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Ministry of Higher Education and Scientific Research
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Faculty of Natural and Life Sciences, Earth and the universe
Department of Natural and Life Sciences

Practical Work Handouts

Laboratory Manual of Cell Biology

Intended For First-Year Common Core Students In Life and Natural Sciences

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Preface

This manual assists first-year Common Core LMD Natural and Life Sciences students on practical work and the acquisition of the fundamental skills necessary for observation, analysis and comprehension of cells.

The practical sessions focus mainly on the microscopic examination of the cell, which is the basic unit of all living organisms. These sessions enable students to recognize the structure of various cell types, both animal and plant. There are five practical sessions planned for the semester.

Each session begins with a brief theoretical overview, followed by the tasks to be completed and a detailed protocol aimed at achieving the following key goals: following an experimental procedure, conducting microscopic observations, and creating precise observational drawings.

Numerous illustrations are provided to assist students in understanding and mastering each step of the practical work, especially to ensure the proper preparation of different microscopic samples.

This manual is intended to complement, not replace, the lecture content. It serves as a guide for laboratory activities, note-taking, interpreting results, and preparing practical reports. I hope that this resource will help students develop greater independence, accuracy, and critical thinking skills throughout their practical sessions.

This handout was prepared using several books and documents; they are all mentioned at the end of this document, without reference to any specific source.

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Introduction:

Cell biology, formerly known as cytology, is a branch of biology disciplines that studies:

- Various types of cells and their organelles.
- The fundamental metabolic and functional cellular processes such as: reproduction, nutrition, respiration, division, etc.
- Cell death, which may be genetically programmed (apoptosis) or caused by external damage (necrosis).

Gaining insights into the cell is crucial, because it is the structural, functional and reproductive unite that makes up all or part of a living organism, ranging from the simplest unicellular bacteria to the very complex multicellular organism like humans.

Cells are too small to be observed with the naked eye, various techniques, particularly microscopy are employed to study their structure, these techniques empower researchers to see cellular features and their components.

Throughout history, advancements in microscopy including brightfield, fluorescence, confocal and electron microscopy have enhanced our ability to analyse or examine cellular shape and molecular arrangement at unprecedented resolutions, facilitating the in-depth study of cell parts and their interactions.

Across the whole cell biology course (see the concept map of the course), only one part can be directly adapted into a practical work: the section dedicated to "methods of studying the cell." Indeed, this part of the program introduces the tools and techniques that allow concrete exploration of the cellular world, notably optical and electron microscopy methods, histochemical, immunological, and enzymological approaches. It is on this basis that the five practical sessions proposed in this document are built to provide students with essential skills in cellular observation and analysis:

The first session focuses on the use of the optical microscope, the main laboratory instrument used to discover the microscopic world.

The second and the third one interested in the microscopic observation of both animal and plant cell, respectively, allowing for a comparison of their organization and main structural features.

The fourth one is devoted to study plastids specific plant organelles, that involved in various plant cell metabolism.

The last one reserved to study methods for separating cellular compounds through centrifugation, a technique used to isolate compounds of an heterogenous mixture.

All these sessions aim to validate the acquired knowledge of the diversity of the cell as well as to enhance both technical competencies and essential experimental skills. Then, the students will be able to acquire the following qualifications:

- Optical microscope use:
    - Understand the different parts of the microscope and know its operating principle
    - Learn the correct and safe handling of the microscope
    - Be able to adjust the focus and observe biological samples at different magnifications.
- Microscopic observation of an animal and a plant cell:
    - Know the essential structural differences between animal and plant cells.
    - Improve the detailed observation skills and representation of the cells through drawing.
- Microscopic observation of plastids:
    - Understand the microscopic structure and the function of plastids.
    - Observe different type of these organelles
- Methods of cellular component separation "Centrifugation":
    - Learning fundamental cell separation techniques.
    - Understanding the physical principle of centrifugation, its steps and its application for separating compounds of an heterogenous mixture of an animal or a plant tissue.

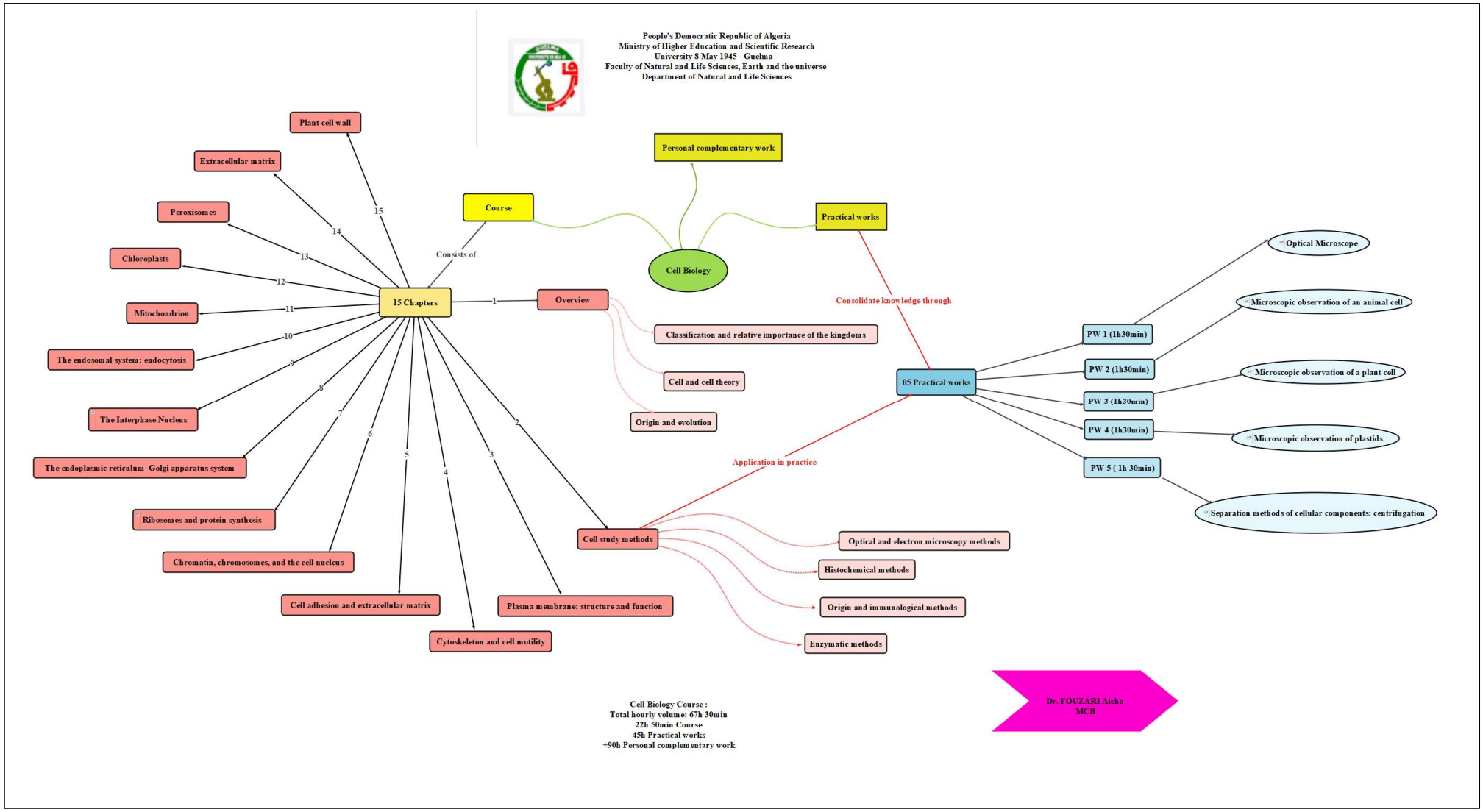


Fig 1: The concept map of the course: Cell Biology

1. **General Laboratory Safety Rules:**

- **Personal Protective Equipment (PPE):** Always wear appropriate PPE, including a clean, non-flammable, closed laboratory coat reserved exclusively for laboratory use, as well as gloves, safety goggles, and masks during all procedures.
- **Personal Hygiene:** Keep fingernails short and avoid wearing jewelry on the wrists or fingers (such as watches, bracelets, or rings).
- **Hand Hygiene:** Wash hands thoroughly with soap and water before and after each manipulation, and upon completion of laboratory work. Use disinfectant solutions when necessary.
- **Prohibited Activities:** Eating, drinking, smoking, chewing gum, or storing food in the laboratory is strictly forbidden. Never place any object in the mouth.
- **Pipetting Practices:** Never pipette by mouth; always use appropriate pipetting devices.
- **Handling Samples:** Avoid direct contact with bacterial or fungal cultures, chemicals, reagents, or biological samples. Use suitable tools such as pipettes or forceps.
- **Labeling and Storage:** Ensure that all chemicals and biological materials are clearly labeled and stored in compliance with safety regulations.
- **Ventilation:** Conduct work in a well-ventilated area or under a fume hood when handling volatile chemicals or solvents.
- **Workspace Organization:** Maintain a clean and organized work area to minimize contamination and accidents.
- **Cleaning Procedures:** Clean and disinfect the workbench and laboratory equipment after each manipulation.
- **Waste Disposal:** Dispose of contaminated instruments, materials, and laboratory waste in designated containers according to established waste management protocols.
- **Safety Compliance:** Follow all safety instructions displayed in the laboratory.
- **Emergency Procedures:** In case of spills, accidents, or exposure, immediately notify the laboratory supervisor and adhere to established emergency protocols.

- **Equipment Maintenance:** Ensure that all laboratory equipment is properly cleaned and turned off after use.

2. Teaching objectives:

- **Develop methodological skills** that empower students to design, plan, execute, and correctly interpret experimental procedures in cell biology.
- **Assist students in identifying and utilizing tools for observing and analysing cells** (such as light microscopes, various staining techniques, sample preparation techniques) so they can apply them in upcoming research projects.
- **Support the development of scientific reasoning** by motivating students to create or formulate hypotheses, asses or analyze obtained results, and draw appropriate conclusions.
- **Assist students in applying their developed methodological and linguistic abilities** (such as understanding guidelines, writing scientific reports, using specialized scientific vocabulary) in the completion of their Master's thesis or other scientific projects.
- **Promote independence and teamwork while carrying** out experimental tasks in the laboratory.

3. Carrying Out Practical Work:

3.1. Students are requested to:

- Arrive on time to avoid disturbing the session;
- Work in pairs;
- Wear a clean white lab coat;
- Bring the required drawing materials: unruled white A4 drawing sheets, pencil, colored pencils, eraser, sharpener, ruler
- Clean their workbench at the end of each session.

3.2. Guidelines for Lab Reports (Drawings):

- All drawings must be done entirely in pencil;
- Each sheet must include:
    - **Top left**: Students full names and groups number
    - **Top right**: the date

- **Center**: the topic and title of the practical session.

- Never draw on both sides of the paper, use only one side for neat presentation;
- A maximum of two drawings per sheet is allowed.

4. Principle of observational drawing:

Observational drawing plays a crucial role in the process of observation: it offers a different approach to reality. Often, capturing what we see through drawing is simpler than to describe it verbally.

Also, when you draw, you are always going back and forth between what you see and what you put down on paper. These repeated actions make you better at noticing things and help you see how things are alike or different. This helps you figure out what's important to focus on in the drawing, even when things are confusing, so you can tell what matters most from what matters less.

Drawing isn't about making a perfect copy of what you see; instead, it's a way to make things clearer. So, drawing what you observe helps you understand what's really there.

4.1.Preparatory steps:

Use a full page or half a page of a blank sheet (remember to choose the sheet orientation: portrait or landscape);

➢ If needed, draw a line 4 cm from the right edge of the sheet (this can help position the labels);

➢ Take time to observe the sample to be drawn in order to identify all structures.

➢ Place the drawing in the centre of the page.

➢ Start by drawing the main structures with light lines (to erase errors more cleanly).

➢ The drawing must have the following qualities: it must be sufficiently large; it must accurately reflect the morphology and spatial arrangement of the observed elements; it must respect real proportions, both in number and in size.

➢ It is unnecessary to represent the same structure more than three times.

➢ If the sketch is satisfactory, go over the lines again to make them thin, clear, and continuous.

