University Center Abdelhafid Boussouf- Mila

Life and Nature Science Institute

Domain: *Life and Nature Science*

SUPPORT DOCUMENT OF PRACTICAL WORKS IN CELL BIOLOGY

Intended to the first year students pursuing a Bachelor's degree,
Common core (LMD)

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Content

I. Introduction

Cell biology is a fascinating field that explores the structure, function, and behavior of cells the fundamental units of life. Understanding cell biology is crucial for comprehending the intricacies of living organisms, as cells are the building blocks that form tissues, organs, and ultimately, entire organisms. In the pursuit of scientific knowledge, hands-on practical work is invaluable, providing students with a unique opportunity to apply theoretical concepts, enhance critical thinking skills, and gain practical laboratory experience.

The Cell Biology Practical Work Support Document serves as a comprehensive guide for both students and teachers engaged in this practical exploration. This document aims to bridge the gap between theoretical knowledge and practical application, offering a structured framework for conducting experiments, making observations, and drawing meaningful conclusions.

Importance for Students

- ✓ **Experiential Learning**: Practical work in cell biology allows students to move beyond textbooks and lectures, engaging with real specimens and instruments. This hands-on experience fosters a deeper understanding of cellular structures and processes.
- ✓ **Skill Development**: The practical work involves various laboratory techniques, such as slide preparation, staining, and microscopy. Students not only learn the theoretical aspects of these techniques but also develop essential laboratory skills that are transferable to future scientific endeavors.
- ✓ **Critical Thinking**: By actively participating in the practical work, students are encouraged to analyze and interpret their observations. This cultivates critical thinking skills, helping them draw connections between theoretical concepts and empirical evidence.
- ✓ **Scientific Inquiry**: The practical work encourages a scientific mindset, as students hypothesize, experiment, and draw conclusions based on their findings. This process mirrors the scientific method and instills a sense of inquiry and curiosity.

Importance for Teachers

- ✓ **Enhanced Teaching Effectiveness**: The Support Document provides teachers with a structured plan for conducting the practical work, ensuring clarity in communication and

consistent delivery of content. It serves as a valuable resource to align teaching objectives with practical outcomes.

- ✓ **Safety Guidelines**: The document outlines safety precautions, promoting a secure laboratory environment. Teachers can use this information to educate students about responsible laboratory practices and adherence to safety protocols.
- ✓ **Assessment Tool**: The practical work serves as an assessment tool for teachers to evaluate students' understanding of cell biology concepts. The document provides guidance on expected outcomes, allowing teachers to assess the effectiveness of the practical session.
- ✓ **Resource for Classroom Discussions**: The document can be utilized as a reference during post-practical discussions. Teachers can refer to specific sections to reinforce key concepts, address common challenges, and facilitate meaningful class interactions.

II. Target Audience

This Practical Work support document was intended for 1st-Year Bachelor's Degree (LMD), Common Core, Department of Natural and Life Sciences

III. Prerequisites

Prerequisites for the Cell Biology Practical Work include a foundational understanding of basic biology concepts. Students undertaking this practical work should ideally have knowledge in the following areas:

- ✓ Cell Structure and Function: A basic understanding of the structure and function of eukaryotic and prokaryotic cells.
- ✓ Microscopy Basics: Familiarity with the principles of light microscopy and an understanding of how to use a microscope.
- ✓ Laboratory Safety: Awareness of laboratory safety protocols and the ability to handle laboratory equipment and chemicals responsibly.
- ✓ Basic Chemistry: Fundamental knowledge of chemical concepts, as some practical work may involve the use of stains and chemical reagents.

IV. General Objectives:

- ✓ The general objective of the Cell Biology Practical Work is to provide students with hands-on experience and practical skills in the field of cell biology. This practical work aims to:

- ✓ Foster Understanding: Deepen students' comprehension of fundamental cell biology concepts, including cell structure, function, and organization.
- ✓ Develop Laboratory Skills: Equip students with essential laboratory techniques such as slide preparation, staining, microscopy, and aseptic handling of cell cultures.
- ✓ Enhance Critical Thinking: Encourage critical thinking and analytical skills by allowing students to make observations, draw conclusions, and relate practical findings to theoretical knowledge.
- ✓ Promote Scientific Inquiry: Instill a scientific mindset by engaging students in the scientific method, where they hypothesize, experiment, and analyze results to understand cellular phenomena.
- ✓ Encourage Teamwork: Provide opportunities for collaborative learning and teamwork, fostering effective communication and cooperation in a laboratory setting.
- ✓ Emphasize Safety: Prioritize and reinforce laboratory safety practices, ensuring students are aware of and adhere to proper safety protocols.
- ✓ Bridge Theory and Practice: Establish a connection between theoretical knowledge acquired in lectures and the practical application of cell biology concepts, promoting a holistic understanding of the subject.

PW 0: Recommendations and Safety Rules

1. Introduction

Practical Work 0 (PW 0) in Cell Biology marks the beginning of your applied exploration into the fascinating world of cell biology. PW 01 is designed to introduce you to essential laboratory techniques and safety protocols, laying the foundation for future experiments throughout the semester.

2. Objective

PW 0 aims to familiarize you with fundamental laboratory techniques used in cell biology. You will learn the proper handling of equipment, preparation of specimens, and use of basic laboratory instruments. Your safety is our priority, so this practical work emphasizes the importance of adhering to safety rules and guidelines in the laboratory. Understanding and following these safety protocols is crucial for a secure and productive laboratory experience.

3. Recommendations

- **Preparation**: Familiarize yourself with the experimental protocol before entering the laboratory. Understanding the procedures in advance will enhance efficiency and comprehension.
- **Collaboration**: Work in pairs as assigned. Encourage open communication and collaboration with your lab partner to ensure smooth workflow and accurate observations.
- **Attention to Detail**: Pay careful attention to measurement units, timings, and specific instructions provided in the protocol. Precision is crucial for reliable results.
- **Note-Taking**: Maintain a detailed record of your observations and steps taken during the experiment. Accurate note-taking is essential for report writing and future reference.
- **Equipment Handling**: Treat laboratory equipment with care. Report any malfunctioning or damaged equipment to the instructor immediately.

4. Safety Rules

- **Lab Attire**: Wear appropriate laboratory attire, including a lab coat and safety goggles, throughout the practical session. This ensures personal safety and helps prevent contamination.
- **Chemical Handling**: Handle chemicals with caution. Follow the provided instructions for the correct usage, storage, and disposal of chemicals. Never taste or smell chemicals.
- **Microscope Usage**: Use microscopes responsibly. Avoid touching the lenses with bare hands and switch off the microscope after use.
- **Emergency Procedures**: Familiarize yourself with the location of emergency exits, safety equipment (e.g., fire extinguisher, eyewash station), and emergency procedures. Report any accidents or spills immediately.
- **Gloves**: Use disposable gloves when handling specimens or chemicals. Dispose of used gloves properly in designated bins.
- **No Food or Drinks**: Do not consume food or drinks in the laboratory to prevent contamination and ensure personal hygiene.
- **Aseptic Techniques**: If applicable, practice aseptic techniques when working with cell cultures. Contamination can impact experimental results.
- **Cleanup**: Clean your workstation before leaving. Dispose of used materials in designated bins and return equipment to its proper place.

Figure 01 : Recommandations and safety rules

PW 01: The light microscope

1. Introduction

The light microscope is an optical instrument that allows the observation of very thin objects (which can be penetrated by light) by magnifying them (15 to 1800 times). The object to be observed, called a specimen, is placed between a glass slide and a coverslip. There are other microscopes, known as electron microscopes that enable higher magnifications.

2. Objective

This practical session aims to familiarize you with the proper usage of a light microscope for observing microscopic specimens. Microscopes are essential tools in biology and many other scientific fields.

3. Light microscope parts

3.1. Eyepiece (Ocular): This is the part you look through. It contains a lens that typically magnifies the image by 10x.

3.2. Nosepiece (Revolving Turret): The nosepiece holds and allows for the rotation of objective lenses. You can switch between different objectives for varying levels of magnification.

3.3. Objective Lenses: These lenses are attached to the nosepiece and come in different magnification powers (e.g., 4x, 10x, 40x, 100x). They are responsible for magnifying the specimen.

