cuboidal, columnar, or squamous). Hematoxylin and eosin staining revealed distinct nuclei and lighter cytoplasm.Closely packed cells form protective layers in animal tissues.





Epithelium cell

7. Size and shape of an organism (prokaryote) – simple staining, use of ocular micrometer

Aim

To observe the size and shape of a prokaryotic organism (e.g., bacteria) using simple staining and measure the cell dimensions with an ocular micrometer.

Principle

Simple staining helps visualize the shape and arrangement of bacterial cells by enhancing contrast. Using an ocular micrometer calibrated with a stage micrometer allows accurate measurement of cell dimensions under the microscope.

Materials Required

Bacterial culture (e.g., *Escherichia coli* or *Bacillus subtilis*), glass slides, cover slips, simple staining reagent (crystal violet, methylene blue), immersion oil, microscope, inoculation loop, sterile water, ocular micrometer, stage micrometer, Bunsen burner, and dropper.

Procedure

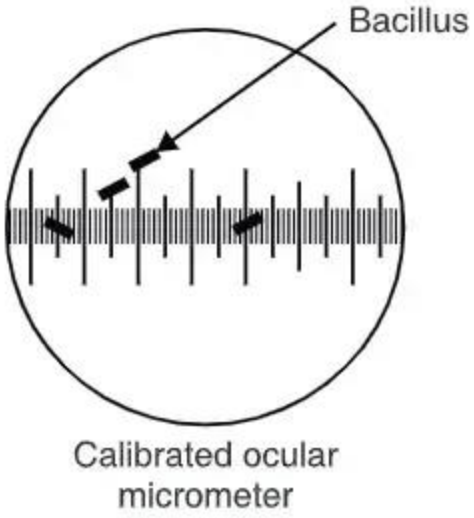
- A clean glass slide was taken, and a small drop of sterile water was added to the center.
- Using a sterilized inoculation loop, a small amount of bacterial culture was transferred to the water drop and spread to make a thin smear.
- The slide was allowed to air-dry completely.
- The slide was passed briefly through a Bunsen burner flame to heat-fix the smear, ensuring the bacteria adhered to the slide.
- A few drops of crystal violet (or methylene blue) stain were applied to the fixed smear.
- The stain was left on the slide for 30-60 seconds.
- The slide was then rinsed gently with sterile water and allowed to air-dry or blotted dry using filter paper.
- The slide was placed on the microscope stage, and immersion oil was added to enhance resolution.
- The bacterial cells were observed under high magnification (100x objective).
- The ocular micrometer in the microscope eyepiece was calibrated using a stage micrometer.
- The size of the bacterial cells was measured by counting the number of divisions on the ocular micrometer corresponding to the length and width of the cells.
- The actual size of the cells was calculated using the calibration factor from the stage micrometer.

Observation

The bacterial cells were observed as rod-shaped (*Bacillussubtilis*) under the microscope. The length and width of the bacterial cells were measured using the ocular micrometer.

Result

The calibrated ocular micrometer enabled the accurate measurement of cell size, with *Bacillus subtilis* exhibiting rod-like structures measuring approximately 2-4 μm in length.



8. Motility of an organism – Hanging drop

Aim

To observe the motility of microorganisms present in curd using the hanging drop technique.

Principle

Curd contains a variety of lactic acid bacteria, such as Lactobacillus species, which are responsible for fermentation. The hanging drop technique allows for the observation of live, motile bacteria in curd, which can be studied for their movement and morphology in a natural environment.

Materials Required:

Curd sample, glass slides, cover slips, inoculation loop, sterile water, immersion oil, petroleum jelly or paraffin, microscope, dropper, and Bunsen burner.

Procedure

- A clean glass slide was prepared, and a small amount of petroleum jelly or paraffin was placed on the edges of the coverslip.
- A small drop of curd was taken using a sterile inoculation loop and placed in the center of the coverslip.
- A concave glass slide was used to accommodate the hanging drop.
- The coverslip, containing the curd sample, was carefully inverted and placed over the depression in the slide, allowing the drop of curd to hang freely in the cavity.
- The coverslip was sealed to the slide using the petroleum jelly or paraffin applied earlier to prevent evaporation and contamination.
- The slide was placed on the microscope stage, and an initial low magnification (10x or 40x objective) was used to locate the sample.

