INTERNATIONAL UNIVERSITY HCMC

SCHOOL OF BIOTECHNOLOGY

June 2014 version.

BIOLOGY (BT155IU)

Labwork Manual

[GROUP NUMBER] _____

[GROUP MEMBERS]

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GENERAL ISSUES

GRADING

Labwork assessments	
Prelab (individually)	20%
Lab report (in group)	30%
Lab examination (individually)	50%
Total	100%

CODE OF CONDUCT

1. Students must understand, remember and follow the **Laboratory safety** (see next page) before starting this course.
2. Absence without permission is not allowed, due to the nature of the labs. For make-up class, join another group having that practical session.
3. Pre-labs must be submitted at 8AM or 1PM on each practical class.
4. Examination is composed of practical performance and questions answering.

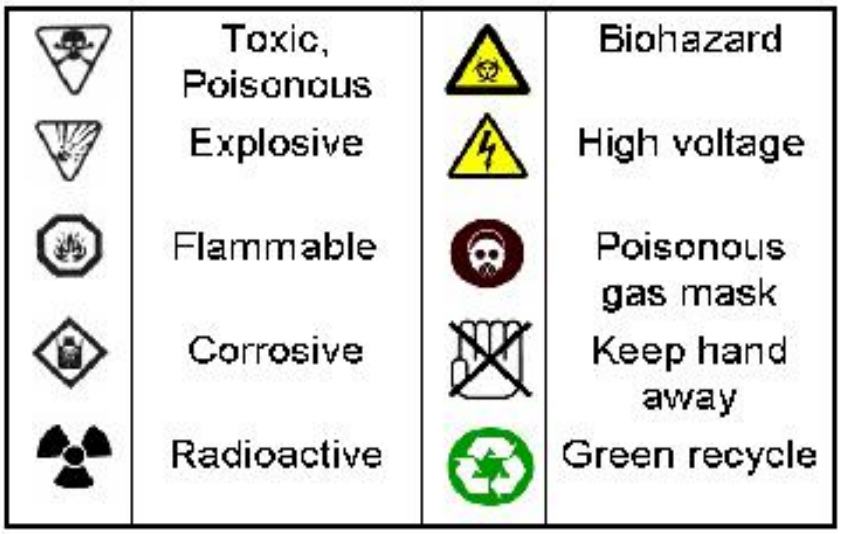
*** More information, please go to:

OUTLINES

LABORATORY SAFETY

Before doing lab-work, students should be aware of the risks, hazards and safety conditions maintained in laboratory. ***Risk*** *is identified as a substance or biological agent that might be harmful under specific circumstances while* ***hazard*** *is the ability of a substance or biological agent to cause harm. In any case, a person working with chemicals, bio-chemicals or other related agents should follow strictly certain principles applied in each laboratory, and all practical works must be carried out with safety in mind to minimize the risk of harm to yourself and to others. The followings are the basic rules for laboratory work.*

1. Make sure that you know what to do in case of fire, including exit routes, how to raise the alarm, and where to gather after leaving the building. Remember that the most important consideration at all time is human safety.
2. Follow tutor's instructions and the laboratory principles while doing lab work.
3. Wear lab-coat and **closed footwear** at all time.
4. Do not smoke, eat or drink in laboratory because of the risks of contamination by inhalation or ingestion.
5. Do not mouth-pipette any liquid.
6. Take care when handling glassware.
7. Know the warning symbols for specific chemical hazards (see below).



8. Use fume cupboard for hazardous chemicals.
9. Work in a logical, tidy manner and minimize risks by planning in advance.
10. Clean up working bench and experimental tools at the end of each lab session.
11. Dispose wastes in appropriate containers.

Practical 1: MICROSCOPY, CELL OBSERVATION & OSMOSIS

AIMS OF THE PRACTICAL

✓ Familiarize students with the use and care of microscope, an indispensible instrument for anyone who works in biological fields.

✓ Introduce students the microscopic sample preparation of plant cells and animal cells.

✓ Examine the osmosis process in plant cells.

1. MICROSCOPY

Microscopy is defined as a study of using microscopes to observe very small objects that are invisible to the human naked eye. Light microscope can enhance our capacity to view detail by 1000 times, so that we can see samples as small as 0.1 µm in diameter. High-tech microscopes, such as Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM), are able to give visual magnification up to 200,000 times and fascinating features in comparison with unaided visibility. In fact, the biological understanding of cell structures and functions would be extremely restricted without microscopes.

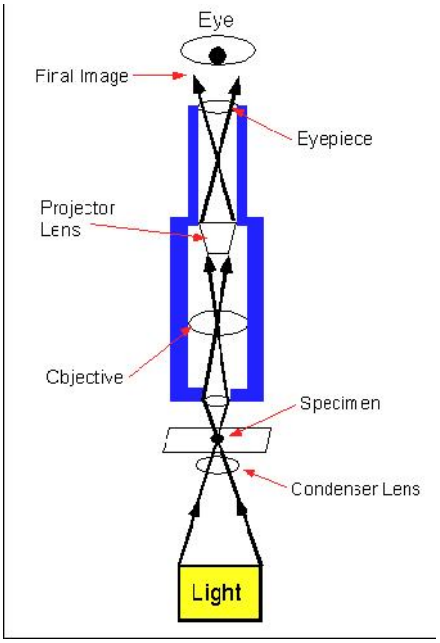


Figure 1.1: Basic principle of a light microscope or optical microscope.

All microscopes consist of a coordinated system of lenses arranged so that a magnified image of the specimen can be seen by the viewer. The main differences among different types of microscopes are the power source to produce the picture, nature and the arrangement of the lens system and maximal resolution (resovling power) they can offer. In general, microscope works base on the magnification of specimen image through a series of lenses.

There are different types of microscope: Compound light microscopes, Stereoscopes, Confocal microscopes, Scanning electron microscopes, Transmission electron microscopes…

Construction of a Compound Microscope

✓ **Eyepieces** (**ocular lens**): are placed at the top of the body tube. They transmit and magnify the image from the objective lens to eyes. Eyepiece has its own magnification power. The eyepieces that you use will have magnification power of 10 (10x).

✓ **Objective lens:** are lens or series of lenses that gather light from specimen and help to magnify the specimen image. This is the most important part of a microscope. To figure the total

magnification of an image that you are viewing through the microscope, take the power of the objective lens (4x, 10x, 40x, 100x) and multiply by the power of the eyepiece.