3.4. Stage: The stage is where you place the specimen slide. It often has a mechanical stage with knobs for precise movement of the slide.

3.5. Mechanical Stage Controls: These controls include knobs for moving the slide left-right (x-axis) and forward-backward (y-axis) on the stage.

3.6. Condenser: The condenser is located beneath the stage and focuses light onto the specimen. It can be adjusted to control the intensity and angle of the light.

3.7. Iris Diaphragm: This is part of the condenser and controls the amount of light passing through the specimen.

3.8. Coarse Focus Knob: The coarse focus knob is used for initial focusing. It moves the stage or the objective lenses up and down in large increments.

3.9. Fine Focus Knob: The fine focus knob is used for precise focusing. It moves the stage or the objective lenses up and down in very small increments.

3.10. Arm : The arm is the curved part that connects the base to the head of the microscope. It is used for carrying the microscope.

3.11. Base: The base is the bottom part of the microscope that provides stability and support.

3.12. Light Source (Lamp): Most microscopes have an adjustable light source, often located at the base, to illuminate the specimen.

3.13. On/Off Switch: This switch controls the power supply to the microscope's light source.

3.14. Eyepiece Tube (Head): The eyepiece tube holds the eyepiece and connects it to the objective lenses.

4. Procedure

4.1. Microscope Setup: a. Place the microscope on a clean, stable, and level surface. b. Plug in the microscope and turn on the light source. c. Adjust the intensity of the light using the diaphragm or light control knob. d. Make sure the objective lenses (usually 4x, 10x, and 40x) are properly attached to the nosepiece. e. Start with the lowest magnification objective (4x) in position.

4.2. Slide Preparation

- Examine your prepared microscope slides and choose one to start with.
- Carefully place a clean glass slide on the stage of the microscope.
- Place the prepared microscope slide (with the specimen) on top of the glass slide.
- Gently lower the coverslip onto the specimen, ensuring there are no air bubbles.

4.3. Initial Observation

- Secure the slide in place with the mechanical stage clips.
- Look through the eyepiece and use the coarse adjustment knob to bring the specimen into rough focus.
- Use the fine adjustment knob to fine-tune the focus until the image is clear.
- Observe the specimen, noting any specific structures or features.

4.4. Magnification Adjustment

- Rotate the nosepiece to switch to a higher magnification objective (e.g., 10x or 40x).
- Refocus using the fine adjustment knob if necessary.
- Continue to observe and take note of any changes in detail.

4.5. Record Observations

- Use your laboratory notebook to record your observations, including the magnification level, the specimen's appearance, and any notable structures.
- Sketch any interesting findings if required.

4.6. Slide Handling and Cleaning

- Remove the slide from the stage and return it to its designated location.
- If you used an oil-immersion objective (usually 100x), clean the objective with lens cleaning solution and paper.
- Always handle slides with care to avoid breakage or contamination.

4.7. Shutting Down

- Turn off the microscope's light source.
- Unplug the microscope.
- Return the objective lens to the lowest magnification (4x) position.
- Wind the power cord and store it neatly.
- Wipe down the microscope with a clean, dry cloth.

5. Conclusion

This PW session has introduced you to the basic operation of a light microscope and the observation of microscopic specimens. Practice and careful handling of the microscope are essential for accurate observations

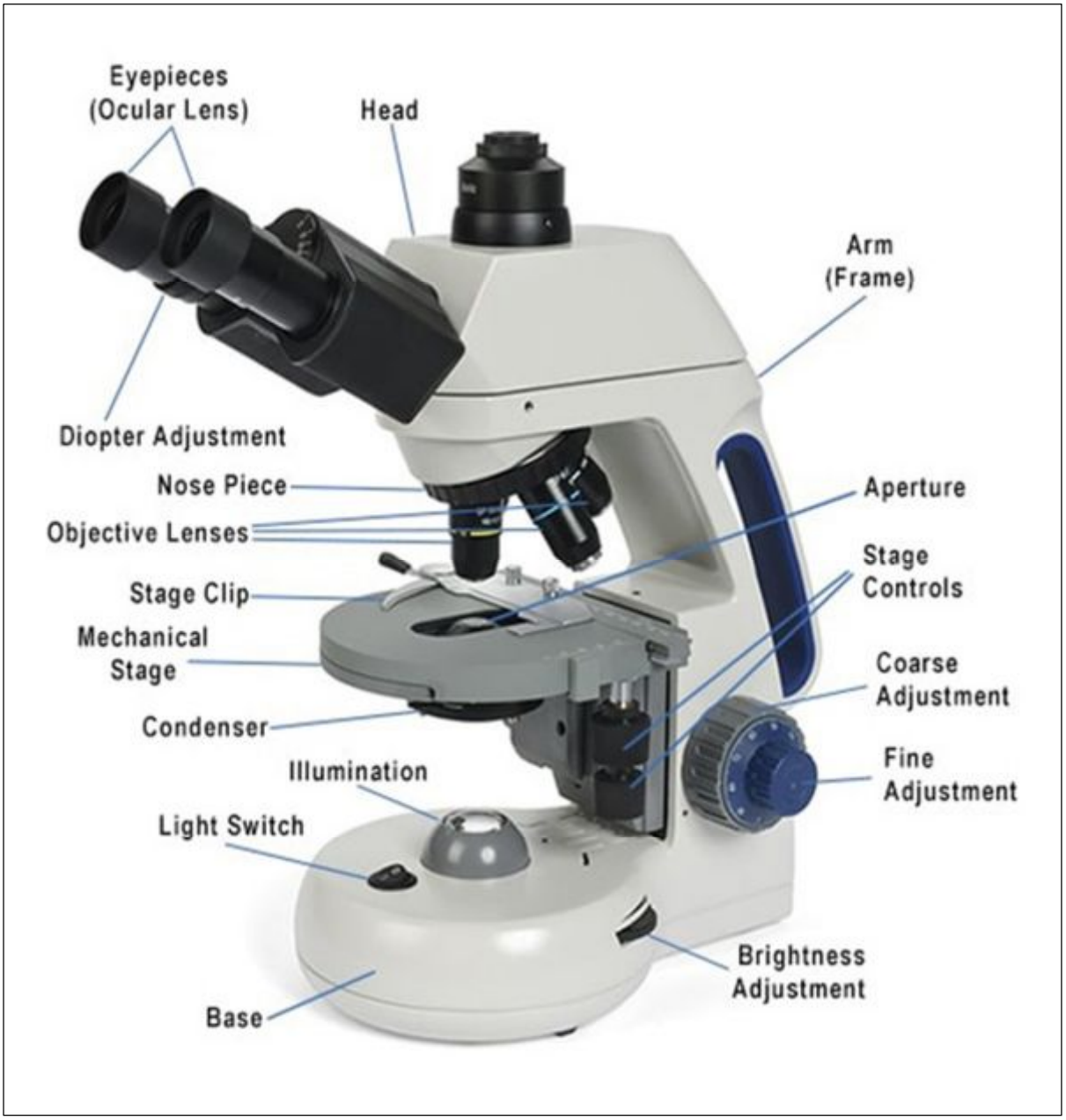

Figure 02 : A light microscope

PW 02: Tissue Processing for Histological Sample Preparation

1. Introduction

Histological examination, a fundamental technique in the field of pathology and biomedical research, allows us to gain valuable insights into the microarchitecture and cellular composition of tissues. Histological examination serves as an indispensable tool for diagnosing diseases, understanding physiological processes, and conducting research across various scientific disciplines.

To ensure the accuracy and reliability of histological analyses, the quality of tissue preparation plays a pivotal role. This tissue processing protocol aims to provide a comprehensive guide for the proper preparation of histological samples, starting from tissue collection to the generation of high-quality tissue sections suitable for microscopic examination.

2. Objective

This practical session aims to prepare histological samples for microscopic examination.

3. Materials

- Tissue specimens
- Formalin (10% neutral buffered formalin)
- Disposable gloves
- Tissue cassettes
- Tissue processing machine
- Embedding molds
- Paraffin wax
- Microtome
- Glass slides
- Microscope

4. Procedure

4. 1. Collection of Tissue Specimens

- Collect the tissue samples of interest using appropriate surgical or biopsy procedures.