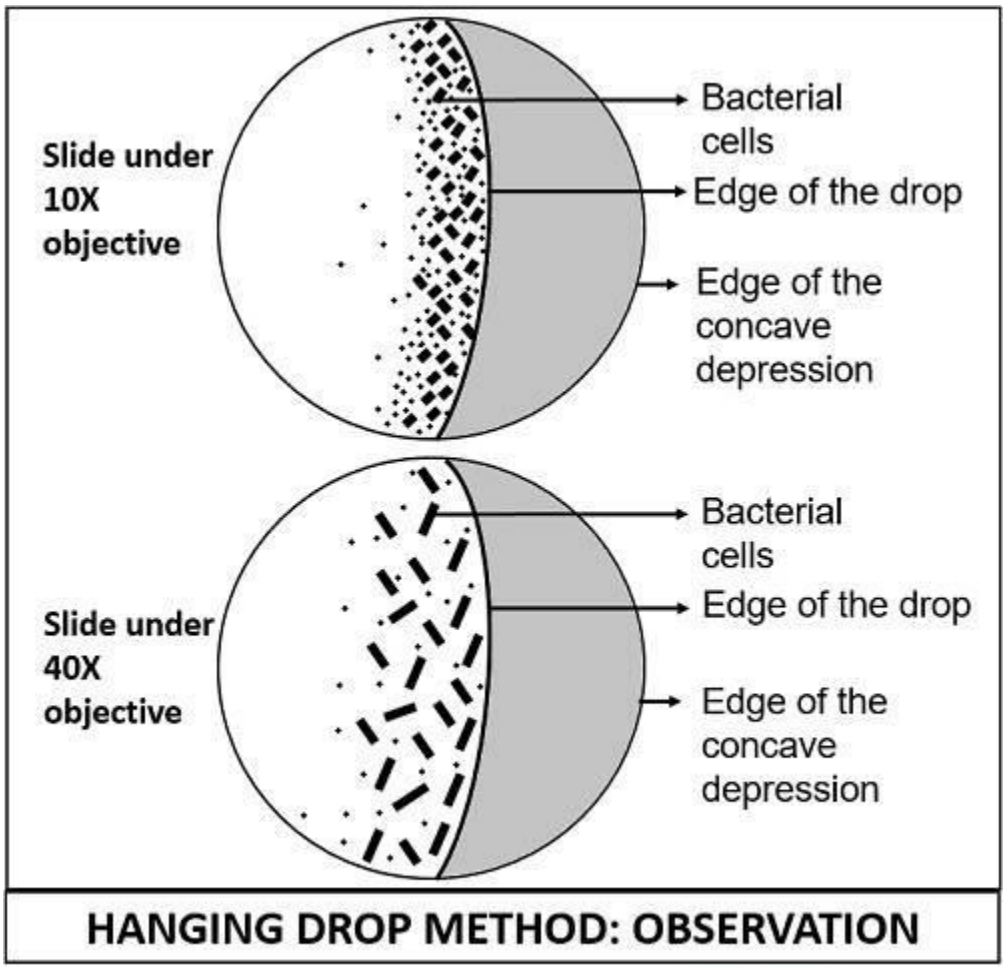
- Higher magnification (100x with immersion oil) was used to observe the motility of the microorganisms in the curd.
- Movements of bacteria within the curd drop were observed and recorded.

Observation

Lactic acid bacteria, such as *Lactobacillus*, were observed under the microscope. These bacteria appeared as long rods, often arranged in chains, and exhibited limited motility due to their role in fermentation.

Result

Lactobacillus species were identified as the dominant bacteria, exhibiting rod-shaped, chain-like structures with minimal movement, consistent with their role in lactic acid fermentation during the curdling process.