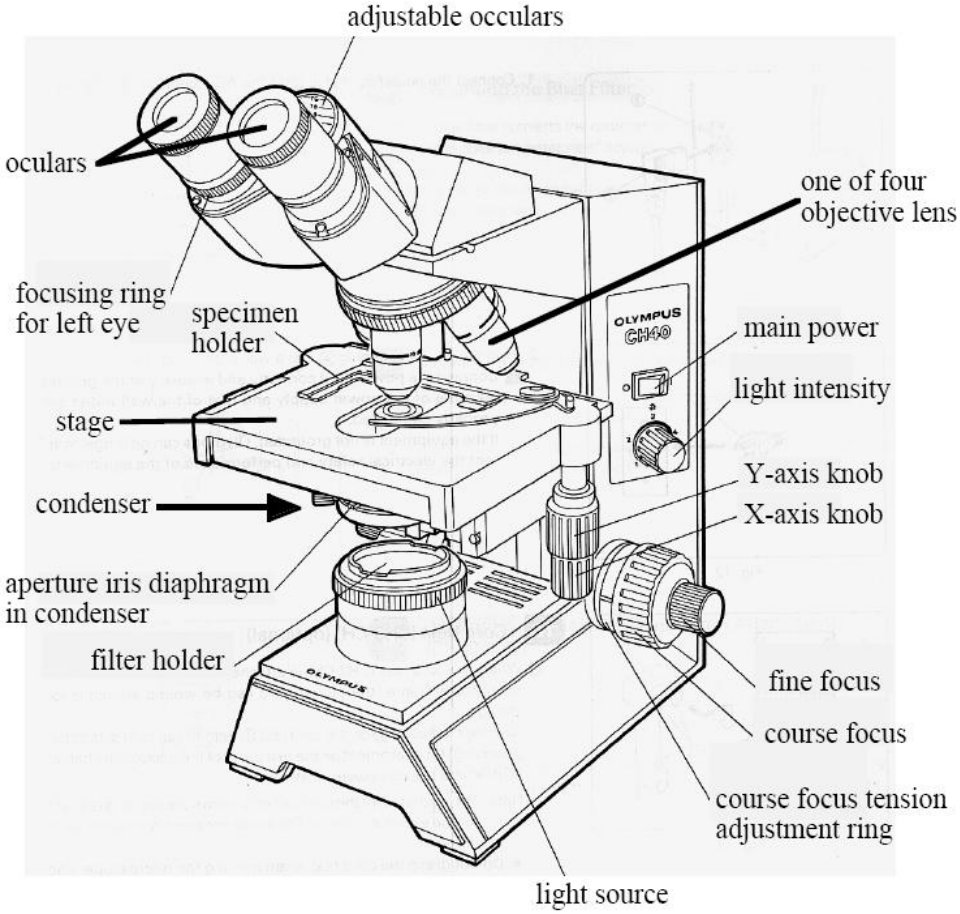


Figure 1.2: Different parts of a compound light microscope.

✓ **Body tube:** allows light to travel from the objectives through a series of magnifying lenses to the eyepieces. In your microscope, the eyepieces are held at an angle for convenient use, and the body tube contains a prism that bends the light rays so that they will pass through the eyepieces.

✓ **Stage:** is the surface or platform on which you can place your specimen. **Stage clips** or **specimen holder** is used to clamp your specimen on the stage. For your microscope, the stage is movable and is called a mechanical stage. The movement is controlled by two knobs (**X-axis** and **Y-axis** knobs) located on the bottom of the stage. They allow the specimen to move vertically and horizontally.

✓ **Condenser**: is a lens system under the stage that gathers light from the light source and focuses it onto the specimen.

✓ **Condenser Adjustment Control:** is to adjust the height of the condenser. Usually, the condenser always will be all the way up.

✓ **Aperture iris diaphragm:** is to control the level of light that can go through the condenser.

✓ **Light source:** the illuminator of most microscopes is built into the base of the microscope and controlled by **on/off switch**. You can control the light intensity by adjusting the voltage of a **transformer** attached to the illuminator.

✓ **Coarse Focus Knob and Fine Focus Knob**: You focus a microscope by using the Coarse and Fine Focus knobs. Both coarse (large) and fine (small) adjustment knobs are found on both sides of our microscopes. Remember that the coarse adjustment is used only with the low-power objective (4x). These knobs control a gear mechanism that raise and lower the stage.

Proper Use of a Compound Microscope

1.1. General instructions

- Avoid dropping the microscope, banging it against a lab bench, or having the eyepieces fall out.
  - Carry the microscope upright using both hands. Keep one hand on the arm and another at the bottom of the microscope.
  - Keep microscope away from the edge of the bench.
  - Switch off the illuminator and remove power cords from the power supply when not in use.
- Avoid breaking a coverslip and microscope slide, and even the objective lens while focusing.
  - First adjust the stage to the lowest position.
  - Locate the ready specimen using the lowest power objective lens, and then switch to the higher power objective lenses.
  - Never focus the high power lenses with the coarse adjustment knob, and never use these lenses to examine thick specimens.

1.2. Focusing

Notice: ***Always use clean microscope slides, and proceed from the lowest power to the highest power objectives***.

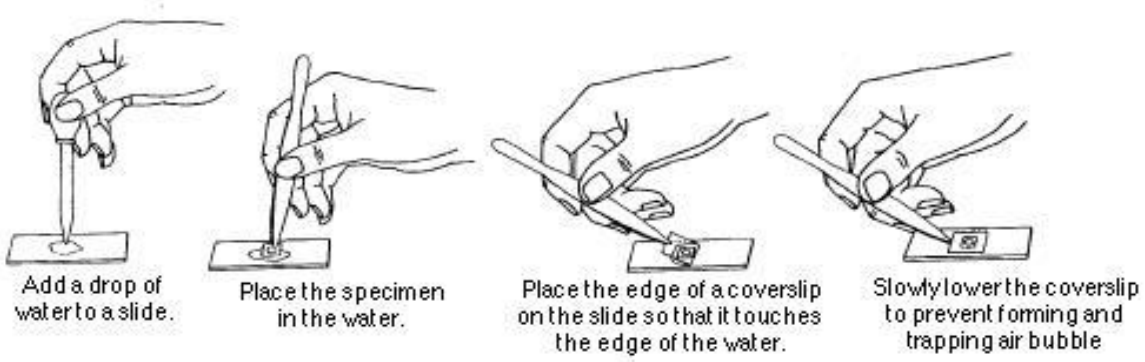


Figure 1.3. Preparing a wet mount slide

1. Clean the eyepieces and objective lens using lens-cleansing papers (if necessary)

2. Cut out a letter *"e"* from a newspaper or other printed page. Clean a microscope slide and prepare a wet mount of the letter, following Figure 1.3.

3. Put the lowest power objective (4x) in position, lowest the stage of the microscope, and place the slide on the microscopic stage.

4. Switch on the illuminator and diaphragm fully, and adjust the condenser level appropriately.

5. Move the specimen into the bright area on the stage using X and Y-axis knobs.

6. Lift the stage up close to the objective lens using the coarse focus (clock-wise direction) while observing through the eyepieces until the specimen comes into focus.

7. Use the diaphragm to adjust the light intensity as necessary as well as center the specimen by moving the slide using X and Y-axis knobs.

8. Switch from the scanning objective (4x) into the high-power objective (10x). Refine the focus by gentle adjustment with fine focus knob only.

9. Switch to the higher-power objective (40x or 100x) and again adjust the focus with the fine focus knob only.

2. CELL OBSERVATION

Objective: Observation different cell types (plant cells and animal cells) under microscope at different objective lens and make comparation (shape, size…)

Materials and Equipments:

- Onion bulb
- Light Microscopes
- Glass slides
- Coverslips
- Water
- Lugol solution
- Blades
- Forceps
- Tooth-pick

2.1. Plant Cells – *Onion Epidermis Cells*

1. Remove a piece of an onion leaf from a section of an onion bulb.

2. Break the piece of onion leaf into half as shown in Figure 1.4. The outer epidermis layer should be easy to separate from the rest of the leaf.

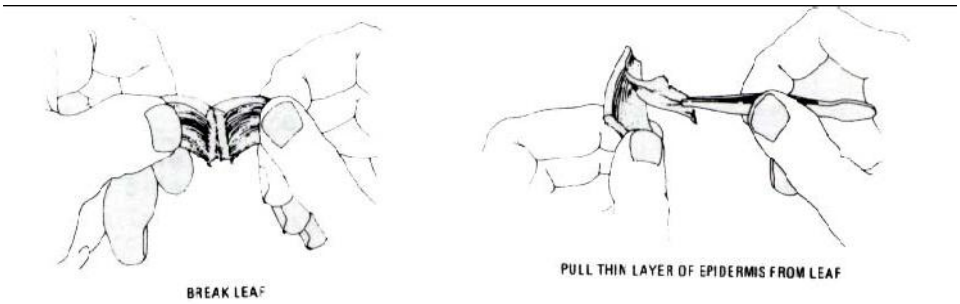


Figure 1.4. How to obtain a piece of onion for slide preparation

3. Place the epidermis layer flat on a slide. Wrinkles will trap air bubbles and obscure your observations.

4. Add a drop of water/Lugol solution and cover with a coverslip.

5. *Observe your slide with your microscope*. Remember to locate a good region of the epidermis with the lowest magnification (4x) lens before observing details of cell structure with higher magnifications up to 100x.