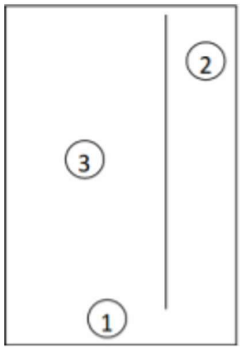
➢ Finish the drawing with the necessary details, always using thin, clear, and continuous lines.

➢ Draw horizontal, parallel labels using a ruler; these lines end either with a discreet arrow or directly on the indicated structure, and they stop at the margin line on the right. Be careful: labels must never cross.

➢ Write the labels in the margin, always in pencil.

➢ Indicate the magnification used for the observation (usually at the bottom right of the sheet). Sometimes it may be included in the title.

➢ Write a title above the drawing. The title must specify: the observation method, the name of the observed structures, their origin, and the view (example: observation with a light microscope).



1 **Title area** (An area including the observed object, the observation method, the staining used, and the magnification)

2 **Legend area**

3 **Drawing area**

Fig 2: Schematic representation of a scientific drawing template

4.2.Evaluation grid:

As follows (**Tab 1**), a standardized evaluation grid is used in order to assess the student's drawings in each report, this grid ensures objective grading by taking in consideration some key criteria: the accuracy of the drawing, the presence and clarity of the legend, the neatness and the organization of the page, respect and adherence to the teacher's instructions.

Tab 1: Assessment Grid for Observational Drawing

Skills	**Producing an Observational Drawing**		
Success Criteria	Conformity	Completeness and Accuracy	Neatness
Level 4: Very Good Mastery	Drawing looks like it's the right size and shape compared to observed object, and it's pretty close	All annotations: title, labels and magnification are right and has been written down.	Pencil lines are exact, thin, the same, straight, and made well.
Level 3: Satisfactory Mastery	Drawing is a little bit like the real observed object in size and shape, but it's not an exact match.	Some annotations are wrong or missing, like the title, labels or magnification	Lines that are not straight, lines that were not drawn with a pencil, or lines that are shaky.
Level 2: Weak Mastery	Drawing barely looks like the right size and shape of the observed object it's supposed to be.	Half of the annotations are missing (title, labels or magnification) or contain errors.	
Level 1: Insufficient Mastery	The picture and the real observed object have nothing in common at all.	There are almost no notes written down.	Lines that are a little off, mistakes that were crossed out, or a ruler was not used.

5. Practical work n°1: Optical Microscope.

5.1. Lesson review:

Cell biology now has better ways, increasingly powerful techniques and analytical instruments to explore the internal processes of the cell. Since cells are tiny (10 to 100 µm), we need microscopes to see them clearly.

The ways we look at cell parts involve using light microscopes and electron microscopes. For example, light microscopy techniques, including brightfield and fluorescence microscopy, enable the observation of live or fixed cells, while electron microscopy provides ultrastructural details at nanometer resolution.

5.2. The Optical Microscope (O.M):

An optical microscope, also called a light microscope, is a scientific instrument that uses visible light and optical lenses to magnify and observe images of microscopic objects or fine details of biological, chemical, or mineral samples. It thus allows the examination of structures invisible to the naked eye.

It is widely used for the visualization of microstructures in biology and medicine. Typical applications include histology, pathology, medical practices, education and training, routine laboratory work, and basic immunofluorescence techniques.

There are many different models of microscopes, with the most commonly used ones listed in the table (**Tab. 2**). Some are equipped with additional accessories such as a micrometric eyepiece or adapters for video and photo cameras.

Tab 2: The different types of optical microscopes and their uses

Type	Usage
Bright-field optics	Viewing the inside parts of cells after they have been stained.
Dark-field optics	Examination of unstained specimens and dynamic, living cells.
Fluorescence optics	Fluorescent labelling of structures and macromolecular compounds.
Phase-contrast optics	Enhancement of optical contrast through variations in refractive indices.
Confocal optics	Generation of 3D reconstructions images of the object

5.3. Objectives:

- Understand the different parts of the microscope and know its operating principle
- Learn the correct and safe handling of the microscope
- Be able to adjust the focus and observe biological samples at different magnifications.

5.4. Operation and Principles:

The optical microscope relies on several physical principles:

- **Transmission of light**: light passes through or is reflected by the sample.
- **Absorption and diffraction**: certain wavelengths are absorbed or deflected, modifying the image.
- **Refraction**: light changes direction as it passes through lenses, enabling focusing.

5.5. Applications and Limitations:

Optical microscopes are widely used in biology to study cells, tissues and various microorganisms, in chemistry to analyse molecular structures, in material sciences to observe surfaces and textures.

However, their resolution is limited by the wavelength of visible light, generally around 200 nm, preventing observation of smaller structures such as proteins or atoms; their observation requires electronic microscope.

5.6. Description of the Optical Microscope:

The optical microscope (**Fig. 3**), consists of four main systems:

5.6.1. Illumination System: It includes:

- **A light source**: an electric lamp with adjustable intensity via a rheostat, the slider of which is located on the right side of the device, along with an on/off switch.
- **A condenser lens**: concentrates the light emitted by the source onto the sample to be observed.
- **A diaphragm**: controls the amount of light illuminating the sample (usually set to a medium value).

5.6.2. Support System: It includes:

- **A base**: that provides microscope stability.
- **A stand** (**arm**): that connects various components such as the binocular (or occasionally monocular) tube, rotating turret holding the objectives, and the stage. The stage has an opening allowing light to pass through the specimen and includes clamps to hold the preparation (the object to be examined placed between a slide and coverslip).

5.6.3. Adjustment System:

- **Lateral screws**: allow horizontal movement of the stage to centre the specimen so that the object aligns with the optical axis through which the light passes. There are two screws: one moves front to back, the other left to right. Lateral screws also enable scanning of the sample to find the appropriate area for observation.
- **Coarse focusing knob**: also called macro focusing knob, the larger knob responsible for rapid vertical movement of the stage, enabling rough focus.
- **Fine focusing knob**: or Micro focusing knob, the smaller knob for very slow and small vertical adjustments, permitting precise focus.

These screws adjust the distance between the objective (on the optical axis) and the specimen to obtain a sharp image, meaning they control focusing.

5.6.4. Magnification System: It includes:

- **Objectives**: The objectives are the key component of the microscope, typically, four objectives are mounted on a rotating turret, each providing a specific level of magnification:

Objective 4x: magnifies the image 4 times actual size.
Objective 10x: magnifies the image 10 times
Objective 40x: magnifies the image 40 times
Objective 100x: magnifies the image 100 times.

- **Eyepieces**: also called ocular lenses, located at the top of the microscope where the observer's eye is placed; they have a magnification factor engraved on the ring (commonly 10x).

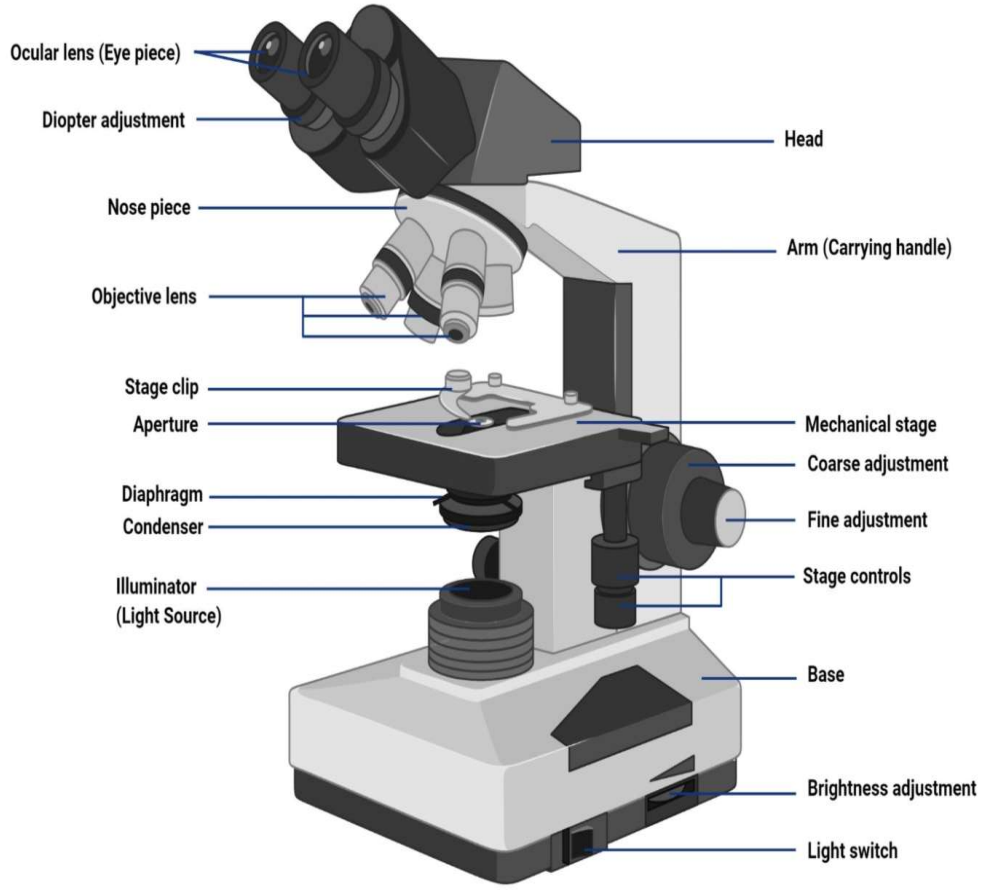


Fig. 3: Parts of an optical microscope**.** Image copyright © Sagar Aryal, www.microbentes.com

5.7. Magnification:

Microscope magnification refers to its capacity to enlarge an object's image relative to its true size, indicating how many times the observed image exceeds the actual dimensions of the specimen. It is calculated by multiplying the magnification of the objective by the magnification of the eyepiece (most often 10X), we obtain the total magnification of the microscope.

$$\mathbf{G} = \mathbf{G}_{obj} \times \mathbf{G}_{oc}$$

Where:

G: Total magnification

$\mathbf{G}_{obj}$: Objective magnification factor

$\mathbf{G}_{oc}$: Eyepiece magnification factor

5.8. Interpupillary Distance Adjustment:

Interpupillary distance refers to the spacing between the centers of the eye pupils. In binocular optical microscopy, this distance must be adjusted by sliding the eyepieces closer together or farther apart to match the user's interpupillary measurement, typically ranging from about 55 mm to 75 mm.

Note:

If the interpupillary distance is not properly adjusted, the user will see two separate images, causing visual discomfort.

Accurate adjustment results in a unified, sharp image, enabling precise observation with reduced visual strain.

5.9. Focusing Steps:

To obtain a clear view of the object, follow these steps:

1. Position the microscope arm facing the observer.
2. Set the objective with the lowest magnification in the optical axis.
3. Turn on the microscope and the lamp.
4. Lower the stage to its lowest position using the coarse focus knob (maximizing distance between stage and objective).

5. Place the slide on the stage with the coverslip facing upwards, securing it with the stage clips.
6. Center the slide using the lateral screws so the object is aligned with the light path.
7. Look through the eyepieces.
8. Slowly raise the stage with the coarse focus knob until the image appears (rough focusing).
9. Refine the image quality with the fine focus knob.

10. To increase magnification, switch to the 10x or 40x objective and fine-tune focus with the fine focus knob until a sharp, enlarged image is obtained.

5.10. Microscope Storage:

1. Set the objective with the lowest magnification in the optical axis.
2. Lower the stage to its lowest position using the coarse focus knob.
3. Remove the slide.
4. Set the rheostat to zero.
5. Turn off the lamp via the power switch.
6. Place the protective cover on the microscope.

Note:

The optical microscope is a delicate instrument. When transporting it, support the arm with one hand and the base with the other, then place it carefully on the workbench.

5.11. Microscope Maintenance:

Even the most basic optical microscope is a delicate device, incorporating precise mechanical systems and fragile optical elements. For safe handling, it should be lifted by the arm with one hand while supporting the base with the other, avoiding the optical tube or stage to protect sensitive components. Dust on optical parts should be removed with a brush, and persistent residues such as immersion oil or fingerprints should be cleaned using lens paper (Joseph paper moistened with alcohol) to preserve the integrity of the lenses.

