

**DEPARTMENT OF BOILOGY   
AND BOICHEMISTRY**

**BIOL11 LAB MANUAL**

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**Lab 1   
The Microscope**

**--**The **Meaning** of the **Microscope:** (micro: small, scope: to see).

-- The **Definition** of the **Microscope:** is an instrument used to magnify and resolve the image of objects that are too small to be seen by the naked eye.

-- **Magnification:** is the ratio of the size of image to the real size of object.

-- **Magnification = image size / object size.**

**--Total Magnification:** **magnification of the eyepiece x magnification of the object.**

**--Resolution:** is a function of three parameters:1. Wave length of the light (λ)  
 2. Angular aperture of a lens (α)  
 3. Refractive index (n)

**r = 0.61λ / n sin α.**

**--Numerical Aperture (NA):** is (n sin α) of the objective lens **and it is specific for each lens.**

**r = 0.61λ / NA.**

**Types of Microscopes**

**1. Light Microscopes.  
2.Electron Microscope.**

**1.Light Microscopes:** uses light (photons) as a source of illumination

There are different types of light microscopes:

**1. Compound microscopes 2. Dissecting microscopes** 3. Dark field microscopes 4. Ultraviolet microscopes  
 5. Phase contrast microscopes 6. Differential interference contrast microscopes  
 7. Confocal microscopes

**1. Compound microscopes:** consiststhe following parts:

**1. Base:** also called the supporting stand rests on the bench and generally  
horseshoe-shaped.

**2. Illuminator:** the source of light.

**3.** **Iris diaphragm:** is located below the condenser, and it consists of circular  
structure with an aperture that can be adjusted to control the amount of light that  
reaches the condenser.

**4. Condenser:** consists of one or system of lenses that focuses light from on to the specimen located on the stage.

**5. Arm:** for use when carrying the instrument.

**6. Stage:** carried on the arm and serves as a support for the microscopic slide being examined. And it consists two clips that are used to hold the slide in place.

**7. Course adjustment:** is a large knob located at both sides of the microscope.

**It is used for:** 1.Moving the stage upwards and downwards quickly.  
 2.Bringing the image of the specimen approximately into focus.

**8. Fine adjustment:** is a small knob located at both sides of the microscope

**It is used for:** 1. Accurate focusing.  
 2. Allowing the details of the specimen to be seen.

**9. Objective lenses:** are lenses attached to the nosepiece

**There are four objective lenses :** 1. Scanning lens (X4).  
 2. Low power lens (X10).  
 3. High power lens (X40).  
 4. Oil immersion lens (X100).

**10. Eyepiece OR ocular lens:** it is a lens inserted at the top of the body tube.

**It is used for:** 1. Further the magnify the image obtained from the objective.  
 2. As a ruler to allow the measurement of specimen dimensions.   
 3 To adjust the interpupillary distance.  
**\* interpupillary distance:** is the distance between the center of the eyepieces.

**11. Body tube:** it is a tube to connect the eyepiece with the revolving nosepiece.

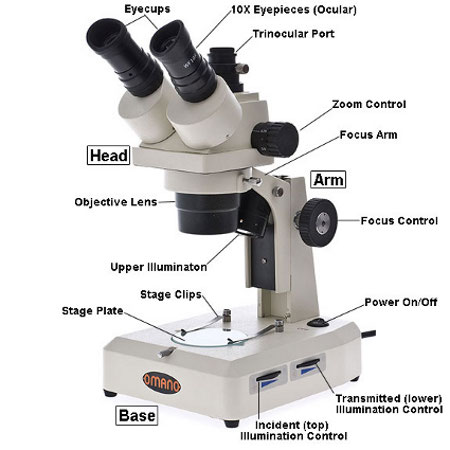
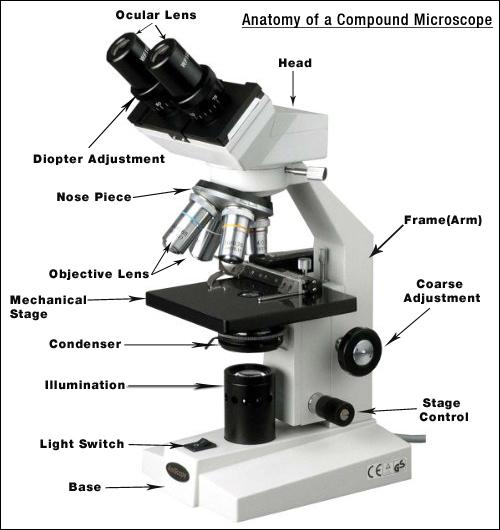
**12. Revolving nosepiece:** is the structure to which the objective lenses are attached.   
 **it is used for:** to bring the desired objective onto position by rotating.