2.2. Animal Cells – *Human Cheek Cell Epithelium*

1. Gently scrape the inside of your cheek with the broad end of a toothpick. (You won't need to puncture your cheek to obtain a good supply of cells.)

2. Smear your cheek scrapings on a clean slide. Wrap your toothpick in a dry paper towel and immediately dispose it into the waste-container provided.

3. Make a wet mount of your cheek cells by adding a drop of water/Lugol solution to the slide.

4. Add a coverslip and observe the slide **at very low light intensity**.

5. When you locate some cheek cells, at 4x objective lense center them in the field of view and move to the next power level (10x) for observation. Re-focus and center your cheek cells and then view them with the high power (40x and 100x) objective lens. *Observation*.

3. OSMOSIS

Diffusion is specified as the net movement of molecules from the regions of higher solute concentration to the regions of lower solute concentration.

Osmosis is a special form of diffusion in which water molecules flow across a differentially permeable membrane from a solution with a lower solute concentration to a solution with a higher solute concentration. This membrane allows only certain types of molecules to pass through it or permeate it freely. Cellular membrane is a type of differentially permeable membrane.

Osmosis does not require expenditure of energy but an energetically "downhill" process. Since the water must lose energy as it moves by osmosis, water must move from an area of greater water potential to an area of lower water potential:

- Hypertonic solution has water potential outside the cell lower than inside the cell, then there will be a net movement of water out of the cell.
- Hypotonic solution has water potential outside the cell greater than inside the cell, then osmosis will be a spontaneous net movement of water into the cell.
- In isotonic solution, the water potential on each side of a cell membrane is the same, there will be no net movement of water across the membrane.

Osmotic pressure of a solution is proportional to the effective concentration of dissolved particles, regardless of the size or chemical nature of the particle.

Objective: Demontrate and observe the osmosis and osmotic pressure using epidemic plant cells from *Zebrina pendula* leaf.

Materials and Equipments:

- A leaf of *Zebrina pendula*
- Distilled water
- 5% Sodium chloride solutions
- 0.85% Sodium chloride solutions
- Glass slides + Coverslips
- Blades + Forceps
- Microscopes
- Paper towel

3.1. Plant cells – *Plasmolysis*

Plant cells always have a strong cell wall surrounding them. When taking up water by osmosis they start to swell, but the cell wall prevents them from bursting. Plant cells become "**turgid**" when they are put in dilute solutions. Turgid means swollen and hard. The pressure inside the cell rises; eventually the internal pressure of the cell is so high that no more water can enter the cell. This liquid or hydrostatic pressure works against osmosis. Turgidity is very important to plants because this is what make the green parts of the plant "stand up" into the sunlight.

When plant cells are placed in concentrated NaCl solutions they lose water by osmosis and they become "**flaccid**"; the exact opposite of "turgid". If you put plant cells into concentrated NaCl solutions and look at them under a microscope you would see that the contents of the cells have shrunk and pulled away from the cell wall: they are said to be plasmolysed. And this phenomenon is call **plasmolysis**.

When plant cells are placed in a solution which has exactly the same osmotic strength as the cells they are in a state between turgidity and flaccidity. We call this incipient plasmolysis. "**incipient**" means "about to be". When one forgets to water the potted plants, their leaves drop. Although their cells are not plasmolysed, they are not turgid and so they do not hold the leaves up into the sunlight.

Procedure

1. Use a scalpel to peel a thin epidermis layer (purple side) of the *Zebrina pendula* leaf.
2. Put a small drop of 0.85% NaCl on a clean glass slide.
3. Place the peeled layer to the saline on the slide. Add a coverslip.
4. Examine the plant cells with the high power lens (40x). Locate the region where the cells are not too dense.
5. Add 2-3 drops of 5% NaCl to edge of the coverslip. *Observe the plant cells and the changes (focus to the cellular content - purple area) that occur as the more concentrated saline solution reaches them.*
6. Make another sample by repeating from step 1 to step 4. This time put 2-3 drops of distilled water to the edge of the coverslip, instead of 5% NaCl. *Observe the plant cells and the changes (focus to the cellular content - purple area) that occur as the less concentrated saline solution reaches them.*

3.2. Animal cells – *Hemolysis*

The cell membrane of **erythrocytes** (red blood cells) is permeable to water but relatively impermeable to salts. If red blood cells are placed in an **isotonic** saline solution (0.85% NaCl), the cells will retain their shape and size.

If the red blood cells are in a **hypotonic** saline solution, water will enters the cells more rapidly than it leaves. As a consequence, the red blood cells swell and ultimately bust, releasing hemoglobin. This phenomenon is called **hemolysis**. Red blood cells if placed in a **hypertonic** saline solution will shrink and appear to have a bumpy, irregular outline. The cells are said to be **crenated**.

Practical 2: ORGANIC COMPOSITIONS OF THE CELL

PRE-LABS

1. What are 4 classes of biological macromolecules and their building blocks?
2. Describe structure of carbohydrate (starch, sugar).
3. What is the difference between Lugol and Iodine solution? How can we prepare them?
4. Describe structure of protein.
5. How would you prepare 100 ml of 0.5% $CuSO_4$ solution from $CuSO_4.5H_2O$ (MW = 250)?
6. Where can we find lipid in plant cells and animal cells?
7. Describe structure of nucleic acid.
8. In the forthcoming practical session, you will have to use a number of different chemical solutions: Lugol solution, concentrated HCl, NaOH, $CuSO_4$, Soudan III, 20% Ethanol and glycerin. List three solutions, which are most potentially toxic and thus require caution while handling, in your opinion. Explain your reason.

AIMS OF THE PRACTICAL

Help students to identify the three main organic compositions of the cell: carbohydrates, proteins and lipids in one sample.

1. CARBOHYDRATES

Carbohydrates make up a group of chemical compound found in plant and animal cells. They have the empirical formula $C_nH_{2n}O_n$ or $(CH_2O)_n$. An empirical formula of carbohydrate tells the atomic compositions of the compound. Carbohydrates are divided into *monosaccharides*, *disaccharides*, and *polysaccharides*.

Glucose (carbohydrate) is the **primary products of plant photosynthesis**. The simplified light-driven reaction of photosynthesis results in the formation of carbohydrates:

$$nH_2O + nCO_2 \rightarrow (CH_2O)_n + nCO_2$$

This type of carbohydrate is found in the structure of plants and is used in the reverse reactions of photosynthesis (respiration) or is consumed as fuels by plants and animals. The excess glucose is stored in form of starch in plants. While in animal, the excess produced glucose is stored in form of glycogen in liver and muscles.

The most stable three-dimensional structure for starch and glycogen is a tightly **coiled helix** stabilized by inter-chain hydrogen bonds.

In amylose (with no branches) this structure is regular enough to allow crystallization and thus determination of the structure by X-ray diffraction. Each residue along the amylose chain forms a 60^{o} angle with the preceding residue, so the helical structure has six residues per turn. For amylose, the core of the helix is of precisely the right dimensions to accommodate iodine in the form I^{3-} or I^{5-} (iodide ions), this complex has the **deep blue color** and this interaction with iodine is a common qualitative test for amylose.