9. Cell Staining – Cytochemical Methods

Aim

To observe and differentiate cellular components by using cytochemical staining methods to highlight specific structures within the cell such as the nucleus, cytoplasm, and organelles.

Principle

Cytochemical staining involves the use of specific dyes to highlight particular cell components or organelles based on their chemical properties. The dyes interact with specific cellular macromolecules such as nucleic acids, proteins, or lipids, allowing for the visualization of these components under a microscope. This method helps in identifying cell structure, morphology, and functions. Common stains include Hematoxylin and Eosin (H&E), which stains nuclei blue and cytoplasm pink, and Giemsa, which stains DNA regions in cells.

Materials Required

Glass slides, coverslips, a fixative such as methanol or formaldehyde, Hematoxylin stain, Eosin stain, Giemsa stain, distilled water, wash bottles, a light microscope, immersion oil for high-power observation, forceps, a microtome, cell or tissue samples, blotting paper, and staining racks.

Procedure

- Tissue sections of approximately 4–5 µm thickness were cut using a microtome and placed on glass slides.
- For cultured cells, the cell suspension was smeared on glass slides and allowed to air-dry.
- The tissue or cell sample on the slides was fixed using a fixative (such as methanol or formaldehyde) for 10-15 minutes to preserve the cellular structure.

Hematoxylin& Eosin Staining:

- The fixed slides were immersed in hematoxylin for 5-10 minutes.
- The slides were rinsed under running water.
- They were then dipped in eosin for 1-2 minutes.
- The slides were rinsed briefly in distilled water.

Giemsa Staining:

- A Giemsa working solution (1:20 in distilled water) was prepared.
- The fixed slides were stained with Giemsa for 10-20 minutes.
- The slides were gently rinsed with distilled water.
- The stained samples were dehydrated through a graded series of alcohol solutions (70%, 90%, 100%) if needed.
- A few drops of mounting medium (or immersion oil for high magnification) were placed on the stained slide, and a coverslip was carefully placed over it.

- The stained slides were observed under a light microscope, starting at low magnification and progressing to higher magnification as needed.

Result

The cytochemical staining effectively differentiates cellular components such as the nucleus, cytoplasm, and other organelles.

10. Sub cellular fractionation.

Aim

To separate and isolate the different components or organelles of a cell, such as nuclei, mitochondria, lysosomes, and cytosol, using differential centrifugation.

Principle

Subcellular fractionation is based on the principle that different cellular components have distinct sizes and densities. By using a series of centrifugation steps at increasing speeds, organelles can be separated from one another. Larger and denser components, such as nuclei, pellet at lower speeds, while smaller organelles, like mitochondria and ribosomes, require higher speeds to sediment. This method enables the study of the function and composition of individual cellular compartments.

Materials Required

Cell homogenate, a cold isotonic buffer (such as sucrose or phosphate buffer), centrifuge tubes, a refrigerated centrifuge, a homogenizer, pipettes, ice, and cold storage containers.

Procedure

- The tissue sample or cultured cells were suspended in a cold isotonic buffer to maintain osmotic balance and prevent organelle damage.
- A homogenizer was used to break open the cell membrane, leaving the organelles intact.
- Low-Speed Centrifugation (800 x g, 10 min): Nuclei and unbroken cells were pelleted, and the supernatant containing smaller organelles was transferred to a fresh tube.
- Medium-Speed Centrifugation (10,000 x g, 20 min): Mitochondria, lysosomes, and peroxisomes were pelleted, with the supernatant moved to another tube.
- High-Speed Centrifugation (100,000 x g, 1 hour): Ribosomes, membranes, and microsomes were pelleted, while the cytosolic fraction remained in the supernatant.
- After each step, the organelle-containing pellets were resuspended in fresh buffer for further analysis, with the cytosolic fraction collected from the final supernatant.

Observation

Each centrifugation step successfully separated distinct cellular fractions. The nuclei were found in the pellet from the low-speed centrifugation, the mitochondria and lysosomes in

the medium-speed pellet, and ribosomal and membrane fragments in the high-speed pellet. The cytosol remained in the supernatant after high-speed centrifugation.