**2. Dissecting microscopes:** is a type of stereo microscope

**It used for:**

**1.** examining an object at low magnification (usually less than 100x).

**2.**its widely used in the dissection and identification of insects, small animals, microsurgery, botanical study, and for examination of different specimens in fresh state without the need of pervious preparation.



**2.Electron Microscope:** a beam of electrons produced by a tungsten filament is used instead of photons to produce images with better resolution (0.2 nm), since the wavelength of electron beams have very short wavelengths.  
A system of electromagnetic lenses is used to focus the electron beam on the specimen, and then the image is formed on a fluorescent screen or photographic film.

**Two main Types of the electron microscope:**

**1. TEM(transmission electron microscope):** the beam of electrons passes through the specimen and two dimensional image is obtained.

**2.SEM(scanning electron microscope) :** the beam of electrons is deflected from the surface of the specimen allowing the formation of three dimensional images.

**Lab2**

**The Cell**

**Cell:** is the "Building Block" of all living organisms.

**cell theory: (**the general principles of cells**):**

**1.** All living organisms are composed of cells.

**2.** The cell is the unit of structure, physiology and organization of living organisms.

**3.** All cells come from pre-existing cells.

**4.** Cells carry their genetic material and pass it to daughter cells through the cell division.

**Two main types of cells based on the membrane:**

**1. Eukaryotic cells:** is enclosed by a membrane-bound nucleus.

**2. Prokaryotic cells:** is not enclosed by membrane-bound nucleus.

**Living organisms can be classified to three domains according to Woese's system:**

1. Eukarya

2.Eubacteria

3.Archaea

**Different cell components:**

**1. Cell Wall:** is a hard layer that surrounds some types of cells, giving the cell support, strength and protection

**2. Plasma Membrane:** a selective permeable membrane that separates the intracellular components from the extracellular environment, it is composed of a phospholipid bilayes with proteins embedded through it (integral proteins or attached to its surface (peripheral proteins).

**3.Cytoplasm:** the entire region between the nucleus and the plasma membrane, it consists of : the cytosol, organelles and cytoskeleton.

**4.plastids:** cytoplasmic organelles found in most plant cell  
 **there are three main types of plastids:**

**a) Chromoplasts:** (colored plastids): encloses orange carotene and yellow xanthophylls, giving flowers, ripened fruits and autumn leaves their colors.

**b) Chloroplasts:** (Green plastids): the site of photosynthesis (anabolism), it contains the green pigment, chlorophyll along with carotenoids.   
 it is bounded by a double, lipid bilayer membrane. Dark reactions take place in an aqueous matrix, the stroma, which contains plate-like thylakoids that are arranged in structures called grana where the light reactions take place.

**c) Leucoplasts:** (leuco: white): Organelles in which  
 -- starch (stored in amyloplasts)  
 -- oils (stored in elaioplasts)   
 --proteins (stored in proteinplasts)

**5.Mitochondiria:** Cytoplasmic organelle, it is the main "powerhouse" of eukaryotic cells, being the site of cellular respiration, in which energy is extracted from food in the form of ATP (catabolism).  
 it is surrounded by two membranes, the inner and outer mitochondria membranes. the inner membrane is infolded forming finger-like projections called cristae, where the electron transport chain takes place. in eddition, the inner membrane encloses with matrix, the site of the TCA cycle(Krebs cycle).