- Ensure proper identification and labeling of each specimen.

Figure 03: Collection of Tissue Specimens

4. 2. Fixation

- Place the tissue specimens in containers filled with 10% neutral buffered formalin.
- Ensure that the volume of formalin is sufficient to cover the specimens completely.
- Fix the tissues for an appropriate duration (typically 24-48 hours) at room temperature.

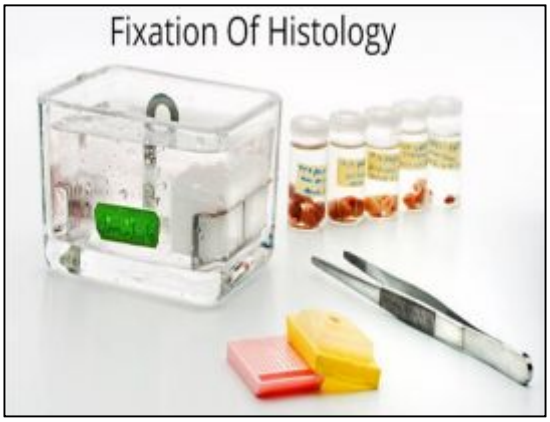

Figure 04: Fixation of histology

4.3. Dehydration

- After fixation, transfer the specimens into a series of alcohol solutions with increasing concentrations (e.g., 70%, 80%, 90%, and 100% ethanol).
- Allow specimens to stay in each solution for a specified duration (e.g., 30 minutes each).
- Ensure proper labeling and tracking of specimens during this process.

Figure 05: Tissue processor (automated instrument)

4.4. Clearing

- Place the dehydrated specimens in a clearing agent (e.g., xylene) to remove the alcohol and make the tissues transparent.
- Monitor and replace the clearing agent as needed until the tissues become fully transparent.

Figure 06: Clearing of specimens

4.5. Embedding

Embedding is the step that follows fixation in a fixative solution. It consists in hardening the sample in a paraffin embedding medium, in order to be able to carry out the sectioning.

- Embed the dehydrated and cleared specimens in paraffin wax.
- Use embedding molds to hold the tissues while the wax solidifies.

- Trim excess wax around the specimens to form a block.

Figure 07: Embedding of specimens in Paraffin wax

4.6. Sectioning

- Use a microtome to cut thin sections (typically 4-5 micrometers thick) from the paraffin-embedded tissue blocks.
- Float the sections on a warm water bath, pick them up on glass slides, and allow them to dry at room temperature.

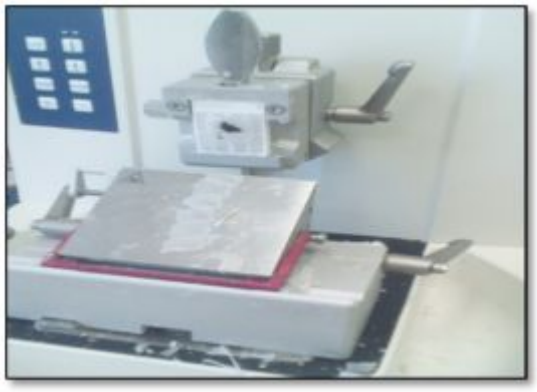

Figure 08: A microtome for sectioning

4.7. Rehydration

If required for specific staining techniques, you can rehydrate the sections by moving them through a reverse alcohol series (e.g., from 100% ethanol back to 70% ethanol) before proceeding with staining**.**

4.8. Staining

Stain the tissue sections using appropriate histological stains (e.g., hematoxylin and eosin) these histochemical stains used to provide contrast to tissue sections, making tissue structures more visible and easier to evaluate.

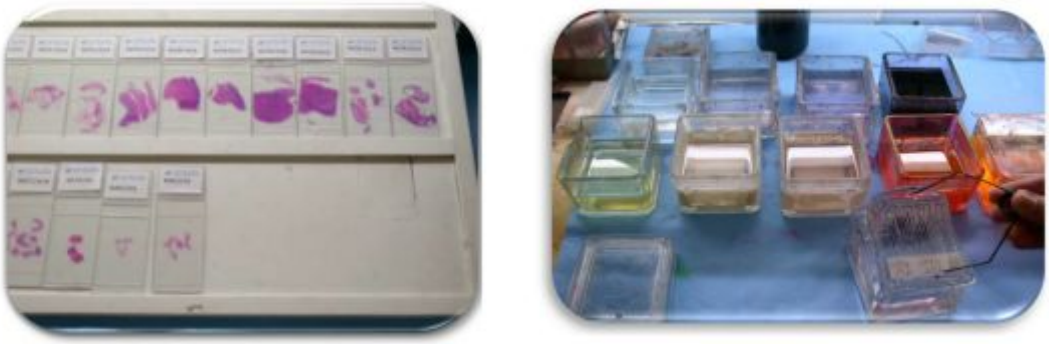

Figure 09: histo-chemical staining

4.9. Mounting

Apply a coverslip over the stained tissue sections using mounting medium to facilitate microscopic examination.

Figure 09: Mounting

4.10. Microscopic Examination

Examine the prepared histological slides under a light microscope to analyze tissue structures and cellular morphology.

5. **Conclusion**: This practical session on preparing histological samples for microscopic examination has provided students with essential hands-on experience and skills crucial for understanding cellular structures.

PW0 3: Microscopic study of plant cells

(Observation of an onion epidermis *Allium cepa*)

1. Introduction

Observing the internal epidermal tissue of an onion bulb provides a remarkable opportunity to delve into the intricate world of plant cell structure. Within an onion bulb, a vertical cross-section reveals a concise stem which supports a network of adventitious roots and nested layers of scales. The outermost scales appear desiccated, while the inner layers are laden with nutrient reserves. At the core, the central bud remains shrouded by delicate scales.

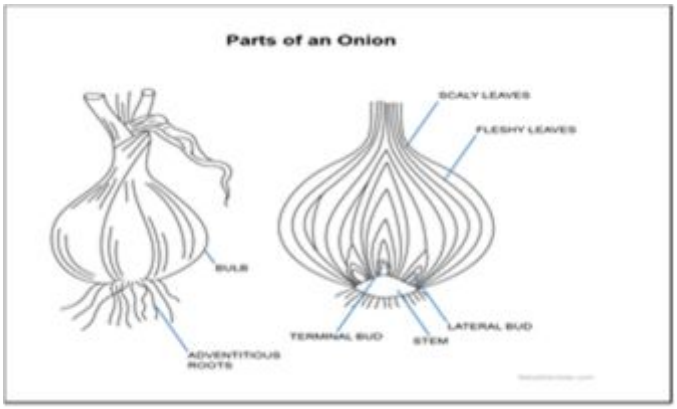

Figure 10: Parts of an anion

2. Objective

The aim of this practical session is to explore and understand the essential characteristics of plant cells through the microscopic examination of onion epidermal tissue. This includes the use of vital and post-vital staining techniques to investigate cell structures and their responses to different staining methods.

3. Materials and reagents

- Microscope
- Glass slides and cover slips
- 1 fresh onion
- 1 sharp knife
- Fine forceps
- 2 watch glasses per workstation

- 1g/L neutral red solution (Prepare by dissolving 0.1g of neutral red in 100 ml of phosphate buffer with a pH of 6.5. Note that neutral red penetration into cells is pH-dependent.)
- Iodine-iodide solution (Prepare by mixing 4g of iodine and 8g of KI in 1L of distilled water)
- Crystallizing dish with bleach for cleaning used slides and cover slips

4. Procedure of slides preparation

- Simultaneously prepare two different dyes in separate watch glasses: Neutral red and Iodine-iodide solution.
- Using fine forceps, carefully collect small fragments of epidermis from the concave side of an onion scale.
- Immediately immerse these fragments in the prepared dye solutions, placing 2-3 fragments in each watch glass.
- On one microscope slide, add a drop of the neutral red solution, followed by the placement of 1-2 epidermal fragments.
- Secure with a cover slip.
- Repeat the same process with the second microscope slide, using the iodine solution.