Result

Subcellular fractionation allowed for the effective isolation of cellular organelles.

11. Osmosis and Tonicity

Aim

To understand the effects of osmosis and tonicity on cells by observing the movement of water across a semipermeable membrane in different solutions.

Principle

Osmosis is the diffusion of water across a semipermeable membrane, driven by differences in solute concentration. Tonicity describes how a solution affects cell volume based on its solute concentration relative to the cell. In a hypertonic solution, water exits the cell, causing it to shrink. In a hypotonic solution, water enters the cell, leading to swelling or bursting. In an isotonic solution, water moves equally in both directions, maintaining cell size.

Materials Required

Potato
Beakers
Distilled water
Sucrose solutions (different concentrations, e.g., 0.2 M, 0.5 M, 1 M)
Weighing balance
Knife
Forceps
Ruler
Marker

Procedure

- Potato tuber pieces were cut into identical cylinders using a knife. Their initial weights were recorded using a weighing balance.
- Three beakers were labeled and filled with distilled water (hypotonic solution), 0.5 M sucrose solution (isotonic solution), and 1 M sucrose solution (hypertonic solution).
- The potato cylinders were placed in each solution and left for 30 minutes to allow osmosis to occur.
- After the incubation period, the potato pieces were removed, blotted to remove excess liquid, and their final weights and sizes were measured.

Observation

- In the distilled water (hypotonic solution), the potato cylinders swelled and became heavier due to water entering the cells.

- In the 0.5 M sucrose solution (isotonic), the potato cylinders maintained their original weight and size, as no net movement of water occurred.
- In the 1 M sucrose solution (hypertonic), the potato cylinders shrank and lost weight, indicating water movement out of the cells.

Result

The experiment demonstrated the effects of osmosis and tonicity. In a hypotonic solution, water entered the cells, causing them to swell. In a hypertonic solution, water exited the cells, resulting in shrinkage. In an isotonic solution, there was no significant change in cell size or weight. This highlights the importance of osmosis in maintaining cell volume and the effects of external environments on cells.

12. Cell Division – Mitotic Stages in Onion Root Tip

Aim

To observe the stages of mitosis in an onion root tip and understand the process of cell division.

Principle

Mitosis is the process by which a cell divides its nucleus and distributes its genetic material equally into two daughter cells. The stages of mitosis include prophase, metaphase, anaphase, and telophase, followed by cytokinesis. Onion root tips are ideal for observing mitotic stages due to their actively dividing cells in the meristematic region.

Materials Required

Onion bulbs, Beaker
Hydrochloric acid (HCl) (1 M)
Acetic orcein stain or Feulgen stain
Distilled water
Microscope
Forceps
Scalpel
Slides and coverslips
Bunsen burner or hotplate
Filter paper
Watch glass

Procedure

- Onion bulbs were grown in water for 3-5 days until roots appeared. Root tips approximately 1 cm long were cut and collected.
- The root tips were placed in a watch glass and treated with 1 M hydrochloric acid for 5 minutes to soften the tissue and break down cell walls for easier staining.

- The root tips were rinsed with distilled water and then stained with acetic orcein or Feulgen stain for 10-15 minutes, allowing the chromosomes to become visible under the microscope.
- A stained root tip was placed on a glass slide. Using a scalpel, the root tip was squashed gently to spread the cells, and a coverslip was placed on top.
- The prepared slide was examined under a light microscope, and the different stages of mitosis were observed at 40x magnification.

Observation and Result

The stages of mitosis—prophase, metaphase, anaphase, and telophase—were successfully observed in onion root tip cells. This experiment demonstrated the process of mitotic cell division, highlighting the organized distribution of genetic material into daughter cells, essential for growth and tissue repair.

13. Cell Division – Meiotic Stages in Tradescantia Flower Bud

Aim

To observe the stages of meiosis in Tradescantia flower buds and understand the process of meiotic cell division.