**6. Endoplasmic reticulum:** a system of membranes distributed inside the cytoplasm. it occurs in two types: **Rough** and **smooth endoplasmic reticulum**. Rough endoplasmic reticulum differs from the smooth endoplasmic reticulum in that ribosomes to its surface

**7. Ribosomes**: are und as units and are not membrane bound. They can be attached to the rough endoplasmic reticulum or free in the cytosol Ribosome to composed mainly of RNA and enzymes that are essential for protein synthesis.

**8. Vacuoles and vesicle:**  membrane bound organelles in the cytoplasm. Vacuoles. larger than vesicles, and occur in different types according to their functions: Food vacuoles that form by phagocytosis, contractile vacuoles that expel water outside the cell of freshwater organisms, and a single large central vacuole found in plant cells.

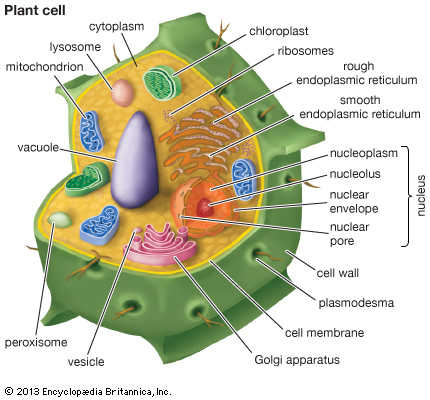
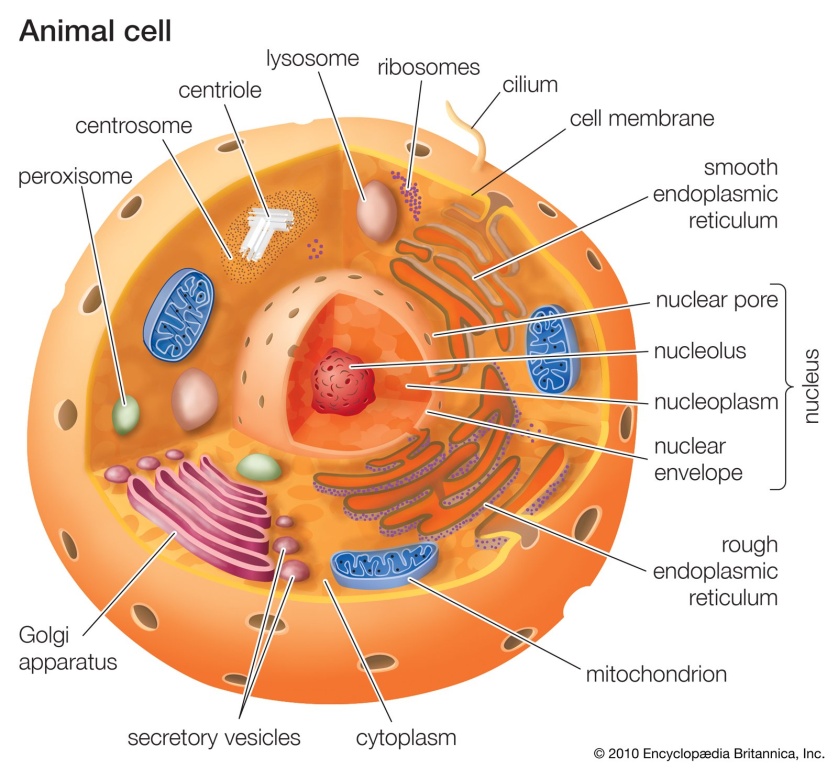
**9. Centrosome:** an organelle associated with the nuclear membrane of animal cells, and is essential in the formation of mitotic spindle in cell division. In each centrosome, there are two central bodies called centrioles,

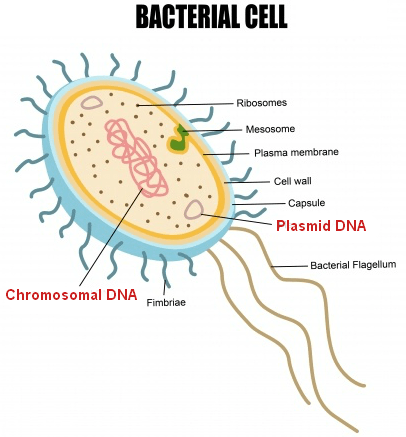
**10. Lysosomes**: small vesicle surrounded by a single membrane It contains hydrolytic enzymes that functions in the hydrolysis, being the site of digestion of cellular macromolecules.