5.12. Tasks assigned to the student for completion:

Task n°1: Getting familiar with the microscope: Identify the parts of the light microscope below

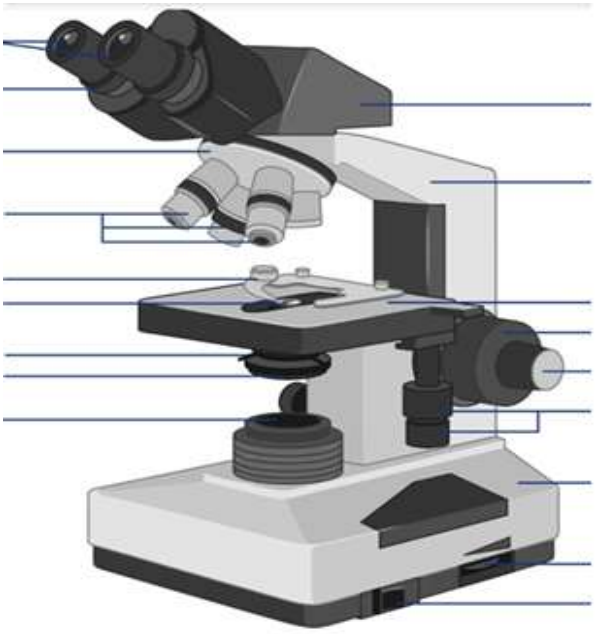


Fig.1 : ……

Task n°2: Check for understanding: Fill in the blanks below the correct part of the microscope.

A. ……..Platform that supports a microscope slide.

B. ……..Device under the stage that controls the amount of light that passes to the specimen and eventually to your eye.

C. ……..The part of the microscope that you look through that magnifies an object ten times (10X)

D. …….Holds the objective lenses and allows you to change magnification

E. ……Knob used only with the scanning and low power objective that bring an object into approximate focus.

F. ……..Lenses located over the stage with different magnifications

G. ……..Knob used with all objectives to bring image into sharp focus.

5.13. Task answers:

Task n°1: Getting familiar with the microscope

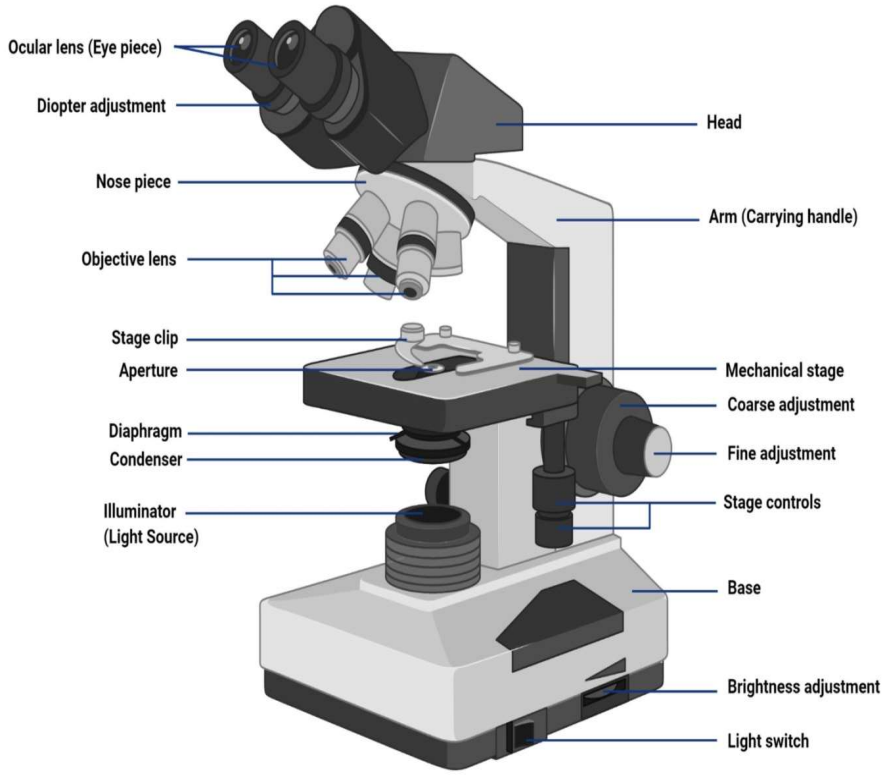


Fig .1: Parts of an optical microscope

Task n°1: Check for understanding

A. **Stage**: Platform that supports a microscope slide.
B. **Diaphragm**: Device under the stage that controls the amount of light that passes to the specimen and eventually to your eye.
C. **Ocular lens (Eyepiece)**: The part of the microscope that you look through that magnifies an object ten times (10X)
D. **Revolving Nosepiece (Turret)**: Holds the objective lenses and allows you to change magnification
E. **Coarse Adjustment Knob**: Knob used only with the scanning and low power objective that bring an object into approximate focus.
F. **Objective Lenses**: Lenses located over the stage with different magnifications
G. **Fine Adjustment Knob**: Knob used with all objectives to bring image into sharp focus.

6. Practical session n°2: Observation of an Animal Cell

6.1. Lesson review:

The eukaryotic domain encompasses unicellular protists as well as multicellular organisms such as fungi, plants, and animals

A defining feature of eukaryotic cells, in contrast to prokaryotes, is the presence of a nucleus (from the Greek karyon), which contains most of the cell's DNA and isolates it from the cytoplasm. The cytoplasm houses diverse organelles, including mitochondria, present in nearly all eukaryotic cells and functioning as the site of respiration and chloroplasts, which are unique to plant cells and serve as the site of photosynthesis.

In animals, the cell represents the fundamental structural and functional unit. As a eukaryotic cell, it possesses a true nucleus enclosed by a nuclear membrane and contains a variety of organelles.

6.2. Main Characteristics:

- Every animal cell belongs to the eukaryotic category.
- Its shape is generally irregular, though often approximately round.
- Unlike plant cells and bacteria, it lacks a rigid cell wall.
- Chloroplasts are absent in animal cells.
- Vacuoles are present but typically small and numerous, rather than one large central vacuole.

6.3. Main Structures and Their Functions:

The table below (**Tab 3**) shows the main characteristic structures of an animal cell along with their main functions.

6.4. Objectives: At the end of this practical session, students will be able to:

- Use the optical microscope, which is the main tool used for all practical sessions;
- Prepare a microscopic slide;
- Understand the structure of human eukaryotic cell.
- Make an observational drawing.

Tab 3: Functions of the main structures of an animal cell

Structure	Function
Plasma membrane	It controls the exchange of various substances between intracellular and extracellular environment
Cytoplasm	It is in form of gel; its main role is to hold various cell organelles in place and allows all chemical and enzymatic reaction to occur
Nucleus	It is the cell organelles where most of the cellular DNA is located, it controls all cellular activities; division, cell death, protein synthesis, …etc
Mitochondria	Produce energy (ATP) through cellular respiration.
Endoplasmic Reticulum	rough ER: is the site of protein synthesis, smooth ER: the site of fats synthesis.
Golgi Apparatus	Organelles responsible of modification, storage and packaging of proteins and other molecules for transport inside or outside the cell.
Lysosomes	Very small organelles full of specific enzymes that break down waste materials and cell debris.
Centrioles	Involved in cell division (mitosis).

6.5. Materials and products used:

- Sterile single-use swab: A cylindrical cotton tip attached to a flexible stick, designed for collecting samples from natural cavities. A standard cotton swab may serve as a substitute when a sterile swab is unavailable.
- Slides and coverslips
- Distilled water
- Dyes (methylene blue, diluted neutral red or iodine water):

- Methylene blue (toxic to cells): stains the nuclei and bacteria.
- Diluted neutral red (vital stain): stains the vacuoles.
- Iodine water: stains intracellular organelles and nuclei.

- Optical microscope.
- Flat sheet of absorbent paper.

6.6. The biological material:

The buccal epithelium serves as the animal tissue commonly employed for microscopic examination of animal cells.

Epithelium: is a covering tissue composed of one or several layers of closely joined cells. The buccal epithelium renews itself through the division of deep cells, while the superficial cells die and detach.

6.7. Procedure:

6.7.1. Preparation of a microscopic slide (Buccal smear):

- Using a sterile cotton swab, gently scrape the inner surface of the cheek without injuring yourself to collect a few cells.
- Place a drop of distilled water in the centre of the slide
- Stir the swab in the drop of water and discard it in the waste bin designated for waste, ensure that the sample of buccal epithelium is well placed in the center of the slide
- To add a dye (Methylene blue), gently press the pipette and dip it into the dye solution
- Release the pressure applied with your fingers so that the stain rises into the pipette.
- Place one drop of Methylene Blue stain, which colours the nuclei due to its affinity with DNA, and if needed, add one drop of distilled water to make the dye more diluted.
- Place the coverslip at the edge of the drop, holding it by the sides to avoid fingerprints
- Tilt the coverslip at a 45° angle, when the drop begins to spread under the coverslip, gently lower it.
- If any liquid overflows, remove it with a flat sheet of absorbent paper.

6.7.2. Prepare the microscope:

- Turn on the light source.
- Select the low-power objective.
- Lower the stage.
- Place the slide so that the sample is centred in the light path.
- Raise the stage to the highest position to observe the human eukaryotic cell under the optical microscope, first at low magnification, then at high magnification.

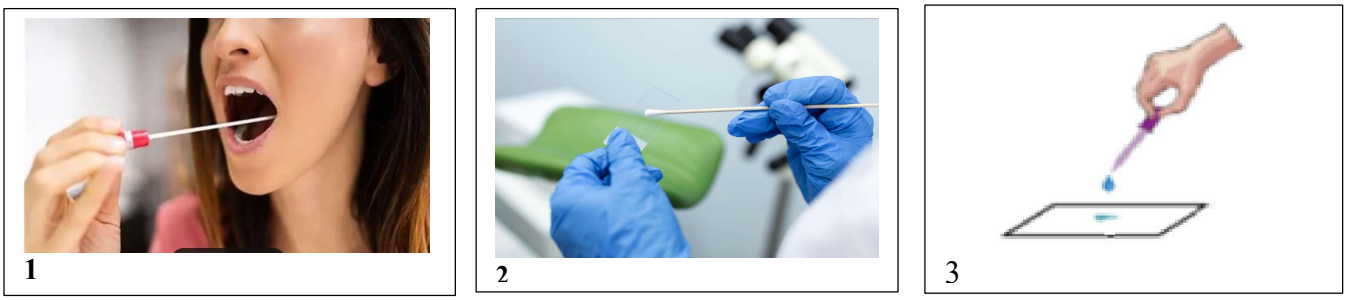


Fig. 4: Experimental steps for preparing the buccal smear

6.8. The tasks assigned to the student:

a) After completing microscopic observation of your specimen, prepare a well-labeled scientific diagram that reflects your findings. The drawing should accurately represent the morphology and structural organization of the cells, with clear identification of the principal components of an animal cell.

b) Which part of the cell is most intensely stained by methylene blue, and why?

6.9. Expected Results:

Cells obtained from the buccal cavity using a swab appear in clusters, overlapping arrangements, or occasionally as isolated units. These are epithelial cells that line the buccal cavity and constitute the mucous membrane. Their surfaces often harbor bacteria, including rod-shaped bacilli and spherical cocci.

Mucous membranes: are membranes made up of epithelial cells that line the natural cavities of the body.

a). We observe a rounded structure bounded by a plasma membrane; this is the **cell**. Within the cytoplasm, there is a centrally located, round, dark blue structure; the nucleus, indicating that it is a eukaryotic cell (**Fig. 5**).

The rounded shape of the cell, the absence of a cell wall surrounding the plasma membrane, and the lack of vacuoles and plastids indicate that it is an **animal cell**.

At these magnifications, small granular inclusions can be seen inside the cytoplasm. These correspond to various cellular organelles, which can only be observed under a **transmission electron microscope.**

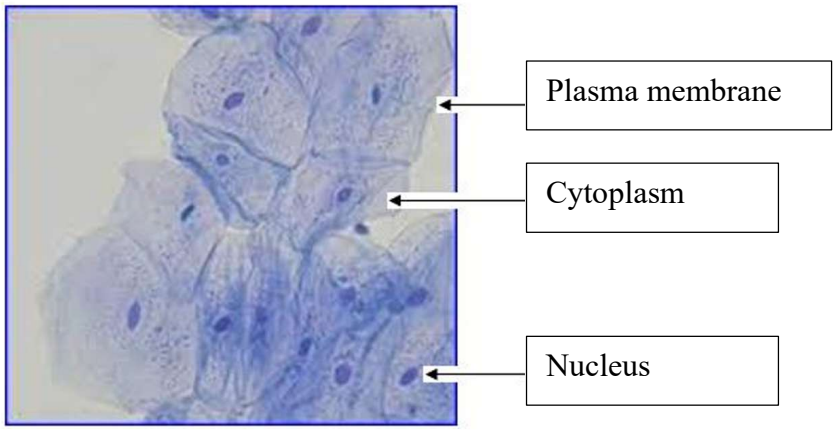


Fig 5: Observation under a light microscope of animal eukaryotic cells from the buccal epithelium stained with methylene blue (X400).

b). The parts of the cell most intensely stained by methylene blue are the nuclei.