Principle

Meiosis is the process by which a diploid cell undergoes two successive divisions (meiosis I and meiosis II) to produce four haploid cells, each with half the chromosome number of the original cell. This process is essential for sexual reproduction, ensuring genetic diversity. The key stages of meiosis include prophase I, metaphase I, anaphase I, telophase I, followed by prophase II, metaphase II, anaphase II, and telophase II. Tradescantia flower buds, due to their meiotically dividing pollen mother cells, are ideal for observing meiotic stages.

Materials Required

Tradescantia flower buds
1% Acetocarmine or Acetic orcein stain
Carnoy's fixative (ethanol
acid, 3:1)
Hydrochloric acid (1 M)
Microscope
Glass slides and coverslips
Scalpel or forceps
Beaker
Filter paper
Watch glass
Distilled water

Procedure

- Young flower buds of Tradescantia, about 3-5 mm long, were collected as they are in the right stage for observing meiosis.
- The flower buds were fixed in Carnoy's fixative for 24 hours to preserve the cells and prevent degradation.
- After fixation, the buds were treated with 1 M hydrochloric acid for 5 minutes to soften the tissue and aid in the staining process.
- The flower buds were stained with 1% acetocarmine or acetic orcein for 15 minutes to make the chromosomes visible.
- A single stained flower bud was placed on a slide and gently squashed with a coverslip to spread the meiotic cells.
- The slide was observed under a microscope at 40x magnification to identify and study the various stages of meiosis.

Observation and Result

The meiotic stages—prophase I, metaphase I, anaphase I, telophase I, and the subsequent phases of meiosis II—were successfully observed in Tradescantia flower buds. The experiment demonstrated the reduction in chromosome number and the genetic variation achieved through crossing over and independent assortment, highlighting the importance of meiosis in sexual reproduction.

14. Cell Division – Binary Fission in Yeast

Aim

To observe and understand the process of binary fission in yeast cells.

Principle

Binary fission is a simple form of asexual reproduction in unicellular organisms like yeast, where a single cell divides into two identical daughter cells. In yeast, binary fission occurs after the replication of genetic material, followed by the division of the cytoplasm. This process ensures that each daughter cell receives a complete set of genetic material, allowing the population to grow rapidly under favorable conditions.

Materials Required

Yeast culture (Saccharomyces cerevisiae), Glucose solution (10%), Glass slides and coverslips, Microscope, Methylene blue or safranin stain, Dropper, Beaker, Sterile distilled water, Incubator or warm water bath.

Procedure

- A small amount of yeast culture was mixed in a beaker containing a 10% glucose solution to provide nutrients for cell growth and division.
- The mixture was incubated at room temperature for 30 minutes to promote active binary fission.
- A drop of the yeast suspension was placed on a clean glass slide using a dropper.

- A coverslip was gently placed over the drop to create a thin layer of the suspension for microscopic observation.
- Methylene blue was added to the slide to make the yeast cells more visible under the microscope.
- The slide was left for 2-3 minutes to allow proper staining of the cells.
- The slide was placed under a light microscope, and the yeast cells undergoing binary fission were observed at 40x magnification.
- Different stages of binary fission were noted.

Observation and Result

Binary fission in yeast was successfully observed. The process began with the formation of a bud on the parent cell, followed by nuclear division and cytoplasmic separation, resulting in the formation of two genetically identical daughter cells. This experiment demonstrated the simple, rapid method of reproduction in yeast through binary fission.

15. Polytene and Diplotene Chromosomes in Chironomus Larva

Aim

To study the structure of polytene and diplotene chromosomes in the salivary glands of Chironomus larva.

Principle

Polytene chromosomes are oversized chromosomes formed by repeated rounds of DNA replication without cell division (endoreplication), resulting in large, visible chromosomes with distinct banding patterns. These chromosomes are typically found in the salivary glands of dipteran larvae, such as Chironomus. Polytene chromosomes provide insights into gene activity and chromosomal structure. Diplotene chromosomes, on the other hand, are a stage in meiotic prophase I, where homologous chromosomes partially separate but remain attached at chiasmata.