**11.Golgi apparatus:** consists of flattened and unconnected sacs called cisternae. Vesicle materials from the endoplasmic reticulum to the golgi apparatus, are sorted, modified stored and shipped to the surface of the cell.

**12. Peroxisomes:** a single membrane organelle that contains enzymes, that transfer hydrogen from different substrates to oxygen producing hydrogen peroxide(H20).

**13. Nucleus**: the center of cellular activities; it encloses the chromatin(DNA and proteins) and nucleoli which is the site of ribosome synthesis It is enclosed by a double membrane, the nuclear envelope, which contains pores allowing the interchange between nucleus and the cytoplasm





**1.Corck dead cells** (cell wall):

 Notes:

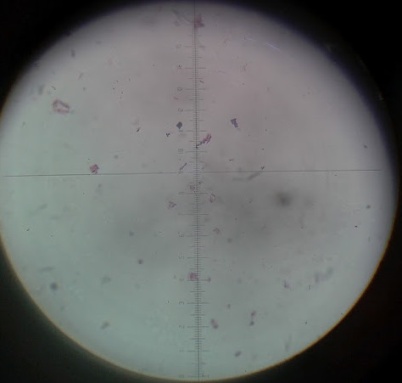
**2.plant cells** (from tomato):

 Notes:

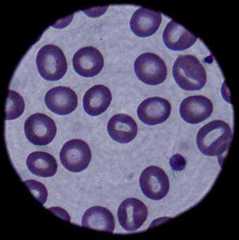
**3. plant cells** (from potato

 Notes:

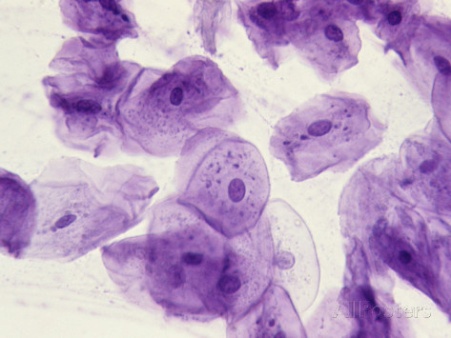
**4.bacteria cells** (lactobacillus):

Notes:

**5. animal cells**(blood cells):

Notes:

**6.animal cells** (cheek squamous epithelium):

Notes:

**Lab 3**

**Biological Membranes: Diffusion and Osmosis**

**Plasma membrane:** is a dynamic barrier that separates the inner contents of the cell from the surrounding environment. Being selectively permeable, it regulates the transport of molecules into and out of the cell

**-**According to the fluid mosaic model proposed by Singer and Nicolson, the plasma membrane consists of proteins embedded bilayer in or attached to the surface of a fluid lipid bilayer.

**-**Solutes can cross the plasma membrane by simple diffusion, facilitated diffusion and active transport.

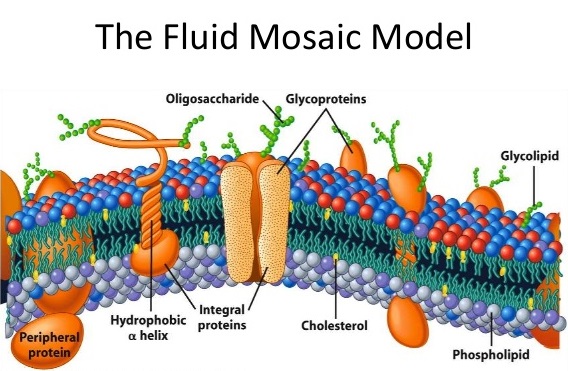
**-**When considering the diffusion of water molecules across a selectively permeable membrane, the process is referred to as **osmosis**. In osmosis, water molecules move from a region of low solute concentration(higher free energy of water/higher water potential) to a region of higher solute concentration (lower free energy of water/lower water potential) through a selectively permeable membrane