5. Observations (Objectives x10 and x40)

A. Neutral Red Staining (Vital Staining)

Neutral red serves as a vital stain, allowing cellular penetration without compromising cell viability. Follow these steps:

- Using the lowest magnification objective (x10), focus on a clear image of the slide preparation, and center a plant cell within the microscope's field of view.
- Switch to high magnification (x40).
- Observe and record your findings, including the staining pattern, cell morphology, the presence of vacuoles, the location of the nucleus and nucleoli, and the characteristics of the pecto-cellulosic cell wall.

B. Iodine Staining (Post-Vital Staining)

Iodine serves as a post-vital stain, effectively fixing cells while imparting a yellow color to specific cellular structures. Proceed as follows:

- Repeat the observation process described in 4.1, but this time using the iodine-stained slide.
- Document your observations, including any changes in cellular structure and the distinctive yellow tint of certain cell components.

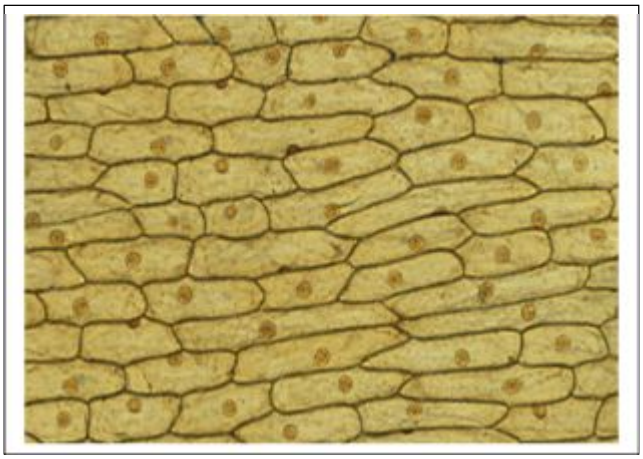

Figure 11: Microscopy of onion cells (X400)

6. Conclusion

This microscopic exploration has not only deepened our understanding of the fundamental building blocks of plants but has also honed our skills in sample preparation, staining techniques, and microscope operation. As we examined cells under different magnifications, we discovered the beauty and diversity inherent in plant cellular structures

PW04. Microscopic study of animal cells

1. Introduction

The microscopic investigation of animal cells provides a valuable opportunity to delve into the details of their structure and function. In this practical session, we will focus on buccal epithelial cells from the inner cheek lining. Through careful preparation and staining, we will gain insights into the characteristics and organization of these cells, allowing for a deeper understanding of their role in the functioning of living organisms.

2. Objective

The aim of this practical session is to explore the microscopic features of animal cells, specifically buccal epithelial cells, using appropriate staining techniques. By observing these cells, we aim to identify their structural characteristics and variations, which will provide valuable insights into their functions within the animal organism.

3. Materials and Reagents

- Microscopes
- Glass slides and cover slips
- Spatula
- Paper towels
- 0.01% methylene blue solution (Dissolve 100 mg of methylene blue powder in 100 mL of distilled water)
- Ordinary water spray
- Dropper
- Staining dish
- Crystallizing dish with bleach for used slides and cover slips
- Clean fingers for buccal cell collection

4. Procedure

- Rinse the mouth with water.
- Gently rub the inner cheek wall with a clean finger to collect buccal cells.
- Place the collected material on a slide and add a few drops of hematoxylin-eosin or methylene blue.
- Rinse with water after 5 minutes and wipe the slide clean.

- Cover the preparation with a cover slip.
- Observe the buccal epithelial cells under high magnification, noting staining patterns, cell size, membrane appearance, and the presence of granules.

5. Conclusion

The microscopic examination of buccal cells has provided valuable insights into the intricate world of animal cells. Through careful observation and analysis, we've gained a deeper understanding of the structure and characteristics of buccal cells. The study has highlighted the importance of microscopy as a tool for exploring the microscopic landscape; enabling us to appreciate the complexity and diversity of animal cells.

Finally, create a summary table of the distinctive morphological and structural characteristics of animal and plant cells.

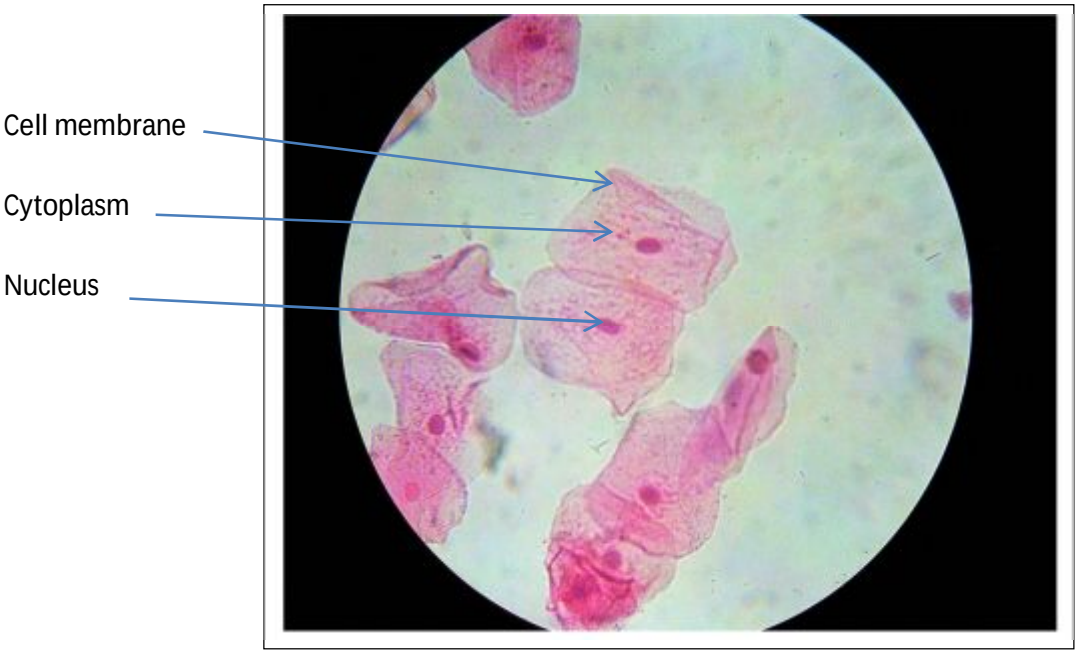

Figure 12: Microscopy of animal cells (Buccal cells X400)

PW 05: Study of a Ciliated Protozoan (*Paramecium caudatum*)

1. Introduction

Protozoa (Protos = First, primitive, and Zoon = animal) are the earliest animals in the evolutionary series. They are microscopic, unicellular, and heterotrophic organisms. Despite being single-celled, they possess the fundamental functions of any animal, with essential organelles such as mitochondria, Golgi apparatus, lysosomes, microtubules, and others.

Depending on the species, protozoa either feed through osmosis (in biological environments as parasites) or through phagocytosis (in aquatic environments as free-living forms). Reproduction occurs through body division (binary fission) and, in certain environmental conditions, sexual reproduction may occur.

Paramecium caudatum

Paramecium caudatum, a unicellular ciliate, offers an intriguing glimpse into the microscopic world of protists. Paramecium serves as an excellent model for studying the physiology, locomotion, and feeding mechanisms of protists. The cell size varies from 50 to 300 µm in length depending on the species. Paramecium utilizes cilia for both movement and feeding. The somatic cilia, covering the cell and beating in a coordinated manner, enable it to move. A distinct oral cilia cover the large funnel-shaped ventral invagination, the peristome, leading to the cytostome (the mouth). It primarily feeds on bacteria through phagocytosis.

Paramecium lives in freshwater environments and was historically categorized as an "infusorian" by early researchers due to its abundance in plant infusions, making its cultivation and study straightforward.

2. Objective

This practical session is designed to provide hands-on experience in observing the behavior and microscopic structure of *Paramecium caudatum*. As a unicellular organism

3. Materials

- Microscope
- Glass slides and coverslips
- Droppers or pipettes

- Culture of *Paramecium*

4. Procedure

- Using a pipette, carefully draw a drop of water from the maceration containing Paramecia.
- Place this drop between a slide and a cover slip, then proceed to observe under low magnification using the microscope.
- You will notice that Paramecia move swiftly, making their observation challenging.
- To limit their movement and facilitate observation, gently introduce a small drop of acetic acid into the water containing Paramecia. Acetic acid will act as a fixative, helping to immobilize the Paramecia on the slide, allowing for a more detailed observation of their structure and behavior.
- After the addition of acetic acid, reobserve the Paramecia under the microscope. You should observe a decrease in their mobility, facilitating the examination of their morphological and behavioral characteristics.