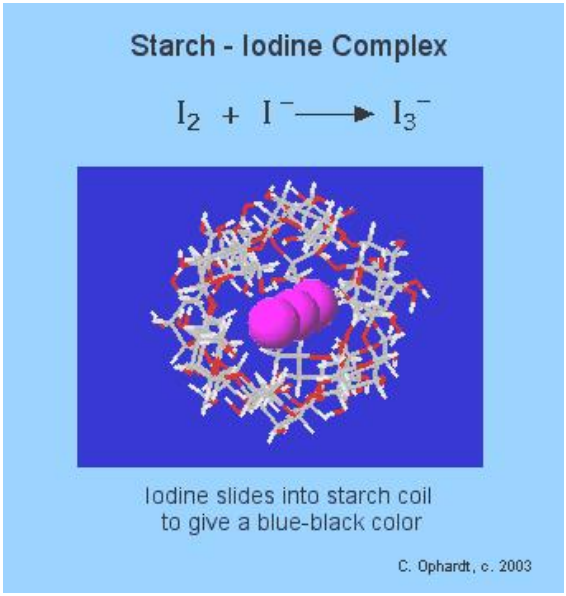


Figure 2.1: Interaction between amylose and iodine ions when starch is treated with Lugol solution

However, this glycosidic bond is easily **wrecked by heat, enzyme, acidic or basic treatment**. Hydrolyzing the starch with acid or exposing to high temperature result in the formation of mixture of saccharides with different lengths (including the one-, two- or number of glucose chains). Once the helix structure of homopolycarbohydrate is broken, the conformation with iodine no longer exists.

Objective: How to detect the starch in the sample/solution by Lugol solution. And understand the effect of temperature to the structure of the starch.

Materials and equipments:

- Potato
- Rice starch suspension.
- Microscope
- Test tubes
- Glass slides, coverslips
- Lugol solution
- Pasteur Pipettes
- Concentrated HCl solution
- Test tube racks
- Test tube clamps
- Toothpicks
- Waterbath

1.1. <u>Task 1 – Microscopic Observation of Starch Granules</u>

1. Prepare the clean glass slide and coverslip.
2. Cut the potato and scratch potato at the edge of this cut.
3. Collect the scratching and place on the slide, add a water drop and cover with coverslip. *Observe under microscope.*
4. After that adding 1-2 drops of Lugol solution to the edge of the coverslip and *observe the phenomenon*

1.2. <u>Task 2 – Chemical Detection of Starch</u>

1. Add 5ml of starch suspension into a test tube.
2. Take out one drop of rice starch suspension and put onto the glass slide. Add 1 drop of Lugol solution and mix well using a toothpick. *Observe* the color change of this suspension (compare with the original color of Lugol solution).

3. After that, add 5 drops of concentrated HCl solution into that test tube containing 5ml of starch suspension. Mix well.

4. Take out 1 drop of starch-HCl mixture onto the slide and test the color with 1 drop of Lugol solution.

5. Place the test tube containing starch and HCl into the rack which has been already submerged in the hot water (the waterbath is set to 100^{o}C) and boil this suspension. **Every 2 minutes**, take out one drop of hydrolysed starch-HCl mixture using Pateur pipette and put onto a glass slide, let it cool down for a while and add 1 drop of Lugol solution. *Observe the change of color intensity.*

6. Continue to boil and test with Lugol solution until no color change is detected. *Mark the time that the color does not change.*

2. PROTEINS

Proteins are complex polymers composed of **amino acids**. Amino acids contain carbon, hydrogen, nitrogen and sometimes sulfur and serve as monomers for making peptides and proteins. Amino acids are linked together by **peptide bonds** in which the carboxyl carbon of one amino acid forms a covalent bond with the amino nitrogen of the other amino acid.

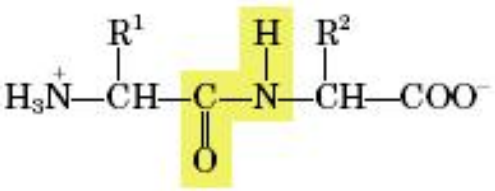


Figure 2.2: A Peptide bond links two amino acid residues

Short chains of amino acid are called peptides, longer chains of amino acids are called oligopeptides or polypeptides.

In the strong basic environment, two nitrogen atoms from two adjacent peptide bonds can coordinate with other two nitrogen atoms on other peptide chain in the conformation with metal ions such as Cu, Zn… if present. The complex gives out the color ranging from purple to red depending on the length of peptide chain (or the number of peptide bonds). This Biuret reaction is used to detect the presence of peptide or protein in sample.

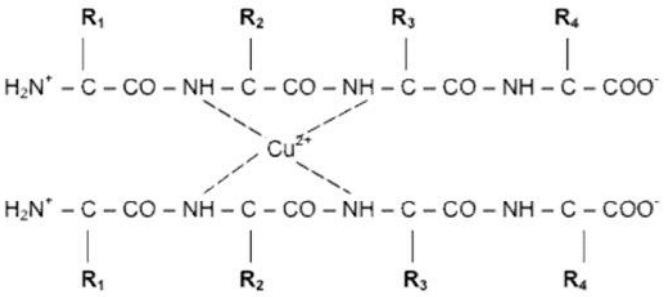


Figure 2.4. Protein-Copper complex formation in Biuret reaction

Objective: Understand the Biuret reaction and the role of chemical reagents in protein detection.

Materials and Equipment:

- Protein suspensions of egg white and fresh cow milk
- Test tubes
- Pasteur Pipettes
- 10% NaOH solution
- 0.5% $CuSO_4$ solution

Qualitative Detection of Proteins

1. Pipette 2 ml of protein suspension into a test tube.
2. Add 2 ml (or 10 drops) of 10% NaOH solution. Mix well.
3. Add one to 2-3 drops of 0.5% $CuSO_4$ solution into the test tube.
4. *Observe the color*.

3. LIPIDS

Lipids are a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or "building-blocks": ketoacyl and isoprene groups. Using this approach, lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits).

The main biological functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment.

Objective: Detect and observe the lipid in plant cells using red Soudan III solution.

Materials and Equipments:

- Peanut (soaked in water)
- Soudan III solution
- 20% Ethanol
- Blades
- Pasteur pipettes
- Microscope
- Glass slides & cover slip

Qualitative Detection of Lipids

1. Slice the peanut (which was soaked in water) as thin as possible.
2. Place it on glass slide; add a drop of Soudan III solution. Keep sample in this solution for staining in 10 minutes.
3. Wash the slide with 20% Ethanol.
4. Add a drop of water/immersion oil to the sample then put the coverslip on.
5. *Observe the lipid granules stained in peanut cells using microscope up to 100x.*

Practical 3: PHOTOSYNTHESIS & TRANSPIRATION

PRE-LABS

1. What are autotrophs and heterotrophs? Give each one example.
2. What is “photosynthesis”? How many stages are there in the photosynthesis? Describe.
3. Where is the chlorophyll distributed in plants and animals?
4. What is the function of chlorophyll?
5. Define the terms “transpiration” and “respiration”. What is the difference between them?
6. What are stomata and guardcells? Describe their distribution on the leaf.

AIMS OF THE PRACTICAL

Help students to understand the process of photosynthesis by examining the products of photosynthetic reaction.

1. PHOTOSYNTHESIS

Photosynthesis is the process by which plants, some bacteria, and some protistans use the energy from sunlight to produce sugar, which can then be converted into ATP, the "fuel" used by all living things via cellular respiration process. The overall photosynthesis reaction can be summarized as follow:

$$6H_2O + 6CO_2 \text{ -----> } C_6H_{12}O_6 + 6O_2$$

Photosynthesis is associated with the activities of the green pigment **chlorophyll**. Several modifications of chlorophyll occur among plants and other autotrophic organisms. **A pigment** is defined as a substance that can absorb light. The color of the pigment comes from the wavelengths of light reflected (in other words, those are not absorbed). Chlorophyll, the green pigment common to all photosynthetic cells, absorbs all wavelengths of visible light except green, which reflects the green colour to be seen by our eyes.