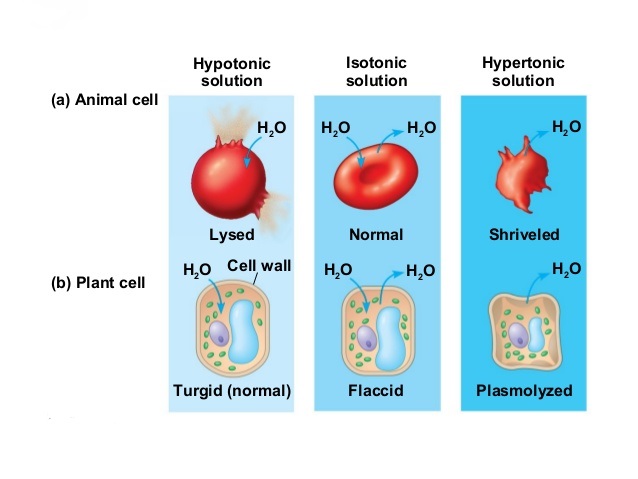
When a cell is immersed in a solution, the solution can be classified to the following according to its concentration when compared to the concentration inside the cell

**1. Hypotonic solution:** a solution of lower solute concentration than that inside the cell Therefore, water molecules tend to move inside the cell causing it to swell and then **burst (animal cell)** cell or become **turgid (plant cell)**.

**2.isotonic solution:** the solute concentration of the solution is **the same** as that inside the cell

**3. Hypertonic solution:** a solution of higher solute concentration than that inside the cell. Therefore, water molecules move outside the cell, causing it to **shrink(animal cell)**. As **(the plant cell)** is surrounded by a cell wall, the cell membrane is partially detached from the cell wall in a Process called **plasmolysis**.





Lab 4

Carbohydrates

**Carbohydrates** are organic compounds often with the general formula Cx(H₂O)n, where x and n could be equal or different integers.

**-** According to **number of molecules** that contain, carbohydrates can be classified into:  
1. monosaccharides  
2.disaccharides  
3.polysaccharides

**1.monosacharides:** are the simplest form of carbohydrates and considered the monomers (building blocks ) of the more complex carbohydrates.

**-**According to the **location of the carbonyl functional group** monosacharieds can be classified into :   
1.Aldoses: terminal carbonyl group   
2.Ketoses: internal carbonyl group

**-**According to the **number of carbons** they contain, monosacharieds can be classified into :   
1.trioses (three carbons sugar)   
2.tetroses (four carbons sugar)   
3.pentoses (five carbons sugar)   
4.hexoses (six carbons sugar)   
5.heptoses ( seven carbon sugar)

**\***five carbon aldose: ribose : aldopentose

**\***six carbon ketose: hexose

**\* Additional examples** of monosacharieds:   
1.Glucose   
2.Fractose   
3.Galactose

**2.Disaccharide:** is two monosacharieds that are joined together by dehydration reaction, and they join by covalent bond called glycosidic linkage.

**-Common examples** of disaccharides are:   
1.maltose (glucose-glucose)   
2.succrose (glucose-fructose)  
 3.lactose (glucose-galactose)

**3.Polysaccharides:** are polymers with hundreds to thousands of monosacharieds joined by glycosidic bond

**-Common examples** of polysaccharides that are polymers of glucose monomer are:   
1.Starch   
2.Glycogen   
3.Cellulose

**1.Starch:** is a helical storage polysaccharide found in plants

**--**there are two forms of starch:   
1.Amylose (simple and unbranched)   
2.Amylopectin (complex and branched)

**2.Glycogen:** is a helical and highly branched polysaccharide found in polysaccharide that is fund in the cell wall of the plant cells.