5. Conclusion

Our exploration into the microscopic world of Paramecium caudatum has unveiled the remarkable complexity and functionality of ciliated protozoans. Through careful observation and experimentation, we have delved into the intricacies of their locomotion, feeding mechanisms, and overall life processes. This practical work has not only enhanced our understanding of protozoan biology but has also underscored the significance of studying these microorganisms in broader ecological and biological contexts.

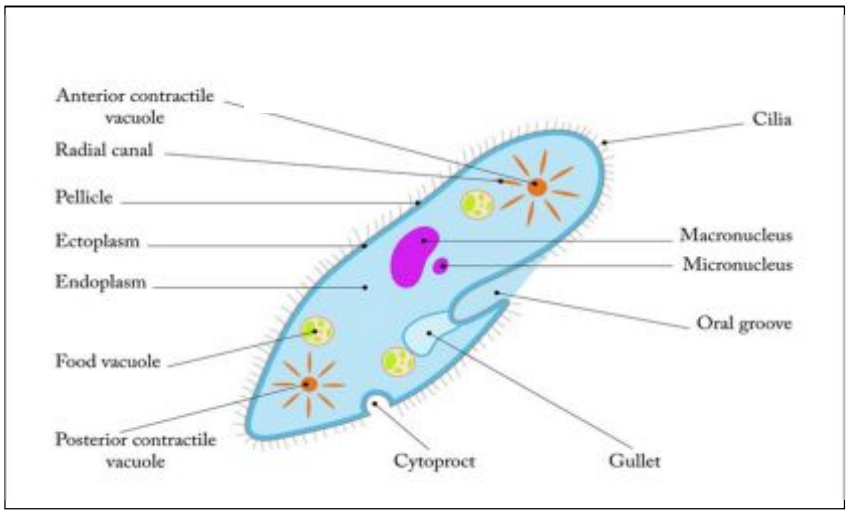

Figure 13: Structure of *Paramecium caudatum*

PW06: Observation of plastids

1. Introduction

Plastids are intracellular organelles exclusively found in plant cells. The observation of plastids, specifically amyloplasts, chloroplasts, and chromoplasts, is a fundamental aspect of understanding the intricate cellular structures present exclusively in plant cells. Plastids play crucial roles in the synthesis and storage of various essential substances, such as starch, chlorophyll, and carotenoid pigments.

2. Objective

This PW aims to delve into the distinct characteristics of amyloplasts, chloroplasts, and chromoplasts, shedding light on their roles within plant cells and enhancing our comprehension of cellular diversity and microscopy skills through detailed observation of cellular components at varying magnifications.

3. Materials

- Microscope, slides, cover slips,
- Cotton, a crystallizing dish (with bleach) for used slides and cover slips.
- Stain: very diluted Lugol's solution.
- 1 lancet needle, 1 porcelain or plastic dish
- 1 spray bottle with tap water.
- Fine forceps, razor blade
- Potato (*Solanum tuberosum*).
- Green bell pepper (*Capsicum annuum*) or spinach leaves.
- Red pepper or tomato.

4. Procedure

4.1. Amyloplasts

Amyloplasts, organelles that store starch, were investigated in this experiment. Starch, a prevalent reserve substance in plants, accumulates in specialized plastids, gradually transforming into starch granules.

❖ **Slide preparation**

- Gently scrape a small piece of potato pulp with a lancet needle.

- Place a drop of water on a slide, dilute the collected product on it.
- Cover with a cover slip (avoiding air bubble formation).
- Observation without staining:
- Draw, title, and label. Starch grains or amyloplasts and their growth striations around a central point, the hilum, are clearly visible.

❖ **Observation with staining**

Prepare a new slide and add a drop of much diluted iodine solution. Amyloplasts stain in violet-blue (a characteristic reaction).

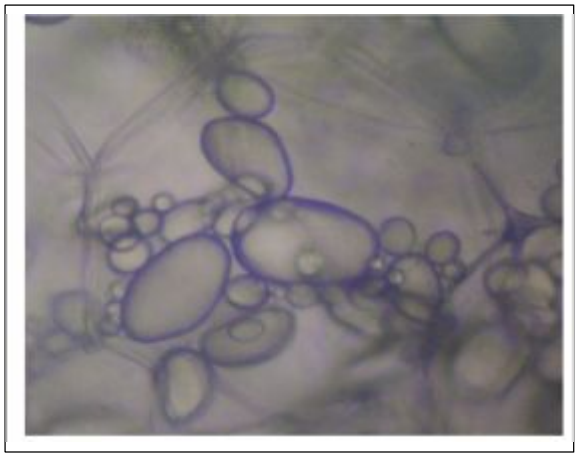

Figure 14: Microscopic observation of Amyloplasts (X400)

4.2.Chloroplasts

Similar to amyloplasts, chloroplasts are plant cell organelles containing chlorophyll. Leaves from the terminal bud of an Elodea plant (common in watercourses and ponds) are suitable for chloroplast observation. However, for this experiment, we used spinach leaves or green bell pepper.

❖ **Slide preparation**

Use fine forceps to make a thin section in the outer layer of the pepper, place it between a slide and cover slip, in a water droplet.

❖ **Observation**

Describe observations at low, medium, and high magnification: cell appearance, observed cellular components, plasma membrane, cytoplasmic aspect, vacuole shape, nucleus position, and chloroplast aspect and abundance.

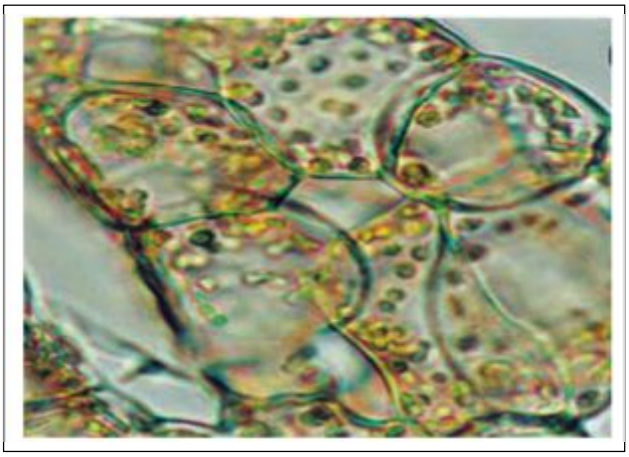

Figure15: Microscopic observation of chloroplasts (X400)

4.3.Chromoplasts

Chromoplasts are cellular organelles containing carotenoid pigments (yellow, red, or orange). Tomatoes, yellow peppers, and carrots are rich in chromoplasts.

❖ **Slide preparation**

Follow the same protocol as the previous manipulation.

❖ **Observation**

Describe observations at low, medium, and high magnification: cell appearance, observed cellular components, plasma membrane, cytoplasmic aspect, vacuole shape, nucleus position, and chromoplast aspect and abundance.

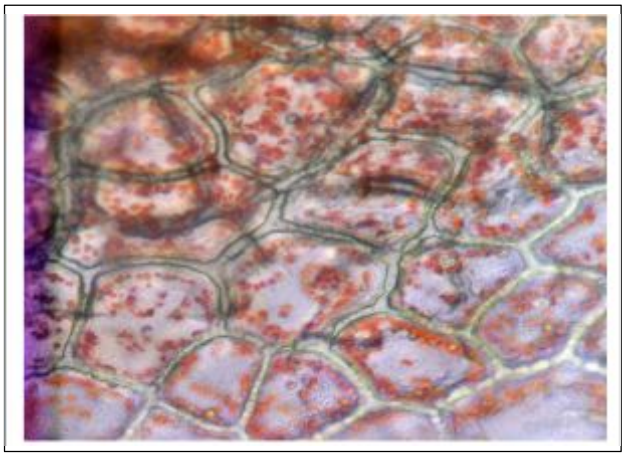

Figure16: Microscopic observation of Chromoplasts (X400)

5. Conclusion

In conclusion, this practical work provided valuable insights into the diverse plastids found in plant cells. The detailed observation of amyloplasts, chloroplasts, and chromoplasts highlighted their unique structures and functions within cellular processes. These observations not only contributed to a deeper understanding of plant cell biology but also honed microscopy skills. As budding scientists, this exploration sets the stage for further investigations into the intricacies of cellular diversity and its implications in the broader context of plant cell biology.