The raison: Animal cells have their nuclei stained blue with methylene blue because: Methylene blue is a basic (cationic) dye that binds specifically to nucleic acids (DNA and RNA). The nucleus contains a large amount of negatively charged DNA. Thus, the positively charged dye (methylene blue) attaches to the DNA-rich regions, making the nucleus appear dark blue or bluish-purple under the microscope.

c). The student's expected drawing (**Fig 6**)

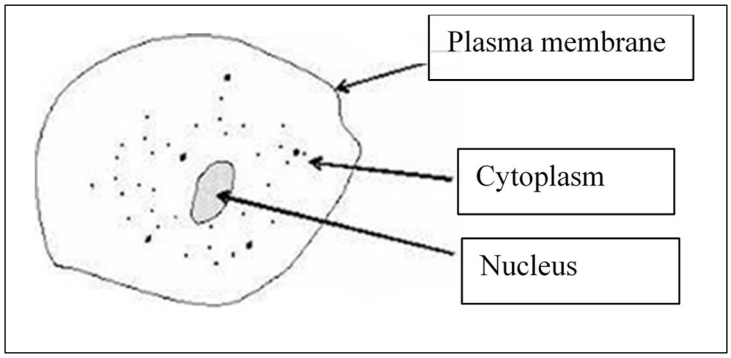


Fig 6: Schematic drawing of a microscopic observation of animal eukaryotic cells from the buccal epithelium stained with methylene blue (X400).

Notes:

1. Optional staining: You can stain the buccal smear with neutral red or iodine water to highlight certain cellular structures, then follow the same procedure as the one described above for methylene blue.

2. At the end of the observation, collect the slides and coverslips to be thrown into the waste bin.

7. Practical session n°3: Microscopic Observation of a Plant Cell

7.1. Lesson review:

Plant cells represent the fundamental structural and functional units of plant organisms. Similar to animal cells, they are enclosed by a plasma membrane and contain a nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, ribosomes, and other organelles essential for vital cellular activities.

Plant cells, which lack centrioles, have specific components:

- **A cell wall**: The plant cell wall is primarily composed of cellulose, a glucose-based polymer. This porous structure envelops the plasma membrane, conferring shape and mechanical rigidity to the cell.
- **Vacuoles**: Although vacuoles are present in animal cells and protists, they are a defining feature of plant cells. In plant cells, vacuoles occupy a substantial portion of the cytoplasm, sometimes up to 80% and are filled with vacuolar fluid.
- **Chloroplasts**: the site of photosynthesis, and therefore absent in animals and fungi.

The structural organization of plant cells enables them to carry out fundamental processes, including photosynthesis, growth, and mechanical support. Examining plant cell architecture under varying conditions provides insight into how plants adapt to environmental changes and maintain internal balance.

7.2. Objectives:

At the end of this practical session, students will be able to:

- Use the optical microscope, which is the main tool used for all practical sessions;
- Prepare a microscopic slide;
- Observe and identify the structure of plant cells (onion epidermal cells) under a light microscope.
- Make an observational drawing.

7.3. Materials and products used:

- Optical microscope
- Slides and coverslips
- Distilled water
- 1 onion
- 1 knife
- Fine forceps
- Watch glasses
- Crystallizing dish with bleach for used slides and coverslips.
- 40% NaCl solution (40 g of NaCl in 100 mL of water)
- Dyes:

  - **Methylene blue** (toxic to cells): stains the nucleus.
  - **Diluted neutral red** (vital stain): stains the vacuoles (1 g/L neutral red solution (Dissolve 0.1 g of neutral red in 100 mL of phosphate buffer at pH 6.5; the penetration of neutral red into cells is only possible at this pH),
  - **Lugol's solution** or **Iodine water**: stains intracellular organelles and nuclei (Lugol's solution, is a solution composed of iodine (I_2) and potassium iodide (KI) dissolved in water: (4 g of iodine, 8 g of KI in 1 L of distilled water)

7.4. The biological material:

The onion is classified as a bulb (**Fig. 7**), functioning as a storage organ for food reserves. Microscopic examination of a fragment from the inner epidermis of the onion bulb (***Allium cepa L.***) reveals the fundamental structural characteristics of plant cells.

The epidermis itself is a continuous cellular layer that envelops the aerial organs of the plant, providing protection against desiccation and external injury, while simultaneously regulating gaseous exchange with the atmosphere. They provide protection against excessive dehydration and are always tightly packed together. Two types can be distinguished:

- **Simple epidermis** (a single layer of cells)
- **Compound epidermis** (several layers of cells)

In higher plants, these cells are living but lack chloroplasts; however, in shade-adapted and certain aquatic plants, chloroplasts are present.

A vertical section of the onion bulb (**Fig.7**) reveals a short stem known as the basal plate, which supports a cluster of adventitious roots and overlapping scales. The outer scales are dry and protective, while the inner scales serve as nutrient reservoirs. At the center lies the central bud, enclosed by delicate, thin scales.

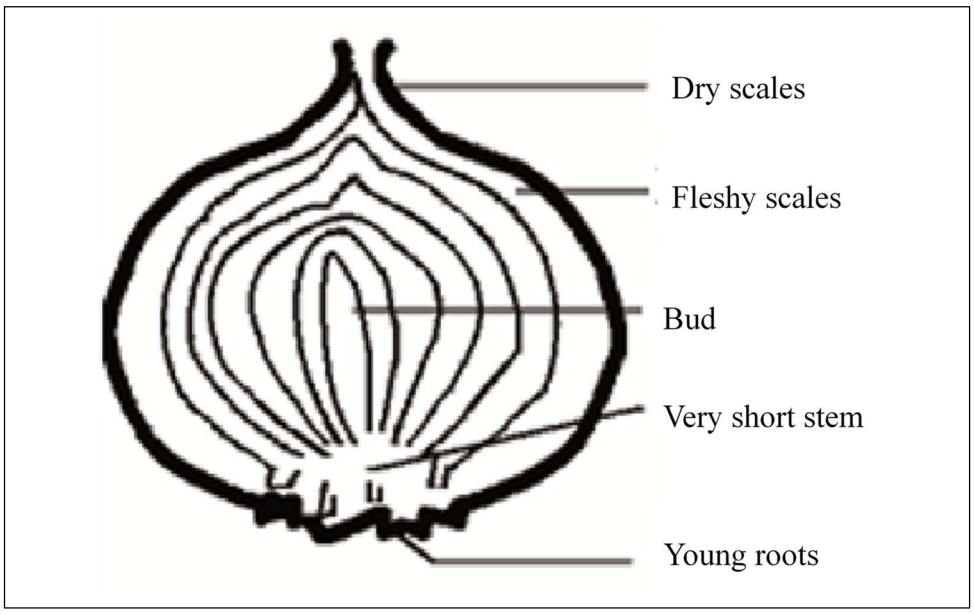


Fig 7: Longitudinal Section Showing the Components of an Onion Bulb

7.5. Procedure:

7.5.1. **Observation 1:** Observation of the cells in their natural stat (**Fig.8**)

- Place a drop of distilled water in the center of the slide.
- Using a knife cut a small rectangle (0.5 cm × 0.5 cm) on the inner epidermis from the concave side of an onion bulb scale, which is made up of thin layers of cells, then carefully remove only the thin film on the surface with fine forceps.

- Spread the tissue in the drop of water.
- Gently place the coverslip over the preparation, avoiding the formation of air bubbles
- Observe under ×10, then ×40 objective.

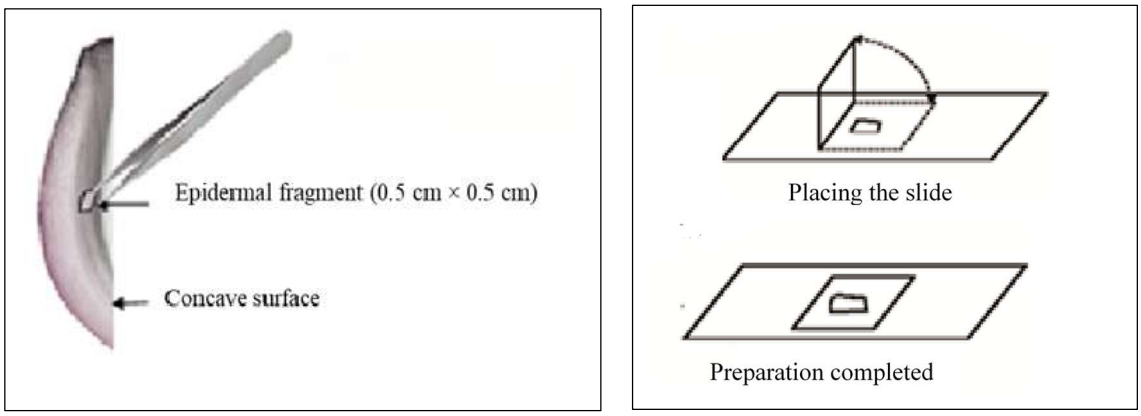


Fig 8: Observation preparation of the cells in their natural stat

7.5.2. Observation 2: Observation of the cells with a simple stain (Using methylene blue).

- To highlight the cell nucleus, you can add a drop of methylene blue to the preparation.
- Observe under ×10, then ×40 objective.

7.5.3. Observation 3: Observation of the cells stained with Lugol's solution

- Cut a small rectangle on the inner epidermis of the onion using a knife, then carefully remove only the thin film on the surface with fine forceps.
- Place the fragment on the slide.
- To make the cells, which are naturally transparent, more visible, we will use Lugol's solution.
- Add a drop of Lugol's solution (Iodine water) to the preparation:
  - Iodine kills the cell by causing the coagulation of the cytoplasm and the nucleus, thus acting as a fixative, which allows for better observation.
  - Iodine water reveals the presence of starch; therefore, all starch grains will be stained brown.

- Gently place the coverslip over the preparation, avoiding the formation of air bubbles
- Then proceed to microscopic observation using the ×10 objective, followed by the ×40 objective.

7.5.4. Observation 4: Observation of cells in their turgid state.

- Place a new fragment on a slide
- Add a drop of neutral red:
    - Neutral red is a vital dye that enters cells without killing them
- Observe under ×10, then ×40 objective.

7.5.5. Observation 5: Observation of the plasmolysis phenomenon in plant cells

- Take another fragment and immerse it for 5 minutes in a 40% NaCl solution (40 g of NaCl in 100 mL of water) kept in a Petri dish.
- Place this fragment in a drop of neutral red
- Cover the preparation with a cover slip.
- Observe under ×10, then ×40 objective.

Note:

To observation of the plasmolysis phenomenon in plant cells, you can place the same sample, previously stained with neutral red, into a highly concentrated solution (e.g 40% NaCl solution)

7.6. Prepare the optical microscope:

- Turn on the light source.
- Select the low-power objective.
- Lower the stage.
- Place the slide so that the sample is centred in the light path.
- Raise the stage to the highest position to observe the plant cells under the optical microscope, first at low magnification, then at high magnification.

7.7. The tasks assigned to the student:

a) Following your microscopic observation of your preparation, produce a well labelled scientific diagram illustrating your observations. Your drawing should accurately represent the shape and structure of the cells, clearly indicating the main parts of plant cell.

b) Explain the state of each observation.