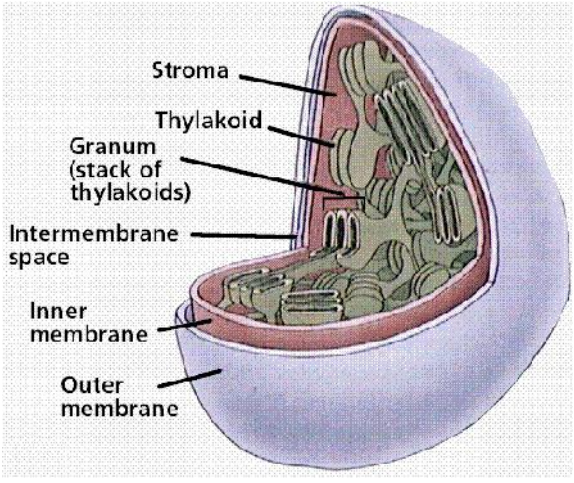


Figure 3.1: Structure of a chloroplast

Chlorophyll is embedded in the membrane of a specific structure called **thylakoid**. This is the unit of photosynthesis. Thylakoids are stacked like pancakes in stacks known collectively as granum.

The areas between grana are referred as stroma. While the mitochondrion has double membrane systems, the chloroplast has three, forming three compartments.

Objective: In this experiment, we will examine the presence of photosynthetic products in plant.

Materials & Equipments

- Waterweed
- Leaf with covered part
- Ethanol 70%
- Lugol solution
- Beaker, petri dish, test tube, forceps
- Absorbent paper

1.1. Task 1 – Examination of Oxygen Formation

1. Prepare one beaker with two thirds of water.
2. Turn down the funnel to the beaker.
3. Put 10 waterweed branches into the funnel.
4. Cover the tunnel end with the test tube filled with water and bring this beaker to the light.
5. *Observe the formation of coming up bubbles and record the level of water goes down every 3 days up to 2 weeks.*
6. After the incubation time, take out the test tube from the funnel while still keep it at the original orientation, cover the test tube by hand and upturn this test tube.
7. Remove hand and immediately test the gas in test tube with the burned match.
8. *Observe what happens with the fire from match.*

1.2. Task 2 – Examination of Starch Formation

1. Choose one leaf on a growing tree. Clean this leaf with water and tissue paper and then dry.
2. Use a piece of black paper or cotton to cover the middle half of this leaf, make sure that no light can penetrate to this part. Leave it for at least two weeks. (*should not use electric tape – too stick to remove without breaking the leaf)
3. On the day of doing the experiment, pick this leaf from the tree.
4. Remove the cover (paper or cotton). *Notice the color difference between two areas of the leaf.*
5. Put this leaf into the boiling water for 5 minutes.
6. Use forceps to take out the leaf from hot water and put it into the test tube with ethanol 70%, put this test tube with leaf into boiling water, continue to boil until the green color disappears.
7. Take out the leaf from test tube, wash with water and stretch it out on a Petri dish, dry the leaf with absorbent paper and then add Lugol solution into the dish.
8. *Observe the color in 2 areas of the leaf.*

2. TRANSPIRATION

Water enters the root and is transported up to the leaves through specialized plant cells known as xylem. Land plants must guard against drying out (desiccation), mainly via the leave system. Adaptation through evolution has made the leaf surface usually covered by a cuticle layer in order to

avoid water evaporation. However, the leaves also contain specialized structures known as **stomata** to allow gas to enter and leave the leaf. This is especially important for photosynthesis process. Carbon dioxide cannot pass through the protective waxy layer covering the leaf (cuticle), but it can enter the leaf through an opening the stoma flanked by two guard cells. Likewise, oxygen produced during photosynthesis can only pass out of the leaf through the opened stomata. Unfortunately for the plant, while these gases are moving between the inside and outside of the leaf, a great deal water is also lost though the same way. Cottonwood trees, for example, will lose 100 gallons of water per hour during hot, sunny days.

Objective: We will examine the evaporation of water in leaf by using the indicator from Co^{2+}. The Co^{2+} ion can form the complex with absorbed water and change its original color (blue) to the color of its conjugate with water (pink).

$$CoCl_4^{2-} + 6H_2O \longleftrightarrow Co(H_2O)_6^{2+} + 4Cl^-$$

blue **pink**

Materials and Equipments

- 3 different kinds of leaves
- 3% $CoCl_2$ solution
- Absorbent paper
- Scissors
- Sticking tape
- Forceps
- Petri dish
- Desiccator

Examination of Water Loss on Leaf Surface

1. Cut the absorbent paper into pieces with size smaller than size of sticking tape and of leaves examined.

2. Soak these paper pieces into 3% $CoCl_2$ solution for two minutes and then dry the paper at 80^oC in desiccator till dried. *Notice the color of paper*.

3. Clean and dry leaves with tissue paper (make sure that the leaf is completely dry)

4. Use forceps to take out the dried piece and place it on a sticking tape.

5. Quickly and tightly apply this tape onto a leaf to ensure that no moisture from the air can enter to the paper.

6. *Check the color of paper frequently and notice the time needed for this color change, compare the results from different kinds of leaves, if possible.*

Practical 4: ENZYMES

PRE-LABS

1. What is a catalyst and a catalysis reaction?
2. Compare the basic difference between chemical catalysts and biological catalysts.
3. What are enzymes? Describe their basic four properties?
4. How many types of enzyme are there? List them and give at least 1 example.
5. Define the term “optimal temperature” of an enzyme.
6. What makes biological catalysts specific? Define the “active site” of the enzyme and the “substrate”

AIMS OF THE PRACTICAL

Familiarize students with the enzyme properties in reactions and understand the effect of temperature on activity of enzyme.

1. AMYLASE

Amylases belong to the hydrolase group enzyme. They all act on α-glycosidic bonds of starch. The actions of amylases on these linkages result in the formation of glucans and glucoses. Amylases are found mostly in animal saliva, pancreas, intestine…, and in sprouting seeds.

There are three types of amylase:

- α-amylase acts on 1,4-α-D-glucan-glucan glycoside
- β-amylase acts on 1,4-α-D-glucan-malto glycoside linkage
- γ-amylase acts on glucan 1,4-α-glucoside and glucan 1-6-α- glycoside linkages

Like other enzymes, amylases are heat sensitive (affected by heat). The change of reaction temperature causes its change of activities.

Materials and Equipments

- Green been sprout
- Starch suspension
- Lugol solution
- Pasteur pipettes
- Test tubes and rack
- Mortar and pestle
- Filter paper
- Two waterbaths

Procedure

1. Select 40-60 green bean sprouts, put all into the mortar, add 20ml of water and grind till homogenous. Filter this suspension and collect the aqueous phase (enzyme - amylase suspension).

2. Prepare 8 test tubes and mark them as indicated below:

Temperature	**4°C**		**RT**		**50°C**		**100°C**	
Marked tubes	4-S	4-E	RT-S	RT-E	50-S	50-E	100-S	100-E

3. Add into each tube with "E" 2ml amylase suspensions prepared from green bean sprouts and place tubes with "4" label in ice, "50" in warm water, "100" in boiled water and "RT" at room temperature for 5 minutes. *cover the "100" test tubes to prevent the evaporation.

4. Then, add into all test tubes 4 ml of starch suspension. Continue to keep all reactions for 10 minutes in the same condition.

5. After the time indicated, take tubes out, add 1-2 drops of Lugol solution into each tube and 2ml of water into each "S" tube. Mix well.

6. *Observe the color of these tubes.*

2. PROTEASE

Proteases (proteinases, peptidases, or proteolytic enzymes) are enzymes that break **peptide bonds** between amino acids of proteins. The process is called **peptide cleavage.** The enzymes use a molecule of water for this and are thus classified as hydrolases.