PW07: Study of cellular organelles ultrastucture using electron microscopy

1. Introduction

Cellular organelles play crucial roles in the functioning of eukaryotic cells, each contributing to specific cellular processes. Understanding their ultrastructure provides profound insights into their functions. Electron microscopy, with its high resolution and magnification capabilities, allows for a detailed exploration of cellular organelles' intricate morphology. In this study, we focus on mitochondria, endoplasmic reticulum, and Golgi apparatus, seeking to unravel their ultrastructural features and establish correlations between morphology and function. By employing electron microscopy, we aim to delve into the microscopic realm and uncover the nuances of these organelles, shedding light on their roles within the cellular landscape.

II. Objective

This PW aims to use electron microscopy for detailed observation and analysis of the ultrastucture of mitochondria, endoplasmic reticulum, and Golgi apparatus in order to gain insights into their cellular functions.

3. Materials

- Thin sections of cells with fixed and stained mitochondria, endoplasmic reticulum, and Golgi apparatus
- Electron microscope
- Specimen holder and carbon-coated grids
- Fixatives (e.g., glutaraldehyde)
- Stains (e.g., osmium tetroxide, uranyl acetate)
- Dehydration and embedding materials (e.g., ethanol, epoxy resin)
- Ultramicrotome
- Laboratory notebook
- Disposable gloves and lab coat

4. Procedure

A. Sample Preparation:

❖ **Fixation**

- Immerse specimens in a fixative solution (e.g., glutaraldehyde).
- Follow recommended fixation times.

❖ **Dehydration**

- Gradually dehydrate specimens using a series of ethanol concentrations.
- Ensure thorough dehydration.

❖ **Embedding**

- Infiltrate specimens with an embedding medium (e.g., epoxy resin).
- Embed specimens in molds for sectioning.

❖ **Ultramicrotomy**

- Cut ultrathin sections (around 70-90 nm) using an ultramicrotome.
- Collect sections on carbon-coated grids.

B. Staining

❖ **Stain sections with heavy metal stains**

- Osmium tetroxide for membrane contrast.
- Uranyl acetate for additional contrast.

C. Electron Microscopy

- Load the specimen grid into the electron microscope.
- Set appropriate imaging parameters (voltage, magnification, focus).
- Capture high-resolution images of mitochondria, endoplasmic reticulum, and Golgi apparatus.

D. Documentation

- Record details of the electron microscopy settings.
- Annotate observations in the laboratory notebook.
- Create labeled diagrams based on electron microscopy images.

6. Conclusion

In conclusion, the utilization of electron microscopy has allowed for a detailed exploration of the ultrastructure of mitochondria, endoplasmic reticulum, and Golgi apparatus. The high-resolution images have provided unprecedented insights into the intricate morphology of these organelles, showcasing the dynamic nature of cellular architecture. The mitochondria reveal their characteristic cristae and matrix structures, emphasizing their pivotal role in energy

production. The endoplasmic reticulum, with its rough and smooth regions, highlights its involvement in protein synthesis and lipid metabolism. The Golgi apparatus, portrayed as stacked membrane structures, underscores its significance in cellular trafficking and modification of biomolecules. This study underscores the importance of ultrastructural analysis in comprehending the functional intricacies of cellular components and paves the way for further investigations into the dynamic world of cellular organelles.

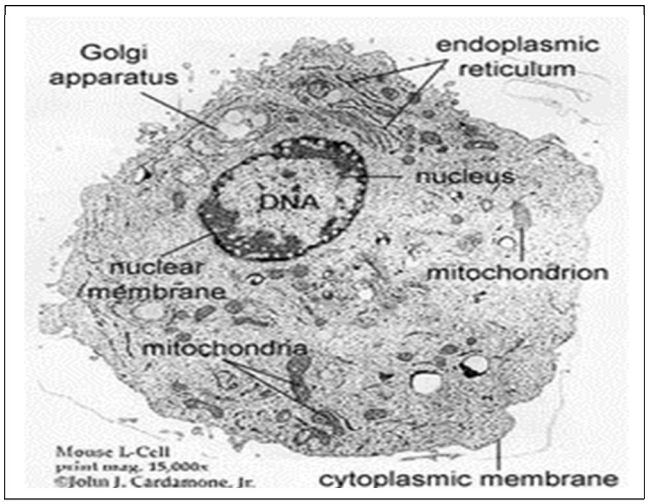

Figure 17: Electron microscopy of pancreatic cell

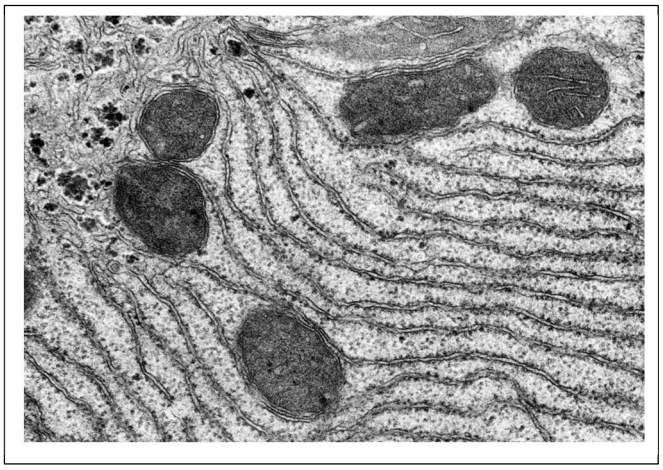

Figure 18 : Endoplasmic reticulum

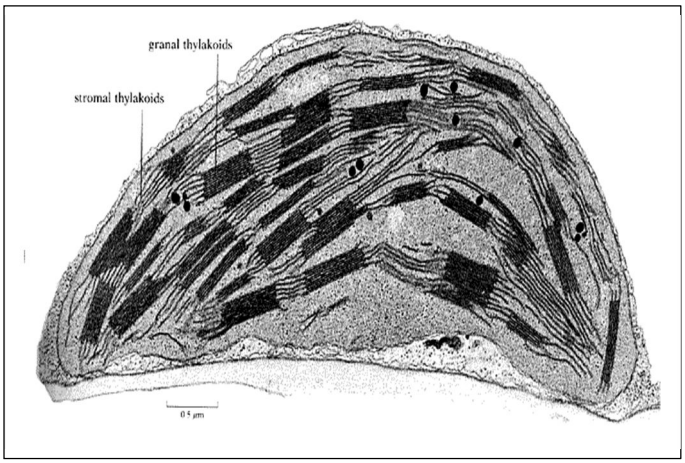

Figure 19 : Chloroplast

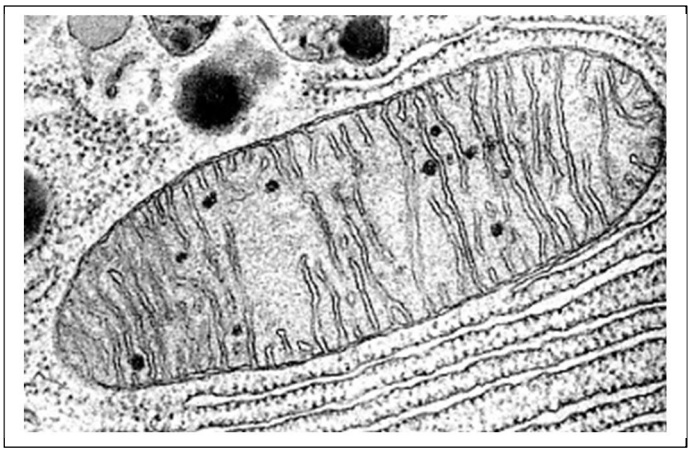

Figure 20 : Mitochondria

Figure 21 : Golgi apparatus

PW08: Study of cell permeability and osmosis in red onion cells

1. Introduction

Cell permeability and osmosis play crucial roles in maintaining the structural integrity and functionality of cells. Osmosis is the movement of water across a selectively permeable membrane, driven by differences in solute concentration. Turgor pressure, the pressure exerted by the cell contents against the cell wall, is a key factor in plant cell stability. This practical work focuses on exploring osmosis, turgor pressure, and plasmolysis in red onion cells, providing insights into how these phenomena shape cellular responses. In the lab you will use a 15% NaCl (salt) solution. This solution has more solutes than the inside of the cell and is hypertonic. You will also use distilled water which is hypotonic when compared to the cell.