7.8. Expected Results:

- **Observation 1:** Observation of the cells in their natural stat

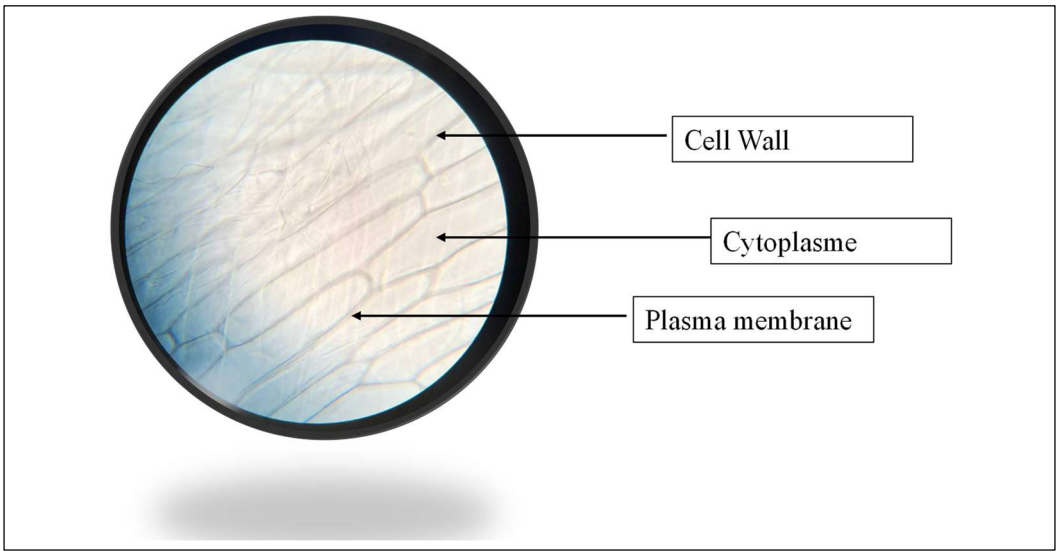


Fig 9: Microscopic observation of onion epidermal cells in their natural state

The cells are completely transparent, the plasma membrane (the envelope that delimits the cell) is difficult to see because it is often pressed against the cell wall (which appears thicker) that separates the cells.

❖ **Observation 2:** Observation of the cells with a simple stain (Using methylene blue).

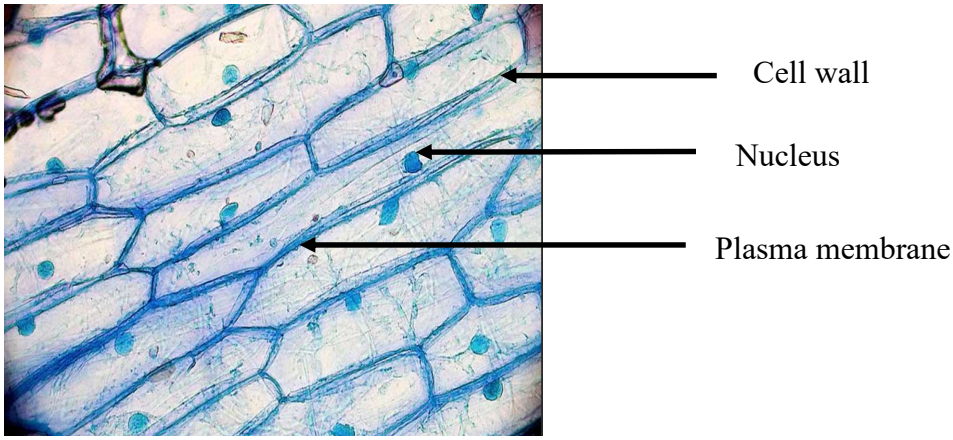


Fig 10: Microscopic observation of onion inner epidermal scale cells stained with methylene blue.

By adding methylene blue to the cells, the parts of the cell most intensely stained by methylene blue are the nuclei.

❖ **Observation 3:** Observation of the cells stained with Lugol's solution

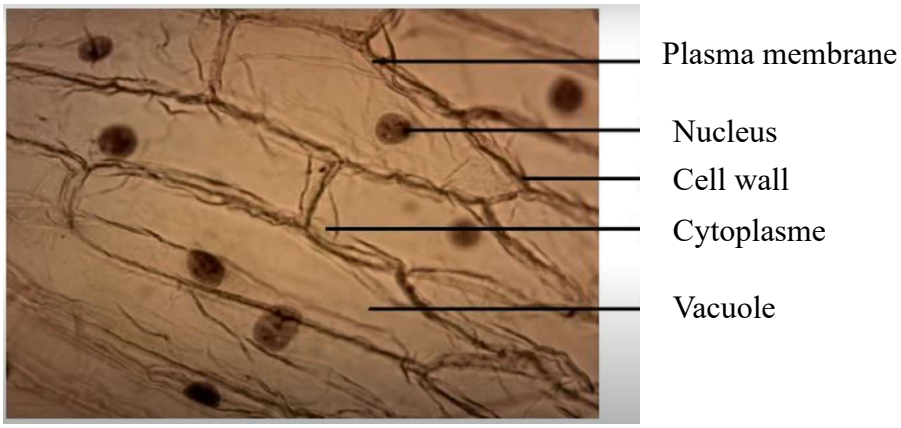


Fig 11: Microscopic observation of onion inner epidermal scale cells stained with Lugol's solution

Under light microscopy, the cytoplasm appears diminished due to the vacuole occupying most of the cell's volume. The nucleus, along with starch grains, takes on a brown coloration.

Application of Lugol's solution results in dark brown to black staining of amyloplasts, confirming their starch content.

In contrast, onion epidermal cells lack starch, so Lugol's solution does not produce significant coloration; the nucleus may remain faintly visible but is not distinctly stained.

❖ **Observation 4: Observation of cells in their turgid state.**

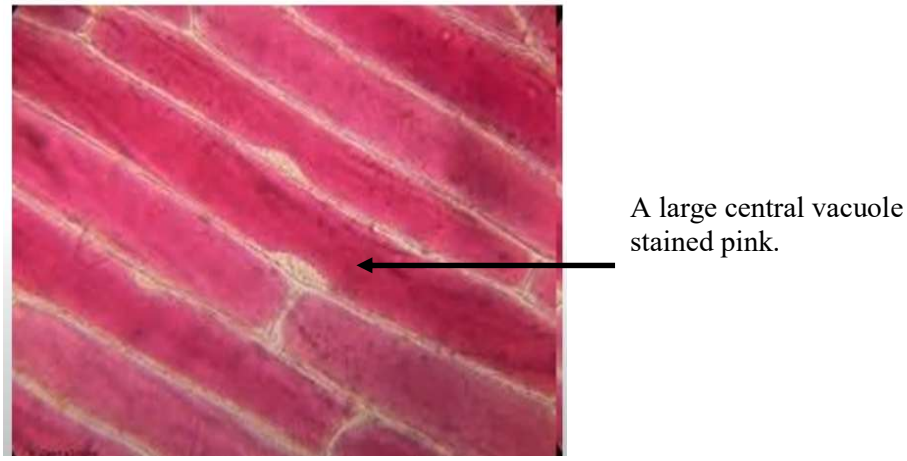


Fig 12: Microscopic observation of onion inner epidermal scale cells **in their turgid state**

Under light microscopy, the cell interior is largely occupied by a vacuole stained pink by the dye. This vacuole fills nearly the entire cellular volume and is swollen with water absorbed via osmosis, rendering the cells turgid.

The cytoplasm appears greatly reduced and is confined to a thin layer between the vacuole and the plasma membrane at the cell periphery, enclosing the nucleus. The plasma membrane is closely appressed to the pectocellulosic cell wall.

❖ **Observation 5:** Observation of the plasmolysis phenomenon in plant cells

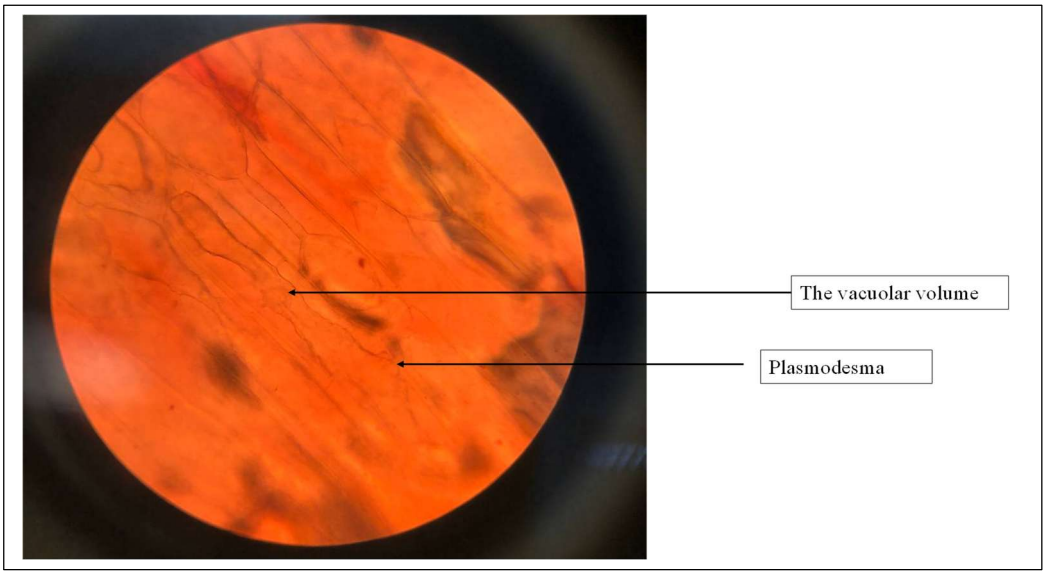


Fig 13 : Microscopic observation of plasmolysis in onion epidermal cells. X100

When the sample is immersed in a highly concentrated solution (40% NaCl), the extracellular medium becomes hypertonic relative to the intracellular environment. Consequently, water exits the cell, leading to a marked reduction in vacuolar volume. This shrinkage causes the plasma membrane to separate from the pectocellulosic cell wall, except at plasmodesmata.

The diffusion of water from the intracellular to the extracellular medium results in plasmolysis, a condition characterized by the detachment of the plasma membrane from the cell wall due to osmotic water loss.

To understand this phenomenon of osmosis, which involves the exchange between two liquid solutions with different concentrations, these solutions are separated by a semi-permeable membrane (the cell wall in our case).

The movement of molecules always occurs from the less concentrated solution (hypotonic environment) to the more concentrated solution (hypertonic environment).

When the cell is placed in a **hypotonic solution** (neutral red solution), the vacuole fills with water, and the membrane pushes against the wall. However, the cell wall prevents the cell from bursting, this state is called **turgidity**.

When a plant cell is placed in a **hypertonic solution** (40% NaCl solution), its large central vacuole loses part of its water, and the cell shrinks. The cell membrane pulls away from the wall; this state is called **plasmolysis**.

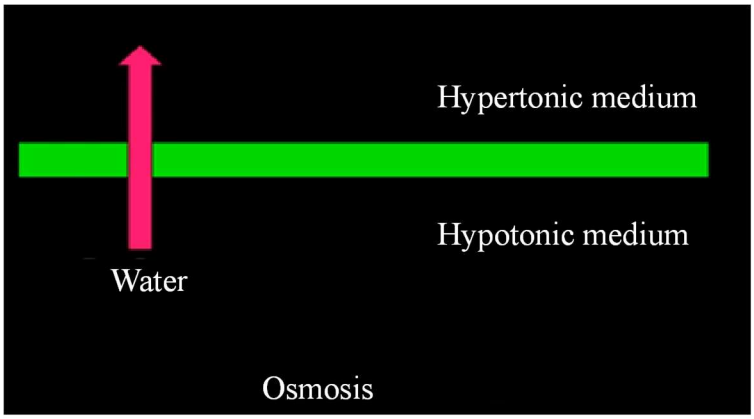


Fig 14: Concentration Gradient and Water Flow in Osmosis

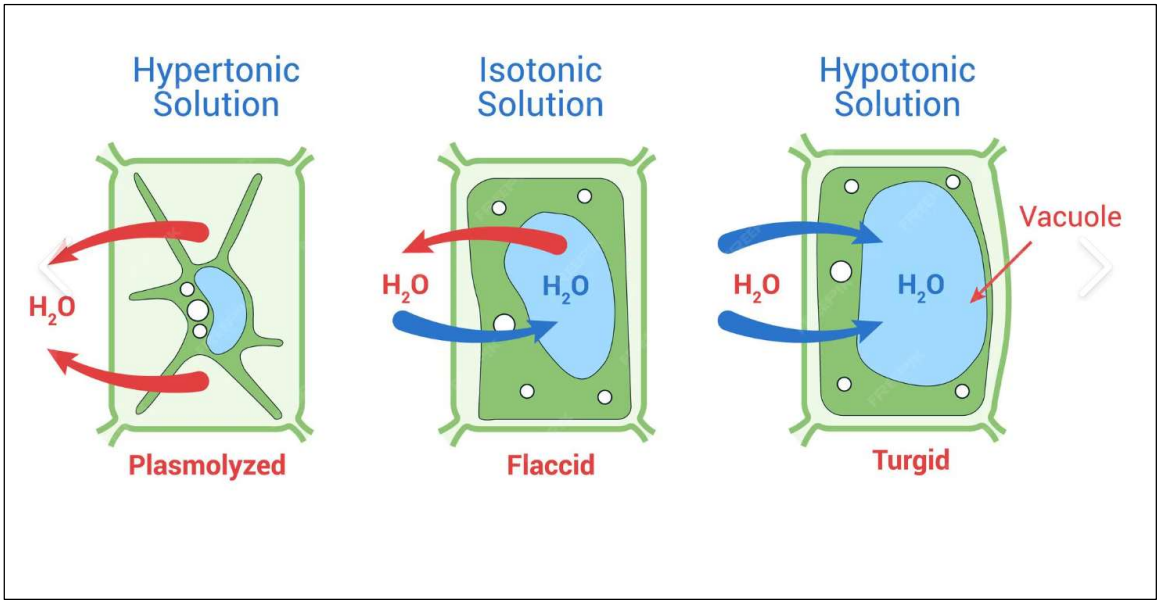


Fig 15: Effects of Osmosis on Plant Cells in Different Solutions

8. Practical session n°4: Observation of plastids

8.1. Lesson review:

Plastids are distinctive organelles with two membranes that are found in plant cells. These plastids have the unique capacity to divide independently inside the cell and contain both their own DNA and a stroma. They play a vital role in essential processes such as photosynthesis and nutrient storage.