The mechanism used to cleave a peptide bond involves making an amino acid residue that has the character of a polarized peptide bond (serine, cysteine and threonine peptidases) or a water molecule (aspartic acid, metallo- and glutamic acid peptidases) nucleophilic so that it can attack the peptide carbonyl group. One way to make a nucleophile is by a catalytic triad, where a histidine residue is used to activate serine, cysteine or threonine as a nucleophile.

Proteases occur naturally in all organisms and constitute 1-5% of the gene content. These enzymes are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades (e.g., the blood clotting cascade, the complement system, apoptosis pathways, and the invertebrate prophenol oxidase activating cascade). Peptidases can break either specific peptide bonds (*limited proteolysis*), depending on the amino acid sequence of a protein, or break down a complete peptide to amino acids (*unlimited proteolysis*).

Materials and Equipments

- One fruit of pineapple
- Boiled white egg
- Toluene solution
- Mortar and pestle
- Pasteur pipettes
- Test tubes & rack
- Waterbath
- Filter paper

Procedure

1. Take one eight of the pineapple fruit, peel off the cover, and cut into small pieces.
2. Put pineapple pieces into mortar, add 15 ml water and grind till homogenous.

3. Filter this mixture and collect the aqueous phase (enzyme - protease extraction).

4. Prepare two test tubes and put into each tube 5 ml enzyme extract

5. Label these 2 tubes: one tube (marked 100) is then put in **boiling water** for 15 minutes, the other (marked RT) left at **room temperature**.

6. Bring two tubes to room temperature; add into each tube the small piece of boiled white egg.

7. Cover tubes, shake lightly and then incubate these tubes at room temperature.

8. After 2 days, pour out the liquid and compare 2 pieces of boiled egg on a petri dish. *Observe results.* ***When finishing, make sure you clean up petri dish and your test tubes****.*

3. CATALASE

Enzyme **catalases**, an anti-oxidant enzyme, are produced naturally in plants, animal and microbial cells. This enzyme functions to convert peroxide into less toxic substances.

The catalase belongs to the group of oxidoreductase. The enzyme can be monitored by its ability to convert hydrogen peroxide (H_2O_2) into oxygen (O_2) and water (H_2O). When the body is infected with high level of hydrogen peroxide, the catalase stored in peroxisome (in plant cell) or mitochondria (in animal cell) will be activated and contribute in the process to convert this toxic compound to oxygen and water. Thus the enzymes play an important role in the cell by mean of its detoxication ability.

Like other enzymes, catalase is also heat inactivated. When enzyme loses activity, the production of oxygen and water is decreased. Potato is known to be the abundant source of catalase.

Materials and Equipments

- Potato
- Distilled water
- H_2O_2 solution (20%)
- Blender
- Pateur pipettes
- Beakers
- Test tubes & rack
- Waterbath

Procedure

1. Put 100 g potato and 250 ml water into blender. Blend this mixture until smooth. Filter this suspension and collect the aqueous phase (this is enzyme - catalase suspension).

2. Prepare 4 test tubes. Mark these tubes as followings:

Temperature	**4°C**	**RT**	**50°C**	**100°C**
Marked tubes	4	RT	50	100

3. Add 5ml of enzyme suspension to each test tube, and mark a line at 5cm above the solution surface. Then bring these tubes to the indicated temperatures, and incubate for 5 minutes.

4. After get tubes out, add 5 drops of hydrogen peroxide into each tube; *observe what happens in each tube. Note down the time needed in each tube for the column of oxygen bubbles forming and reaching to the marked line.*

Practical 5: CELL DIVISIONS: MITOSIS & MEIOSIS

PRE-LABS

1. Compare the basic differences between mitosis and meiosis.
2. If an organism has a diploid number of 16 (2n=16), how many chromatids are visible at the end of mitotic prophase? How many chromosomes are moving to each pole during anaphase of mitosis?
3. Why are mitosis and meiosis important for organisms?
4. Discribe how to use the microscope properly.

AIMS OF THE PRACTICAL

Provide students the basic information and events happen during the division of the cell. Students will take chance to observe the chromosomes at different stages of division using microscope.

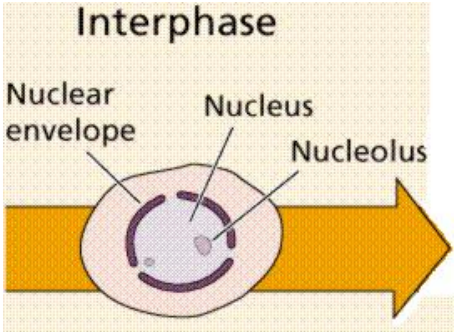
We know that the cell cycle occurs with the cooperation of number of events that involve in the contribution of all the cell composition. The cell division is needed for growth, replacement of cells that are lost by wear and tear or by programmed cell death; or to produce the elements for the sexual reproduction. Mitosis and meiosis are two processes of cell division that are different in nature and purpose.

1. MITOSIS

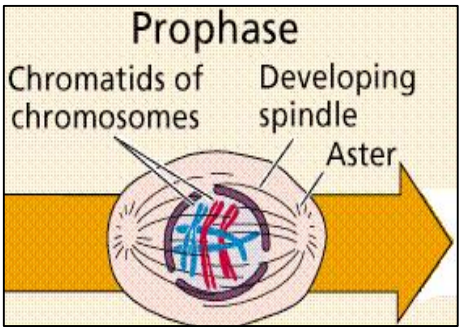
Mitosis produces two daughter cells that are identical to the parent cell. If the parent cell is haploid (N), then the daughter cells will be haploid. If the parent cell is diploid, the daughter cells will also be diploid. This type of cell division allows multicellular organisms to grow and repair damaged tissue.

Events happen in mitosis process can be divided into different phases as summarized below:

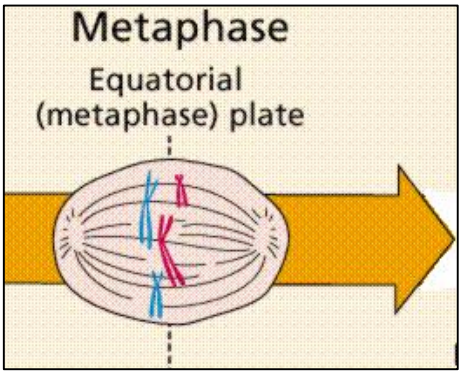
Interphase (G_1 and G_2): Chromosomes (Chromatin) are not visible because they are uncoiled.



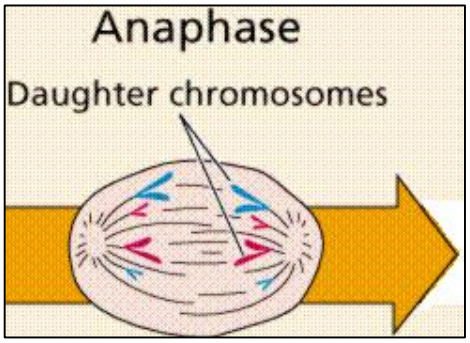
Prophase: The chromosomes coil and condense. The nuclear membrane disintegrates and the spindle apparatus forms. At this phase, each chromosome, which is in duplicated form, attaches to the spindle via kinetochore. If you squash the cell and view under microscope, it is possible to see chromosomes and count them. For example, 46 chromosomes can be seen in a normal human cell sample. This phase may take over one hour.



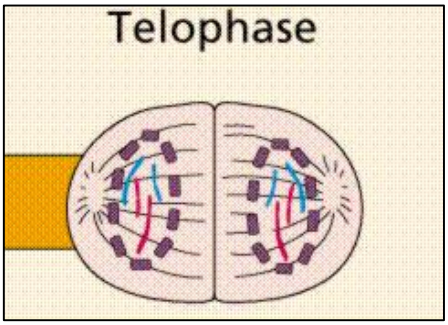
Metaphase: The chromosomes are mostly condensed and become aligned in to one row at the equatorial plate of the spindle. This phase lasts for approximately 15 minutes.