2. Objective

The primary objectives of this experiment are to investigate the impact of osmosis on red onion cells and to observe and understand turgor pressure and plasmolysis in response to hypotonic and hypertonic solutions.

3. Materials

- Red onion bulbs
- Microscope slides
- Microscope
- Distilled water (hypotonic)
- Sodum chloride (NaCl)(hypertonic solution)
- Dropper
- Cover slips
- Stopwatch
- Razor blade

4. Procedure

- Peel thin layers of red onion cells and place them on microscope slides.
- Observe the cells under the microscope to establish their normal state.
- Apply a drop of distilled water to the specimen and observe any changes in cell morphology (turgor pressure).

- Prepare a hypertonic solution using NaCl by dissolving it in distilled water.
- Introduce the hypertonic solution to observe the effects on cell plasmolysis.
- Document the observations, noting cell shape, turgidity, and plasmolysis.

5. Observation

5.1.Normal State

- ✓ Cells are turgid and exhibit a well-defined structure.
- ✓ Vacuole is visible.
- ✓ Cell membrane is pressed against the cell wall.

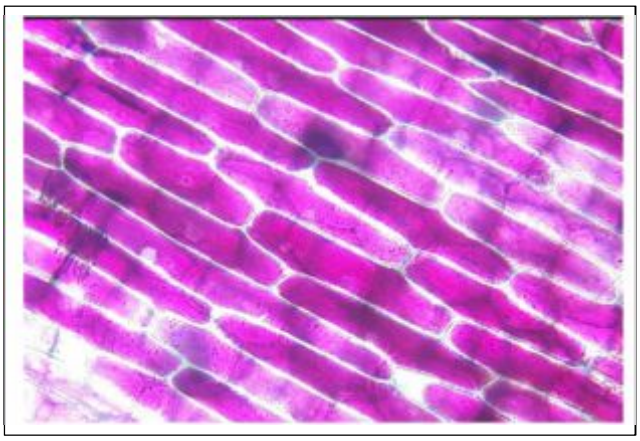

Figure 22: Red onion cells in normal state (X400)

5.2.Hypotonic Solution

- ✓ Cells absorb water.
- ✓ Increased turgor pressure.
- ✓ Cells become more turgid.

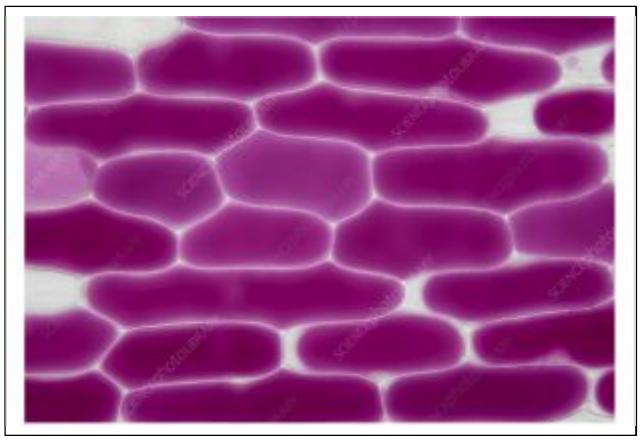

Figure 23: Red onion cells in Turgidity state (X400)

5.3.Hypertonic Solution

- ✓ Water moves out of the cells.
- ✓ Decreased turgor pressure.
- ✓ Plasmolysis occurs.

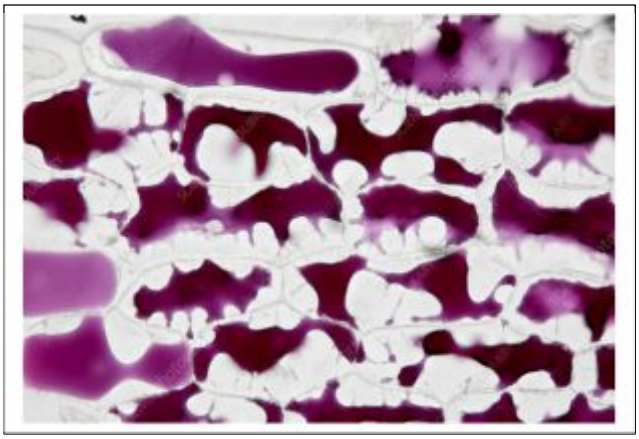

Figure 24: Red onion cells in plasmolysis state (X400)

6. Conclusion

This experiment demonstrates the dynamic responses of onion cells to osmotic changes induced by hypertonic and hypotonic solutions. The use of NaCl solution provides a practical approach to studying cell permeability, osmosis, and the associated turgor pressure and plasmolysis in plant cells. These findings contribute to our understanding of cellular dynamics and have practical applications in various biological and agricultural contexts.

PW09: Microscopic observation of mitosis in onion root tip cells

1. Introduction

Mitosis is a fundamental process in eukaryotic cell division, where a single cell gives rise to two genetically identical daughter cells. The onion root tip is an excellent specimen for observing mitosis due to its actively dividing cells. The root tip contains a region called the meristem, where cell division occurs, making it an ideal area for studying different stages of mitosis. By examining onion root tip cells under an optical microscope, we can gain valuable insights into the dynamic process of cell division.

2. Objective

The primary objective of this practical work is to observe and document the various stages of mitosis in onion root tip cells using an optical microscope. By doing so, we aim to enhance our understanding of the cell cycle and the intricate events that lead to the formation of new cells.

3. Materials

- Onion bulbs
- Microscope slides
- Coverslips
- Forceps
- Hydrochloric acid (HCl)
- Acetic orcein stain
- Dropper or pipette
- Microscope
- Lens cleaning solution
- Microscope light source

4. Procedure

❖ Collection of Onion Root Tips

- Obtain fresh onion bulbs.
- Peel off the outer layers until you reach the white, growing region at the root tip.
- Use forceps to carefully extract small root tips.

❖ **Preparation of Root Tips for Observation**

- Place the root tips in a small dish containing hydrochloric acid (HCl) for 5-10 minutes. This helps soften the tissues.
- Transfer the root tips to a drop of acetic orcein stain on a microscope slide.
- Gently squash the root tips with a coverslip to create a thin layer of cells.

5. Observation and Documentation

- ✓ Identify cells in interphase, prophase, metaphase, anaphase, and telophase.
- ✓ Document the characteristic features of each stage, such as chromatin condensation, spindle fiber formation, and nuclear envelope breakdown.
- ✓ Record the number of cells in each stage.

6. Conclusion

The observation of mitosis in onion root tip cells provides a visual representation of the stages involved in cell division. By carefully documenting the characteristics of each stage, we gain valuable insights into the intricacies of mitosis. This experiment contributes to our broader understanding of cellular processes, emphasizing the importance of studying model organ isms like onion root tips in cell biology research.