They include a variety of pigments as well as materials including lipids, proteins, and carbohydrates. Plastids come in a variety of forms (**Fig 16**):

- A plastid containing green pigment (chlorophyll for photosynthesis) is called chloroplast
- A plastid containing pigments apart from green is called a chromoplast.
- A plastid that lacks pigments is called a leucoplast and is involved mainly in food storage of starch and oils.

Plastids play a crucial role in the metabolic processes of autotrophic eukaryotes, contributing to their ability to produce and store energy.

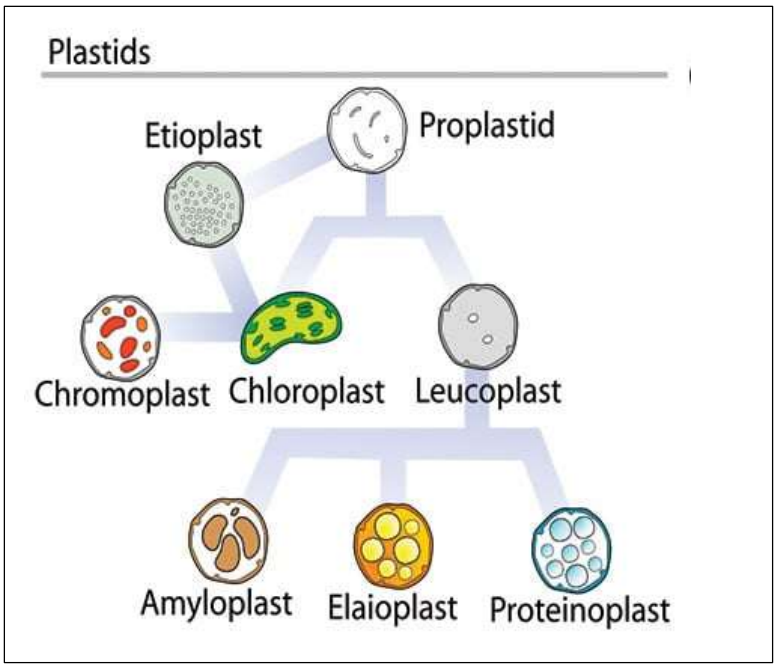


Fig 16: Diagram of plastids

8.2. Main Characteristics:

The table below (**Tab 4**) shows the main characteristics of various plastids.

Tab 4: Characteristics of various plastids

Chloroplasts	Chromoplasts	Leucoplasts
The most widely recognized type of plastid is the chloroplast.	Organelles in which pigments are both synthesized and stored within plant cells.	Non-pigmented organelles.
Responsible for photosynthesis.	These pigments are present in flowering plants, fruits, and senescent leaves.	These plastids are located in the non-photosynthetic tissues of plants, including the roots.
The organelles are densely packed with thylakoid membranes, the sites of photosynthetic activity and chlorophyll localization.	Carotenoid pigments are responsible for the diverse coloration observed in fruits and autumn foliage. These pigments contribute to the formation of structures whose vivid colors serve, in part, to attract pollinators.	In certain cases, they serve almost exclusively as storage compartments for starches, lipids, and proteins.

8.3. Objectives:

At the end of this practical session, students will be able to:

- Use the optical microscope, which is the main tool used for all practical sessions;
- Prepare a microscopic slide;
- Observe different types of plastids (Chloroplasts, leucoplasts and chromoplasts)
- Make an observational drawing.

8.4. Observation of plastids :

8.4.1 Chloroplasts :

Chloroplasts are plant cell organelles that house chlorophyll (from the Greek chloros = green, phullon = leaf). They impart the green coloration to leaves, young stems, bracts, stipules, sepals, and certain flowers. They are in charge of photosynthesis and the color green.

- ✓ **Structure**: Chloroplasts contain DNA and ribosomes, lipid globules, double membrane, stroma (chemical reaction site), and occasionally starch grains, Grana are formed by thylakoids.

- ✓ **Form**:

- **Algae:** Highly diverse shapes (ribbon-like, star-shaped, cup-shaped, spiral), often large and fewer in number.
- **Higher plants:** Typically small, numerous, and ovoid or lens-shaped; each mesophyll cell may contain 30–40 chloroplasts.
- **Variation:** Size ranges from 4 - 6 µm in higher plants to much larger in some algae

- ✓ **Function**: Photosynthesis releases oxygen while synthesizing carbohydrates from CO_2, water and light. There are two phases: light phase: ATP and $NADPH_2$ are produced and pigments absorb light. Dark phases (Calvin cycle), ATP and $NADPH_2$ are used to create carbohydrates, which serve as energy storage (starch).

The aquatic plant ***Elodea***, which is frequently found in ponds and streams, has termin al bud leaves that are especially good for seeing chloroplasts.
However, the samples in our trial are either green pepper or spinach leaves.

a). Materials used:

- Optical microscope.
- Slides and coverslips
- Crystallizing dish containing bleach for the disposal of used slides and coverslips

- Fine forceps
- Scalpel
- Distilled water
- Green pepper (***Capsicum annuum***)

b). Procedure:

- Using a scalpel make a thin cut in the outer layer of the pepper
- Using fine forceps, take a small fragment of the thin outer layer of the pepper
- Place the fragment between the slide and coverslip, in a drop of distilled water.
- Observe the sample using the 10×, then the 40× objective.

8.4.2. Leucoplasts (Amyloplasts):

Amyloplasts are specialized plastids that are used for the manufacture and storage of starch (from the Greek amylon, which means starch).

These are non-pigmented organelles that are mostly present in storage tissues like roots, tubers, and seeds.

Starch, a significant storage polysaccharide found in plants but missing in animals, gradually builds up within amyloplasts to produce separate starch grains.

Depending on the type of plant, these grains differ in size, shape, and quantity.

Additionally, amyloplasts are involved in the conversion of glucose to starch and have the ability to transform stored starch back into sugars when the plant needs energy.

Potato tubers, wheat grains, rice endosperm, and corn kernels are common examples of starch rich foods.

a). Materials and reagents

- Optical microscope
- Slides and coverslips
- Cotton
- Crystallizing dish with bleach for used slides and coverslips
- Stain: very dilute Lugol's solution

- Lance-tip needle, Bottle of distilled water
- Potato (*Solanum tuberosum*)

b). Slide preparation

➢ First case: without staining

- On a small piece of potato, gently scrape the pulp with a lance-tip needle.
- Place a drop of water on a slide, then mix in the collected material.
- Cover with a coverslip, taking care to avoid the formation of air bubbles.
- Observe the sample using the 10×, then the 40× objectives.

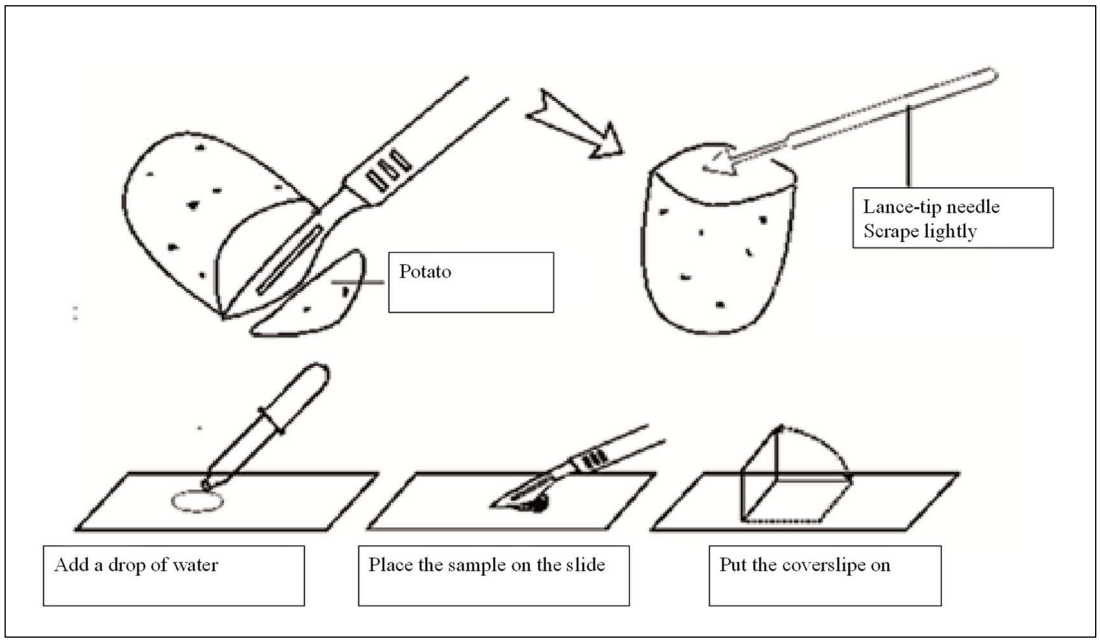


Fig 17: Observation preparation of Amyloplasts

➢ Second case: with staining using Lugol's solution

Prepare the sample again and add a drop of very diluted iodine solution (Lugol's solution), cover the preparation with a coverslip, and observe using the 10×, then the 40× objectives.

8.4.3. Chromoplasts:

These are cell organelles that contain carotenoid pigments (yellow, red, or orange pigments). Tomato, yellow bell pepper, and carrot are rich in chromoplasts.

a). Materials

- Optical Microscope
- Slides and coverslips
- Cotton
- Crystallizing dish with bleach
- Fine forceps
- Scalpel
- Red bell pepper, Carrots or tomato.

b). Slide preparation:

- Using a scalpel make a thin cut in the outer layer of the red pepper, carrots or tomato.
- Using fine forceps, take a small fragment of the thin outer layer of the plant tissue.
- Place the fragment between the slide and coverslip, in a drop of distilled water (avoiding the formation of air bubbles).
- Observe the sample using the 10×, then the 40× objectives.

8.5. The tasks assigned to the student:

a). Based on your microscopic observation describe your observations at all magnifications (low and medium magnification):

- Appearance of the cells
- Observed cell components
- Plasma membrane
- Appearance of the cytoplasm
- Shape of the vacuole

- Position of the nucleus
- Appearance and abundance of plastids.

b). Produce a well labelled scientific diagrams illustrating your observations. Your drawing should accurately represent the shape and structure of the cells, clearly indicating their main parts.