Materials Required

Chironomus larvae, Dissecting microscope, Glass slides and coverslips, Acetic orcein stain, Dissecting needles, Forceps, Scalpel, Dropper, 45% acetic acid solution, Microscope, Distilled water

Procedure

- Larvae of Chironomus (midge) were collected from ponds or aquatic environments, where they are commonly found.
- Under a dissecting microscope, the larvae were placed on a clean glass slide, and the salivary glands were dissected out carefully using forceps and a scalpel.
- The dissected salivary glands were treated with a few drops of 45% acetic acid for 1-2 minutes to fix the tissue and make the chromosomes easier to observe.

- A few drops of acetic orcein stain were added to the salivary glands on the slide. The glands were gently squashed using a coverslip to spread the cells, ensuring that the polytene chromosomes were well-distributed.
- The prepared slide was observed under a light microscope at 40x and 100x magnification to study the polytene chromosomes. The distinct banding patterns and structure of the chromosomes were noted.

Observation and Result

Polytene chromosomes with characteristic banding and puffing patterns were successfully observed in the salivary glands of Chironomus larvae. These large chromosomes provided a clear view of gene expression and chromosomal structure.

16. Microtome – Preparation of Temporary and Permanent Slides

Aim

To prepare temporary and permanent slides of tissue sections using a microtome for microscopic observation of tissue structure.

Principle

Microtomy is a technique used to cut extremely thin sections of tissue, allowing for detailed microscopic examination of cells and tissues. The tissue sections are sliced using a microtome and can be prepared as either temporary or permanent slides. Temporary slides are prepared for immediate observation, while permanent slides involve the embedding of tissues in paraffin wax and staining for long-term preservation and analysis.

Materials Required

Microtome, Tissue specimen (e.g., plant stem, animal tissue), Fixative (e.g., formalin), Paraffin wax (for permanent slides), Embedding cassettes, Microtome blades, Glass slides and coverslips, Staining solutions (e.g., eosin, hematoxylin), Xylene and ethanol (for dehydration), Canada balsam or DPX (for mounting permanent slides), Water bath
Forceps and scalpels, Microscope

Procedure

A. Temporary Slide Preparation

- The tissue specimen was fixed in a fixative solution (10% formalin) for several hours to preserve the structure and prevent decomposition.
- The fixed tissue was placed on the microtome, and thin sections (5–10 μm) were carefully cut using a sharp microtome blade.
- The thin sections were floated on a warm water bath to flatten them and remove wrinkles before transferring to glass slides.
- The tissue sections were transferred to clean glass slides using forceps and water. A drop of water or glycerin was added to the section, and a coverslip was placed on top.
- The prepared slide was immediately observed under a microscope at 40x magnification for temporary analysis.

B. Permanent Slide Preparation

- The tissue was dehydrated by passing it through a series of ethanol solutions with increasing concentrations (50%, 70%, 90%, and 100%).
- After dehydration, the tissue was cleared using xylene to make it transparent, removing any remaining alcohol.
- The cleared tissue was placed in molten paraffin wax and embedded in a wax block. This block was allowed to solidify for sectioning.
- The paraffin-embedded tissue block was mounted on the microtome, and thin sections (5–10 µm) were cut.
- The tissue sections were transferred to a water bath to flatten and then placed onto clean glass slides.
- The sections were treated with xylene to remove paraffin wax and rehydrated through a descending series of ethanol concentrations (100%, 90%, 70%).
- The slides were stained using hematoxylin (to stain nuclei) and eosin (to stain cytoplasm and extracellular matrix), enhancing the contrast between cellular structures.
- After staining, the sections were again dehydrated through ethanol series and cleared with xylene.
- A drop of Canada balsam or DPX mountant was placed on the section, and a coverslip was applied. The slides were allowed to dry.
- The permanent slides were observed under the microscope to study the tissue structure, which was now preserved for long-term analysis.

Observation and Result

Both temporary and permanent slides were successfully prepared using the microtome. The temporary slides allowed for quick observation, while the permanent slides provided detailed, long-lasting specimens for in-depth tissue study. The staining in permanent slides enhanced the visibility of cellular structures and allowed for better differentiation between various components of the tissue.