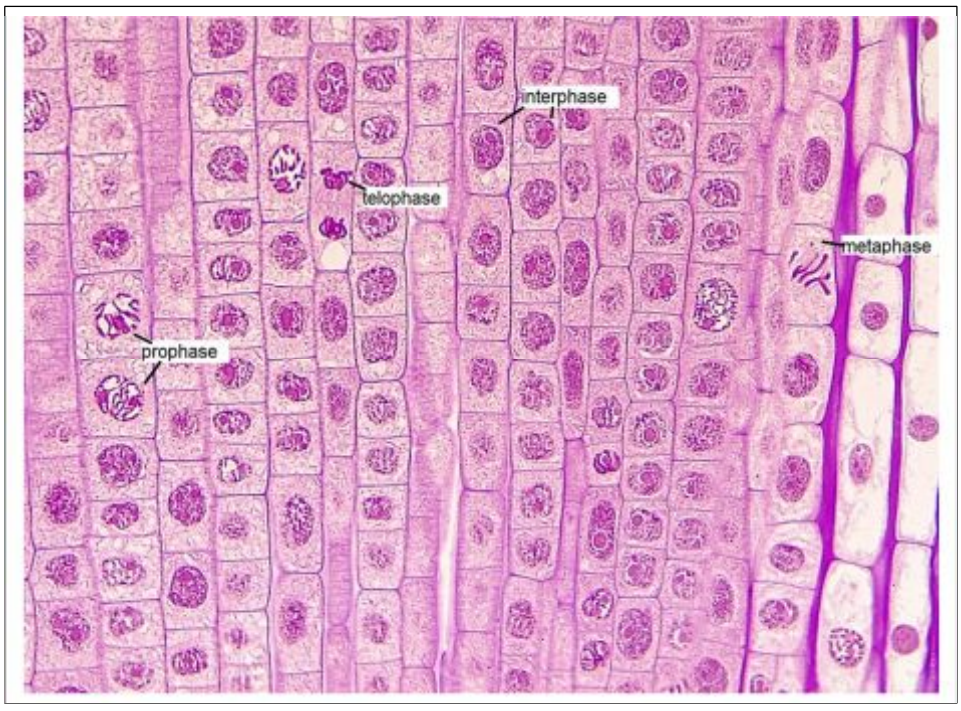

Figure 25: Mitosis in onion root tip cells (X400)

PW 10: Cell Culture

1. Introduction

Cell culture is a fundamental technique in cell biology that involves the maintenance and propagation of cells outside their natural environment. This technique has revolutionized research by allowing scientists to study cellular behavior, function, and responses to various stimuli in a controlled setting. In this practical work, we aim to explore the principles of cell culture, focusing on the cultivation of lymphocyte cell and investigating their growth characteristics under different conditions.

2. Objectives

This practical work aims to understand the basic principles of cell culture and the importance of maintaining a sterile environment and to cultivate the lymphocyte cell and monitor their growth under standard culture conditions.

3. Materials

- Lymphocyte cell line or primary lymphocytes
- RPMI-1640 medium or other suitable lymphocyte culture media
- Fetal bovine serum (FBS)
- Penicillin-Streptomycin solution
- L-Glutamine
- Phytohemagglutinin (PHA) or other stimuli for lymphocyte activation
- Trypan blue or other viability dyes
- Hemocytometer or automated cell counter
- Cell culture flasks, plates, and pipettes
- CO2 incubator

4. Procedure

4.1. Preparation of Culture Environment

- Sterilize all equipment and work surfaces.
- Prepare RPMI-1640 medium supplemented with FBS, Penicillin-Streptomycin, and L-Glutamine.
- Isolate and seed lymphocytes into culture flasks.

4.2. Cultivation of Lymphocytes

- Maintain lymphocytes under standard culture conditions (37°C, 5% CO2).
- Monitor lymphocyte growth and morphology using a microscope.
- Perform subculturing as needed to maintain cell viability.

4.3. Experimental Treatments

- Divide lymphocytes into different groups for treatments.
- Introduce specific stimuli or treatments to activate lymphocytes.
- Monitor and record cellular responses to treatments over a specified time.

4.4. Cell Counting and Viability Assays

- Harvest lymphocytes for counting using a hemocytometer or automated cell counter.
- Perform viability assays (trypan blue exclusion, for example) to assess lymphocyte health.

4.5 Data Analysis

- Compile and analyze data obtained from cell counting and viability assays.
- Compare results between different experimental groups to draw conclusions about the impact of stimuli on lymphocyte behavior.

5. Conclusion

In conclusion, this practical work has provided valuable insights into the principles and techniques of cell culture. The cultivation of lymphocyte under various conditions allowed us to observe and analyze their growth characteristics and responses to specific treatments. The data obtained contributes to our understanding of cellular behavior, laying the foundation for further research in cell biology. This practical experience has equipped us with essential skills in cell culture and data analysis, essential for future endeavors in the field of cell biology.

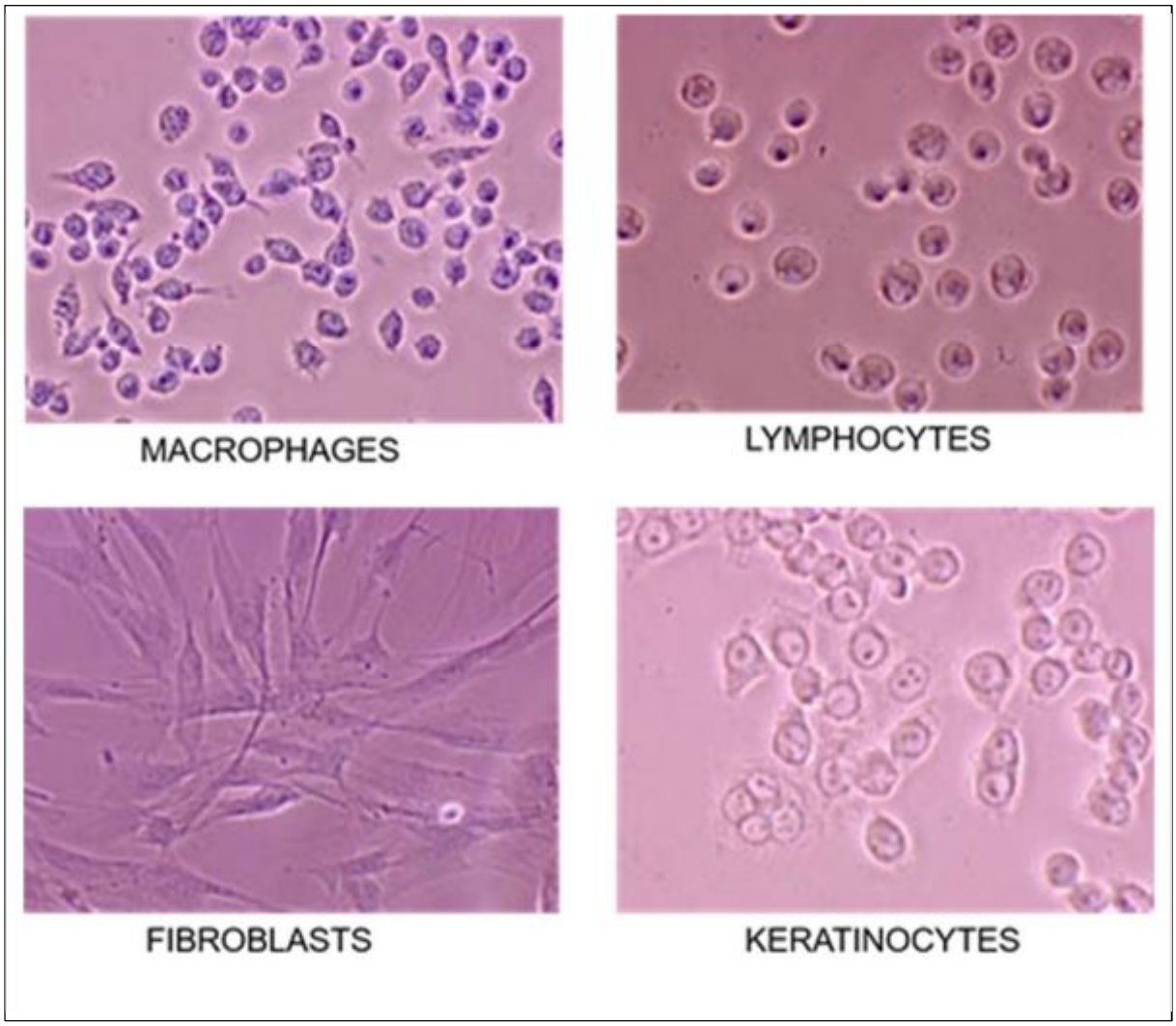

Figure 26: Primary cultures of human cells

V. Conclusion

In essence, the Cell Biology Practical Work Support Document is a collaborative tool that empowers both students and teachers, fostering a dynamic learning environment where theoretical knowledge seamlessly integrates with practical application. It is a gateway to discovery, exploration, and a deeper appreciation for the wonders of cell biology.

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