8.6. Expected Results:

Chloroplasts:

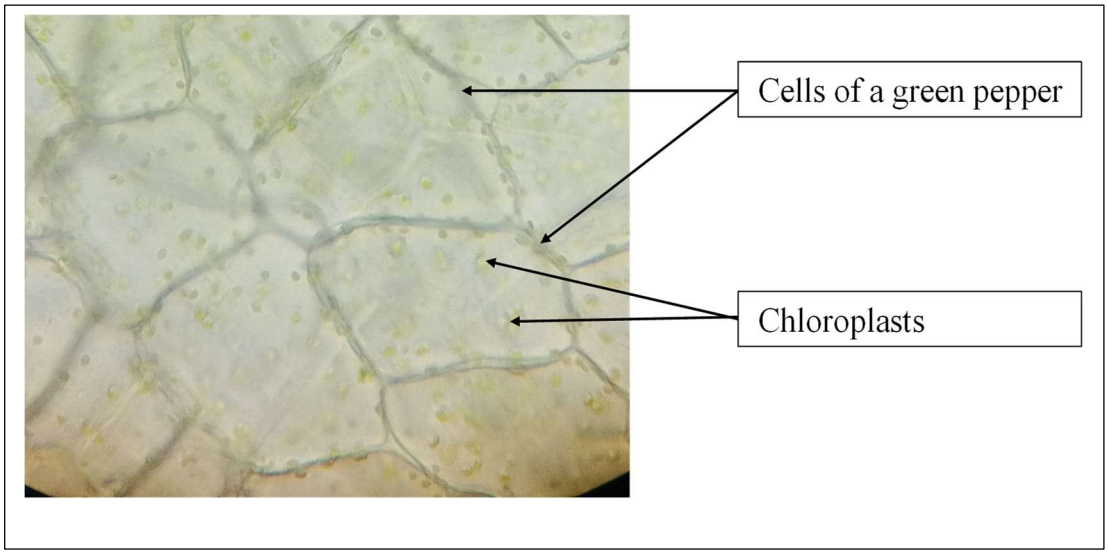


Fig 18: Microscopic observation of Chloroplasts of a Green pepper X400

Leucoplasts (Amyloplasts):

First case: without staining:

The starch grains or Amyloplasts and their growth striations around a central point, the hilum, are clearly distinguishable.

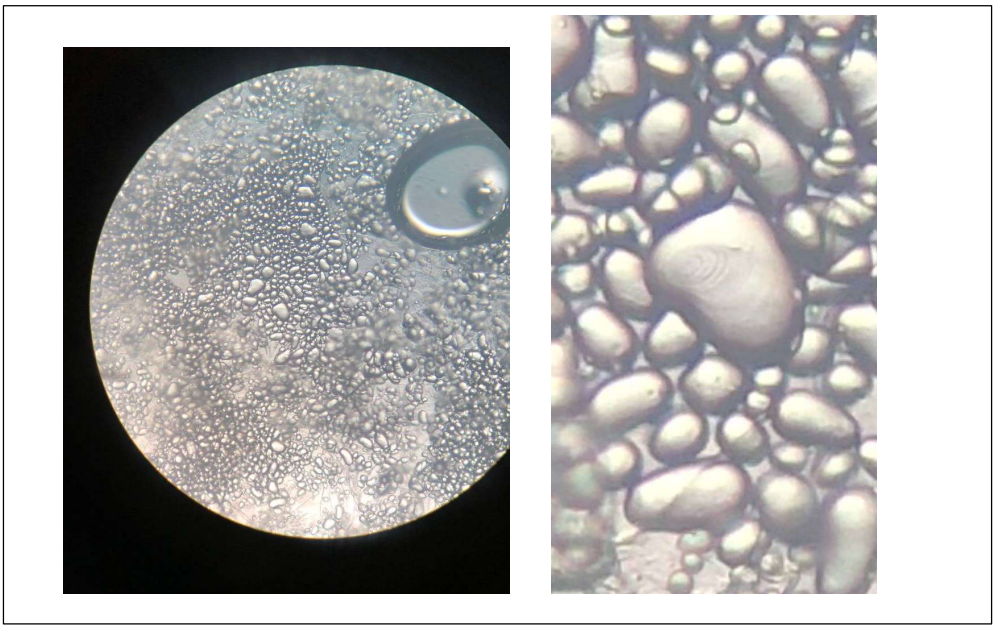


Fig 19: Microscopic observation of Amyloplasts with magnification x 100 (on left) and x400 (on right)

➢ Observation the Amyloplasts with staining:

The amyloplasts stain blue-violet (this is a characteristic reaction).

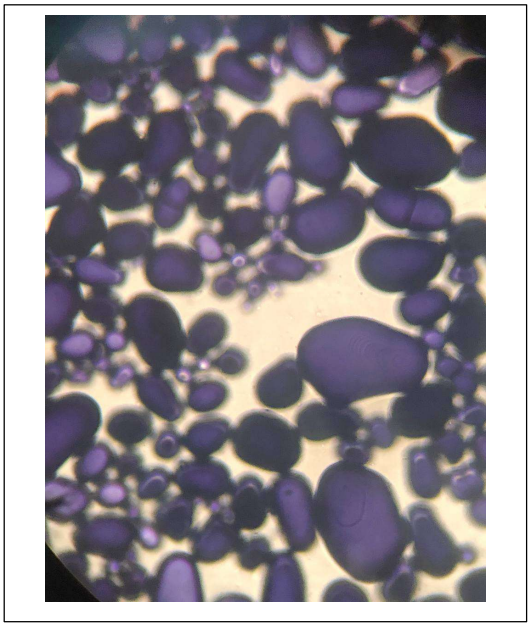


Fig 20: Microscopic observation of Amyloplasts stain in Lugol's solution with magnification x400

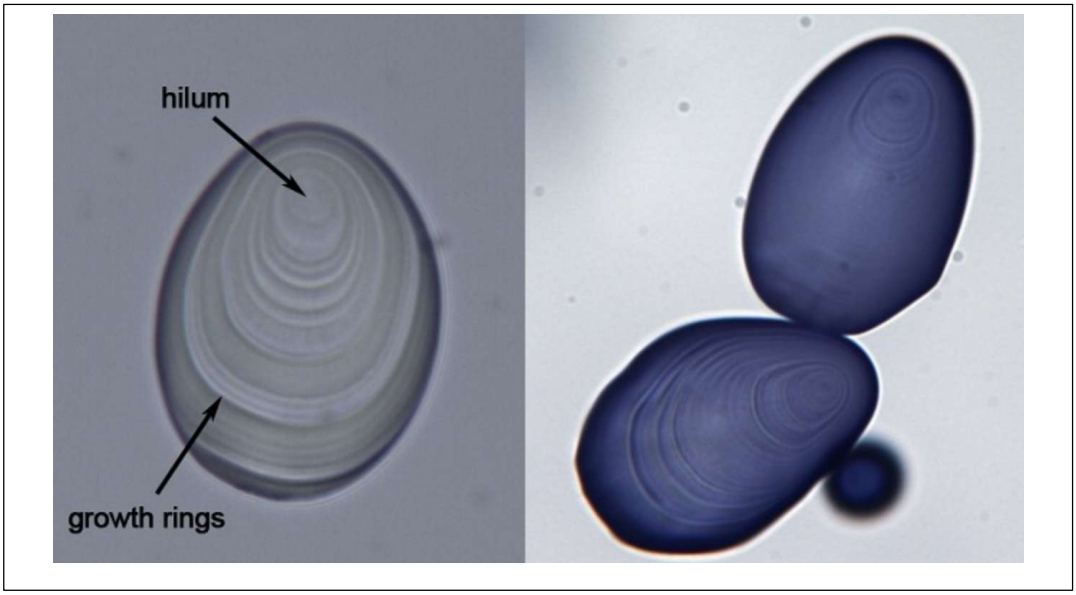


Fig 21: Schematic drawing of a microscopic observation of Amyloplasts (X400).

- **Chromoplasts**:

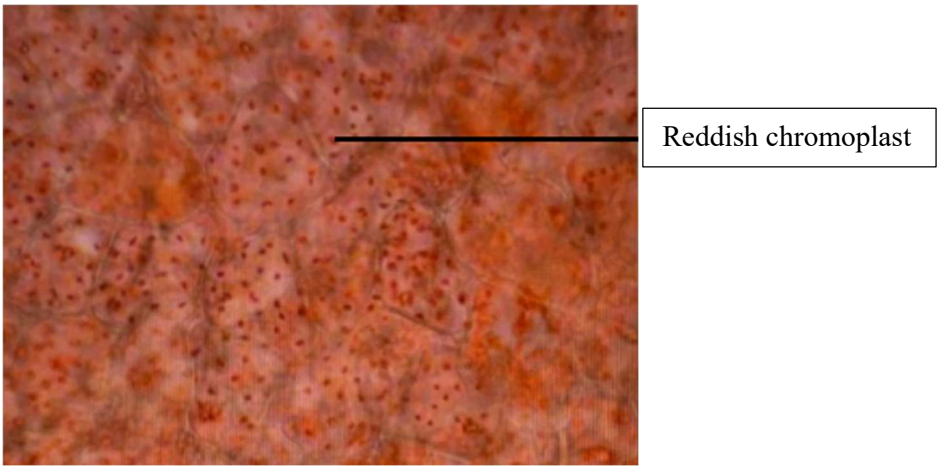


Fig 22: Microscopic observation of reddish chromoplasts of tomato with magnification x400

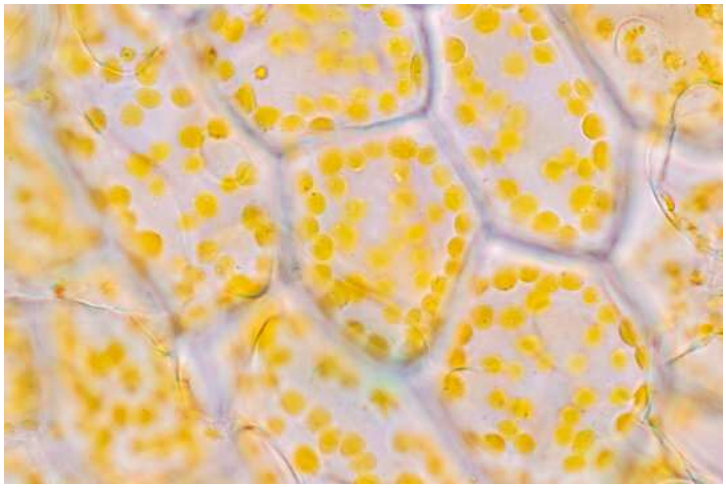


Fig 23: Microscopic observation of yellow chromoplasts of yellow pepper with magnification x400

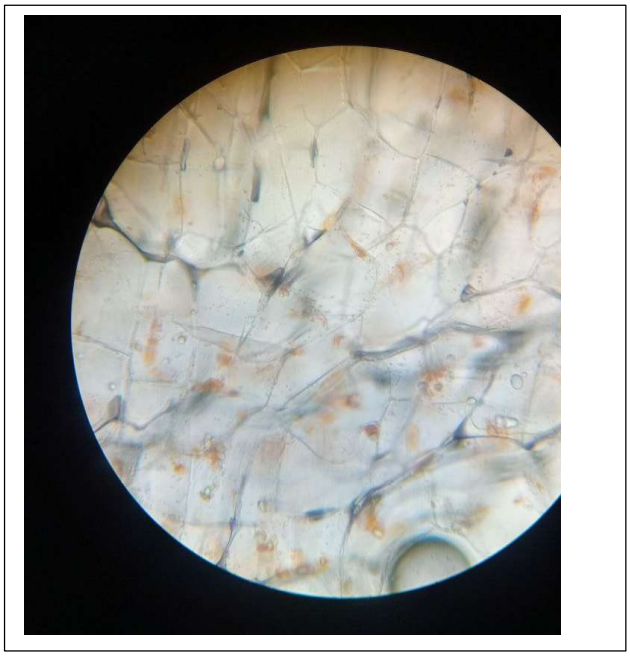


Fig 24: Microscopic observation of Orange chromoplasts of carrots with magnification x400

9. Practical session n°5: Methods for separating cellular components -Centrifugation-

9.1. Lesson review:

The study of cells relies on diverse methods designed to observe and analyze their structural and functional elements:

- **Microscopy:**

• Allows visualization of structures that cannot be seen with the naked eye.

• Main types include**:** Light (photonic) microscope, Transmission Electron Microscope (TEM), and Scanning Electron Microscope (SEM)

• Modes of observation: reflection (SEM), transmission (LM, TEM).

•Requirements: adequate contrast, thin sample.