Anaphase: The sister chromatids of each duplicated chromosome separate and are pulled apart towards opposite poles of the spindle, due to the assistance of the spindle fibers. At this phase, the total number of chromosomes present in the cell has become doubled. This stage only takes place within 10 minutes or so.



Telophase:. The chromosomes relax again. The spindle apparatus breaks down; the nuclear membrane reappears surrounding each group of separated chromosomes and the cell divides into two cells via a process called cytokinesis. Now each daughter cell possesses a chromosome set identical to each other and also to the parent cells.



2. MEIOSIS

Meiosis produces daughter cells (haploid) which have a half of the number of ***chromosomes*** present in their parent cell (diploid). This process leads to the reduction in the number of identical chromosomes from two into one. As a result, each daughter cell produced by meiosis still possesses a single full set of chromosome. 2N → N

Meiosis enables organisms to reproduce ***sexually*** because the fusion of two haploid gametes (sperm and eggs) via fertilization process restores the diploidy in the zygote.

Meiosis includes two rounds of division, called Meiosis I and Meiosis II. Each round also goes through phases which are named similarly in mitosis process. Number I or II appearing along the name of a division phase can help you to recognize whether that phase belongs to Meiosis I or II:

Meiosis I: prophase I, metaphase I, anaphase I, and telophase I;

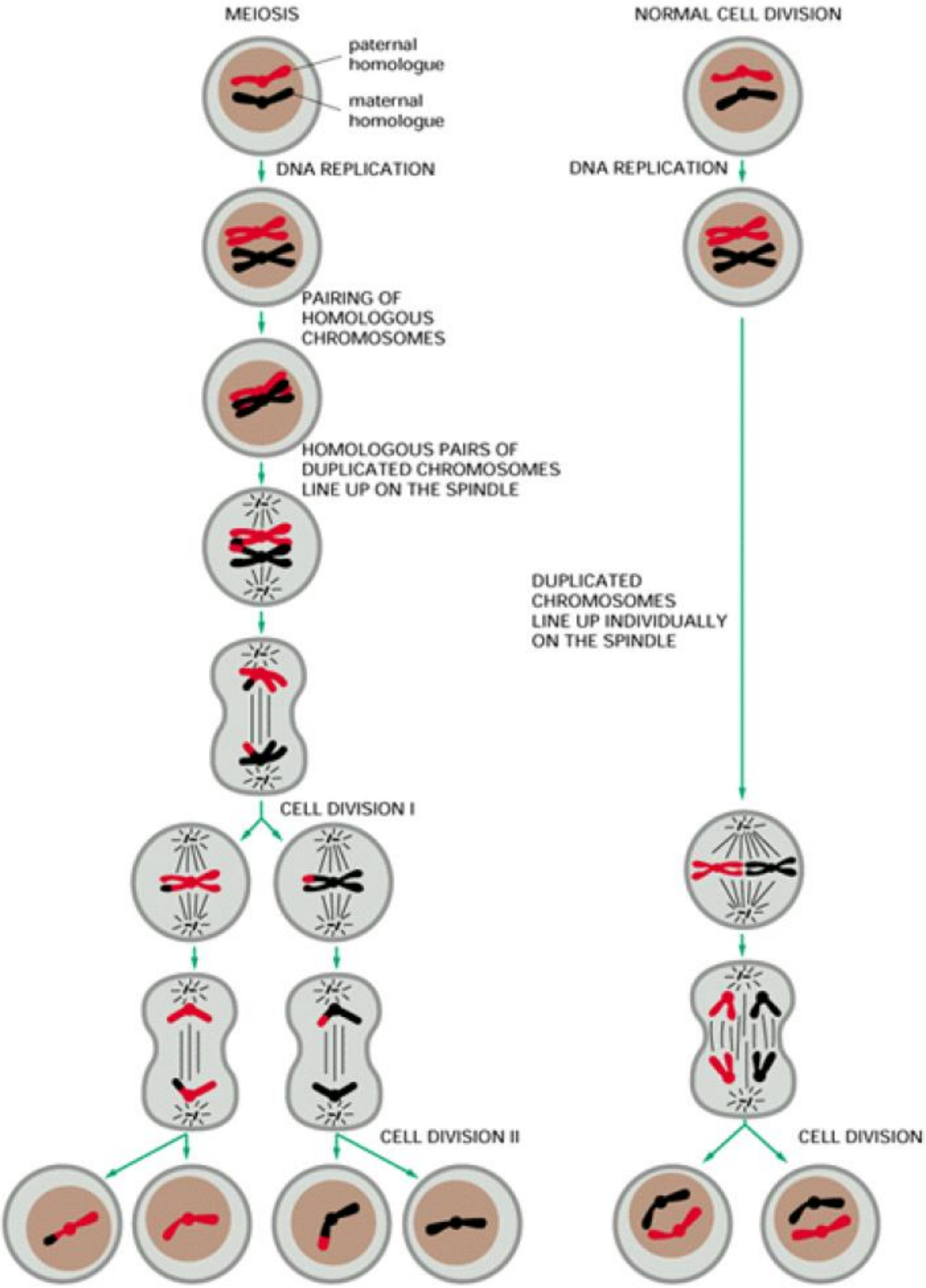
Meiosis II: prophase II, metaphase II, anaphase II, and telophase II.

The first meiotic division involves the separation of identical chromosomes, which have been in duplicated form, into two daughter cells.

The second meiotic division involves the separation of sister chromatids and each daughter cell is further divided into two cells. At the end of the meiosis, therefore, four haploid daughter cells will be produced.

Events happen in each phase are summarized below:

- **Prophase I:** The chromosomes coil up and appear as duplicated chromosomes. The nuclear membrane begins to disintegrate and the spindle forms. Crossing over between homologous chromosomes can take place during this phase.
- **Metaphase I:** Bivalents of homologous chromosomes (tetrads) become aligned in the center of the cell and are attached to spindle fibers.
- **Anaphase I:** begins when homologous chromosomes separate, whereby chromosomes of each identical pair will move towards different poles of the spindle.
- **Telophase I**: The nuclear envelope reforms and nucleoli reappears. This stage is absent in some species.
- **Interkinesis:** Interkinesis is similar to interphase except DNA synthesis does not occur.
- **Prophase II**: The duplicated chromosomes recondense. Nuclear membrane disintegrates again while formation of spindle is seen in each daughter cell.
- **Metaphase II**: The duplicated chromosomes line up into one row at the equatorial plate of each spindle.
- **Anaphase II**: Sister chromatids start to separate towards opposite poles of the spindle.
- **Telophase II**: Nuclear envelope reforms around each single set of chromosome at each cell pole. Cell is further divided and finally four daughter cells are produced. The chromosomes return to relax.


MEIOSIS
paternal homologue
maternal homologue
DNA REPLICATION
PAIRING OF HOMOLOGOUS CHROMOSOMES
HOMOLOGOUS PAIRS OF DUPLICATED CHROMOSOMES LINE UP ON THE SPINDLE
CELL DIVISION I
CELL DIVISION II
NORMAL CELL DIVISION
DNA REPLICATION
DUPLICATED CHROMOSOMES LINE UP INDIVIDUALLY ON THE SPINDLE
CELL DIVISION

Practical 6: EXAMINATION: SKILLS & WRITING

This is the last session of the Practical Class in Biology. You will have a 15-minute examination, which focuses on both laboratory skills gained through microscopic usage and practical knowledge obtained through question answering. Each part will take 50% of your examination score.

You must carefully review all microscopic practical sessions, introduction, all prelab questions and laboratory safety before attending the examination. Hence, you can be confident during the examination.

Good luck!