**3.Cellulose:** molecules are arranged in parallel chains stabilized by hydrogen bonds.

**The qualitave tests** :   
1.Molish Test   
2. I₂/kI Test   
3.Barfoed's Test   
4.Benedict's Test

**1.Molish Test:** it's a sensitive test for the identification of carbohydrates. Nucleic acids and glycoproteins are positive for this test. Since both contain a carbohydrate protein.

**Purple compound:** is a compound that branched by a concentrated sulfuric acid (H So) solution that dehydrates the carbohydrate form a compound that interacts with -naphthol in the molish regent.

**-Starch** +H₂SO₄ →**positive result**(is indicated by the formation of a **purple ring** between the two layers)   
**-Sucrose** + H₂SO₄ →**positive result**(is indicated by the formation of a **purple ring** between the two layers)  
**-Glucose** + H₂SO₄ →**positive result**(is indicated by the formation of a **purple ring** between the two layers)  
**-Water** + H₂SO₄ → **Negative result**

**2.I₂/KI Test:** many polysaccharides can be identified by their reaction with IKI reagent, IKI reagent interacts with the helices of amylose and glycogen, and the chain of cellulose forming a colored complex.

**-Starch** +IKI → **positive result** (**deep blue-black color**)   
**-Glycogen** + IKI → **positive result** (**red-brown color**)  
 **-Cellulose** +IKI → **positive result** (**violet-brown** to **red-brown** **color**)   
**-Water** +IKI → **negative result** (**yellow color**)

**-Note:** the colors fade away by heating and returns by cooling.

**3.Barfoed's Test:** is used to distinguish between reducing monosacharieds and reducing disaccharides. -The Barfoed's reagent consists of copper (II) ions on acidic medium copper (II) ions are reduced by the sugars to insoluble brick read cuprous oxide CU₂O.

**-Note:** hearing for long time could break the glycosidic linkage of the disaccharide molecule .

**-Barfoed's Test equation:** R-CHO +2CU² +2H₂O --> R-COOH + CU₂O +4H⁺

-Ketose are reducing sugars due to the conversion ketoses to aldoses by **tautomerization**

**By heating for 3 minutes :**   
**-Glucose** → **positive result**(red-precipitate)   
**-fructose**→ **positive result**(red-precipitate)   
**-sucrose** → **negative result**   
**-lactose**→ **negative result**   
**-Maltose**→ **negative result**   
**-Starch**→ **negative result**   
**-Water**→ **negative result**

**4.Benedict's Test:** it is a test to identify reducing sugars -Benedict's reagent differs from the Barfoed's reagent in that it consists of mainly copper (II) ions in a strongly alkaline solution.

**-Benedict's equation:** R-CHO +2CU² +5OH⁻ → R-CO₂⁻ + CU₂O +3H₂O

**-By heating for 3 minutes:**

**-Glucose** → **positive result**(**brick red**)   
**-fructose**→ **positive result**(**brick red**)   
**-sucrose** → **negative result**   
**-lactose**→ **positive result**(**brick red**)  
**-Maltose**→ **positive result**(**brick red**)  
**-Starch**→ **negative result**   
**-Water**→ **negative result**

**-why it gets red from blue?**  
 Free aldehyde group reducing, then the agent Reduced

**-Permination of the performance of Benedict's reagent with different concentrations of sugar solutions:**  
 **0.2% Glucose** +2ml Benedict's → **positive result** (**brown color**)   
**0.02% Glucose** +2ml Benedict's → **negative result**  
**0.4% Glucose** +2ml Benedict's → **positive result** (**dark orange color**)

-How to test if a sugar has an aldihyde group:  
7drops of HCL, 10 drops of Sucrose+ boiling for 3 minutes   
to make free aldihydric group  
then cool it (because reagent doesn't work in heat)   
and then add 3ml of Benedict's Test +1ml NaOH (To adjusts the focus of the PH)   
and then heat for 3 minutes   
it gave **positive result**, then we have **free aldihyde groupe**