- **Histochemistry**:

•Using particular stains to draw attention to in situ lipids, carbohydrates, calcium, amyloid, microbes, pigments, and connective fibres.

• Makes it possible to identify certain materials or tissue structures.

- **Immunological methods:**

• In accordance with the antigen-antibody response.

• Precipitation: a gradual, observable process caused by a soluble antigen.

•Particulate antigens cause agglutination, which is a quick reaction.

• Highly sensitive and quantitative marker.

• Based techniques: radioimmunology, ELISA, and immunofluorescence.

- **Enzymatic methods**:

• Histoenzyme techniques: identifying where enzymatic activity occurs in cells.

• Immunoenzymology (ELISA): quantitative detection of antigens or antibodies using an enzyme that produces a color reaction.

In cell and molecular biology, a variety of techniques are employed to separate organelles and cellular components. These methods enable the isolation and detailed study of structures such as the nucleus, mitochondria, ribosomes, lysosomes, microsomes, membranes, and the endoplasmic reticulum.

The principal approaches are summarized in **Table 5**, which outlines the methods used for organelle separation.

Tab 5: Various methods used for separating organelles

Method	Purpose	Techniques
Homogenization (Cell Disruption)	Break cells to release their contents without destroying organelles.	Several techniques ca be used in this purpose: **Physical techniques:** either we use ultrasound or high pressure. **Chemical techniques:** we can make osmotic shock, or use detergents or enzymes. **Mechanical techniques:** pistons and cylinders or homogenizer.
Centrifugation	Separates components based on their size or density.	Tere are two main techniques: Differential centrifugation Density gradient centrifugation
Filtration	Separation of organelles based on their size.	Filtration through: Porous filters, Filtrate membrane Vacuum Filtration
Chemical and Enzymatic Methods	Release specific organelles (e.g., nucleus).	Techniques based on mild detergents or enzymes
Techniques based on chemical properties	Separate specific components (proteins, lipids, nucleic acids) according to their chemical properties (affinity, polarity, charge, and size).	Chromatography, Electrophoresis

In this practical session we are going to study one of the separation techniques, which is centrifugation.

9.2. Objectives :

- Describe the technique used to separate the different types of cells from a tissue fragment.
- Understand the principle of centrifugation.

9.3. The centrifugation separation technique:

9.3.1. Definition:

Centrifugation is a technique used to separate the components of a heterogeneous mixture according to their sedimentation rates under a gravitational field. By applying centrifugal force through a device known as a centrifuge, the separation process is greatly accelerated.

In medical bacteriology, centrifugation is useful for collecting and concentrating bacteria or mineral elements contained in a pathological sample into a pellet, or, on the contrary, for removing interfering suspended elements.

9.3.2. The principle of the technique:

A centrifuge operates by spinning tubes rapidly around a central axis. The resulting centrifugal force acts on the mixture, driving denser particles toward the bottom of the tubes. Both the magnitude of the force and the sedimentation rate increase proportionally with rotation speed.

9.3.3. Centrifugation steps:

a). Homogenization:

The homogenization of cells isolated or dissociated from tissues involves placing them in suspension in solutions with appropriate pH and osmotic pressure, after which the cell membrane is disrupted either by physical methods (ultrasound or high pressure), chemical methods (osmotic shock, detergents, or enzymes), or mechanical methods (pistons and cylinders such as a Potter tube).

Notes:

The phase obtained after homogenization, called the homogenate (**Fig 25**), or tissue grind, is a liquid phase containing various cellular components in suspension: nuclei, mitochondria, endoplasmic reticulum, ribosomes, etc.

The particles present in a liquid have highly variable sizes and densities and are all subjected to different forces:

• **Gravitational force**, which acts from top to bottom and causes the sedimentation of dense or heavy particles.

• **Buoyant force** (**Archimedes' principle**), which acts from bottom to top and causes the flotation of light particles.

• **Molecular agitation**, which is the constant and disordered motion of molecules due to their thermal energy. This movement prevents the sedimentation or flotation of suspended particles because of their small size.

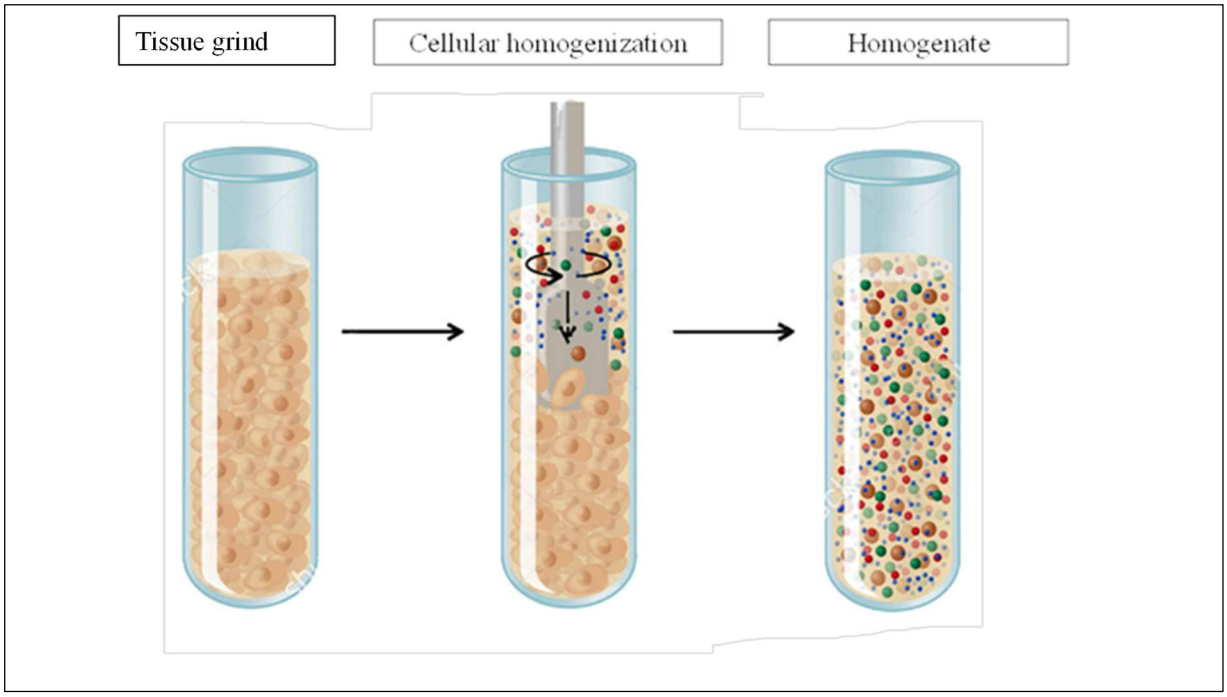


Fig 25: Homogenization process

b). Separation by Centrifugation:

Centrifugation allows the separation of constituents with highly variable sizes and masses contained in a liquid, ranging from simple molecules to entire cells.

A tissue is mechanically ground in a sucrose solution to obtain a homogenate, which is then subjected to centrifugal force at a well-defined speed and duration (**Fig. 26**). This introduces a new force, the **centrifugal force**, which is an acceleration acting radially outward from the axis of rotation. Two fractions are obtained:

- **A solid fraction, called the pellet:** contains the heavy particles, located at the bottom of the tubes.
- **A liquid fraction, called the supernatant:** contains the lighter particles, which remain at the surface.

The sedimentation rate of particles in a gravitational field is influenced by their size, shape (whether globular or elongated), and density. This property enables the calculation of a **sedimentation coefficient**, denoted as **S** (**Svedberg unit**), which is directly proportional to particle size.

S: is the unit of measurement for the sedimentation rate.

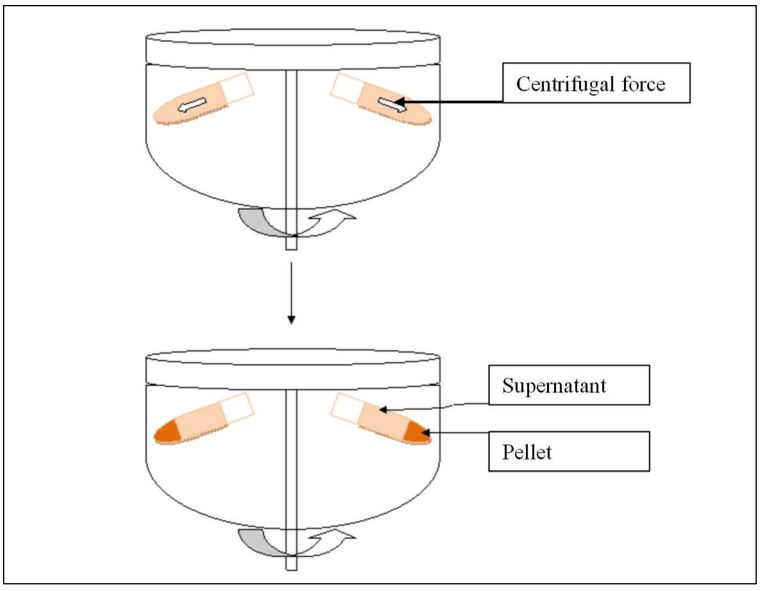


Fig 26: Separation by Centrifugation

9.3.4. Materials used:

- pH 7.5 buffer (can be replaced with distilled water)
- Homogenizer or Grinder
- Centrifuge
- Beaker
- Conical tubes
- Centrifuge tubes
- Tissue fragment (e.g., liver, spinach, parsley, etc.)
- Ice

9.3.5. Procedure:

- Cut the biological material into small pieces.
- Place the conical tubes in a beaker filled with ice (to prevent the destruction of protein molecular structures due to the heat generated).
- Immerse the tissue fragment in a volume of water (or buffer) in each conical tube.
- Perform homogenization using a mechanical grinder to obtain a homogenate.
- Transfer a volume of the homogenate into centrifuge tubes.
- Balance your centrifuge and centrifuge the homogenate for a few minutes.
- Draw the results.

9.3.6. Risks and precautions for using a centrifuge:

The use of centrifuges involves several hazards, including rotor rupture, injury from contact with rotating components, and potential explosion in flammable environments.

To ensure safety, centrifuges must be fitted with locking mechanisms that prevent operation when the lid is open and block access while the rotor is in motion.

Loads placed in centrifuge buckets must be symmetrically arranged and precisely balanced by weighing.

Furthermore, cleaning, adjustments, maintenance, and repairs must only be carried out when the device is switched off and disconnected from the power supply.

9.3.7. Expected Results:

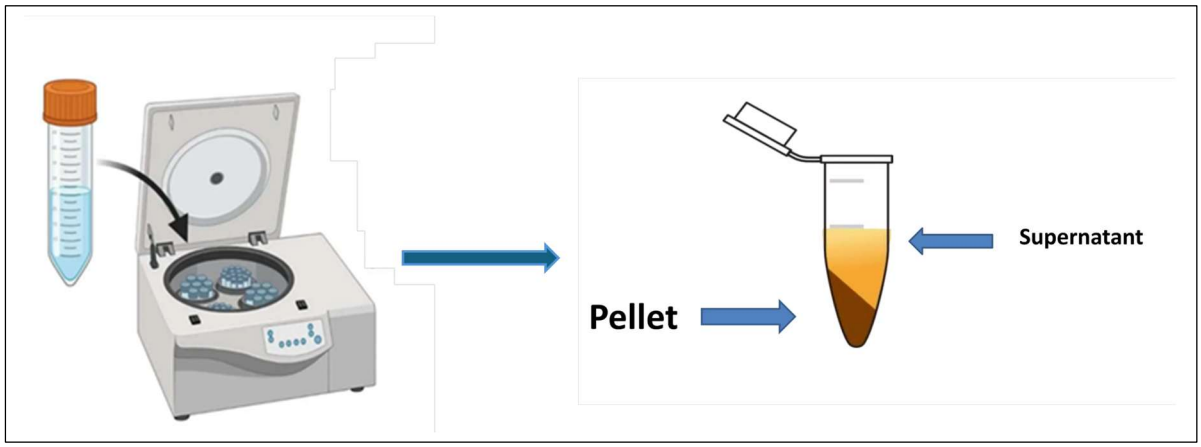


Fig 27: Separation of cellular components by centrifugation

Fig 28: State of centrifuge tubes contain heterogenous mixture of parsley before and after separation by centrifugation

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