GUIDELINE FOR THE LABWORK REPORT

1. LAB REPORT PRESENTATION

- On the front page of the report, state:
    - Course name (and course's ID)
    - Instructor
    - Group number
    - Group member (name and ID)
    - Experiment number and title (optional)
    - Date of submission
- Lab reports should be typed. Print on both 2 sides of a paper.
- Page number.
- Lab reports should consist all of data presentation, data analysis and possibly questions. The information should be presented exactly as requested.

2. GUIDELINE FOR REPORT

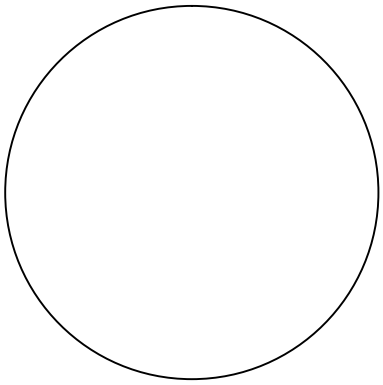
REPORT 1

I/ PLANT CELLS AND ANIMAL CELLS OBSERVATION

1/- Introduction: *Should be short and general, max 150 words.*

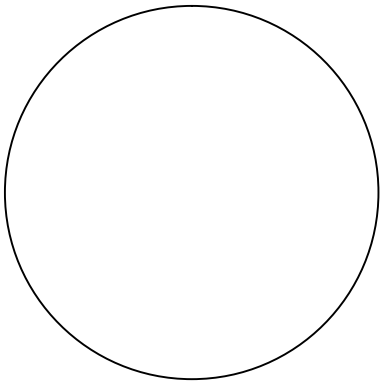
2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Identify cellular membrane, nucleus and cytoplasm.*



Name of sample

Observed at objective lense?



Name of sample

Observed at objective lense?

4/- Discussion:

a) What is the function of Lugol solution in these experiments?

b) What is the difference between plant cells and animal cells?

II/ OSMOSIS IN PLANT CELLS

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Describe the purple area (size and level of color)*

0.85% NaCl ----------> **5% NaCl**

0.85% NaCl ----------> **Water**

4/- Discussion:

a) Explain the phenomenon.

b) When putting plant cells in concentrated NaCl, plasmolysis happened. When putting animal cells in water, hemolysis occurred. What makes the phenomenon in plant cells different from in animal cells?

REPORT 2

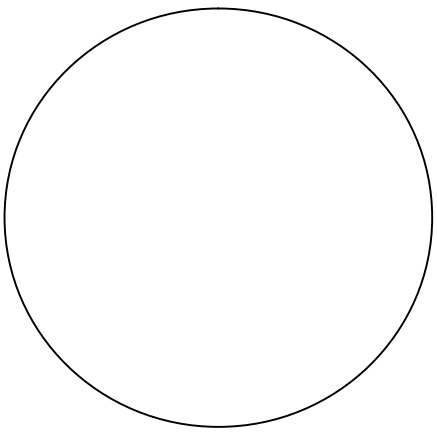
I/ CARBOHYDRATES:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results:

a) Microscopic observation: *identify observed objective lense and starch granules*



b) Effect of temperature to the structure of starch: o*bserve the change of color intensity*

		Color of spot
Lugol		
Starch only		
Starch – HCl mixture	0 min	
	2 min	
	4 min	
	6 min	
	8 min	
	10 min	
	? min	*No color change*

4/- Discussion:

a) Explain the phenomenon when adding Lugol solution to potato starch granules?

b) Explain the different color in Starch – HCl mixture after time of boiling. Based on the color of spot, why can we say that the structure of starch is affected by temperature?

II/ PROTEINS:.

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: o*bserve the change of color*

		Color Observation	
Protein solutions	**Original Color**	**After 10% NaOH**	**After 0.5% $CuSO_4$**
1. Egg albumin			
2. Fresh cow milk			

4/- Discussion:

a) Explain the function of 10% NaOH and 0.5% $CuSO_4$ in Biuret reaction?

b) After adding 10% NaOH, the phenomenon in egg white is different from in cow milk, why?

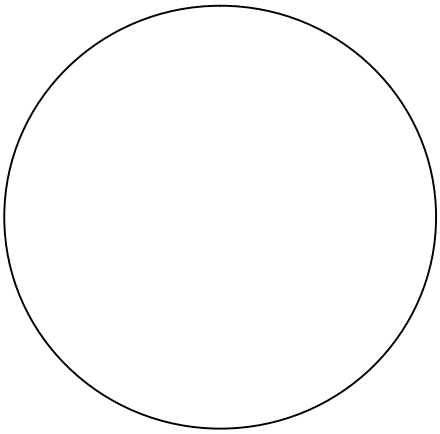
c) Why is the color intensity in egg white different from in cow milk?

II/ LIPIDS:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *identify observed objective lense and stained lipid in cells*



4/- Discussion:

a) Why is Soudan 3 used to detect lipid?

b) Why do we have to wash the stained sample with 20% Ethanol before observation under microscope?

REPORT 3

I/ PHOTOSYNTHESIS:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results:

a) Examination of Oxygen formation: *Observe the level of water in the test tube*

Duration (days)	Level of water (mm)
0	
3	
6	
9	
12	
14	

b) Examination of Starch formation: *Observe the color of 2 areas after staining with Lugol*

	Color Observation			
Leave sample	**Original Color**	**After 1-week Covering**	**After Boiling**	**After Lugol**
Uncovered part				
Covered part				

4/- Discussion:

a) Explain the phenomenon in lowering level of water in experiment 1.

b) What will happen if we get the burned match to meet the O_2, CO_2, H_2?

c) Explain why the color of 2 areas (covered and uncovered) is different.

II/ TRANSPIRATION:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Notice the time and observe the change of color of the paper*

		Color Observation
Absorbent paper	Original color	
	After 3% $CoCl_2$	
	After drying	
Duration after applying on leaf	0 min	
	5 min	
	10 min	
	15 min	
	20 min	
	30 min	

4/- Discussion:

Different trees will have different level of water-out through transpiration. Based on the character(s) of the leaf, we can tell the level high or low. What is that character(s)? Explain your answer.

REPORT 4

I/ AMYLASE:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Report the observed color in different test tubes after adding Lugol solution*

Temp. (^{0}C)	Marked Tubes	Color Observation	
		Before Lugol	After Lugol
4	4-S		
	4-E		
RT	RT-S		
	RT-E		
50	50-S		
	50-E		
100	100-S		
	100-E		

4/- Discussion:

a) Compare “S” with “E” tubes in each condition and explain the phenomenon.

b) Compare all “S” tubes in all conditions and all “E” tubes in all condition. Explain the phenomenon.

c) What is the optimal range of temperature for amylase activity.

II/ PROTEASE:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Report the observation of egg pieces’ shape after 2 days*

Temp. (^{0}C)	At the beginning	After 2 days
RT		
100		

4/- Discussion:

a) Do 2 pieces of egg have different shape after 2 days of incubation? Explain.
b) What is the optimal range of temperature for protease activity.

III/ CATALASE:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Report the time for gas column reach the 5-cm line*

Temp. (^{0}C)	Time Recorded (seconds)
4	
RT	
50	
100	

4/- Discussion:

a) Why is there different in time when bubbles columns reach the 5-cm line between different conditions?
b) What is the optimal range of temperature for catalase activity.

REPORT 5

I/ MITOSIS:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Report or draw different phases of Mitosis*

Phases of Mitosis	Drawings/ Picturing
Interphase	
Prophase	

Metaphase	
Anaphase	
Telophase	

II/ MEIOSIS:

1/- Introduction: *Should be short and general, max 150 words.*

2/- Procedure: *What did you do? Max 50 words.*

3/- Results: *Report or draw different phases of Mitosis*

Phases of Meiosis	**Drawings/ Picturing**
Interphase	
Prophase I	
Metaphase I	
Anaphase I	
Telophase I	
Prophase II	
Metaphase II	
Anaphase II	
Telophase II	