Essential Biology Lab Manual

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Chapter 1 Laboratory Safety and instruments

Learning Outcomes: At the end, after the completion of the current lab, the student should be able to:

- To understand the principle of lab safety and regulation
- To define the biohazards chemical and biohazards living cell
- To understand the use of Personal Protective Equipment (PPE).
- To describe the basic importance of the lab instruments

Introduction

Laboratory safety regulations will be strictly enforced. Repeated infractions will result in points lost. All instruments, chemicals and organisms should be handled with proper aseptic technique and universal precautions manner when working in the laboratory. In the interest of safety and accident-prevention, there are general laboratory rules to be followed by all students in laboratory rooms at University of Arkansas Rich Mountain. Faculty and staff members are authorized to deny the use of any laboratory to students who do not adhere to the rules mentioned below.

- Wash your hands with soap and water before entering or leaving the lab.
- Lab experiments have been designed to minimize unnecessary exposure to any hazardous substance. A list of all chemicals, specimens, and/or microbes used in the laboratories is available for your benefit.
- If you have any medical condition or concern, including but not limited to the following: compromised immune system, seizures, epilepsy, severe allergies, or pregnancy, it is your responsibility to consult with your medical care provider regarding any medical issue associated with taking this lab. If you have concerns about a health risk, please communicate accordingly with the Director of Risk Management, Occupational Health & Safety, so that reasonable accommodations can be determined.
- It is the responsibility of the individual using chemicals, operating equipment or manipulating organisms, to know the associated hazards of each and to handle them

accordingly. Safety Data Sheets and container labels provide hazard information that must be followed. Materials must be disposed of appropriately per faculty instructions.

- Unauthorized work is prohibited. This includes working after hours without proper authorization and performing procedures that have not been expressly authorized by laboratory staff or faculty. Some areas are designated as off-limits to the students and should not be entered without supervision.
- No food or drink is allowed in the lab.
- Cell phones are not allowed in lab under any circumstances.
- Personal Protective Equipment (PPE); Safety goggles must be worn when working with chemicals. Lab coats/aprons and protective gloves are required when handling chemicals. Footwear impermeable to liquid spills is recommended. You must limit exposed skin as much as possible, and faculty may enforce more stringent clothing guidelines than those outlined here. If you wear inappropriate clothing to the lab, you will not be allowed to participate. PPE should not be worn outside the lab.
- It is recommended that contact lenses should not be worn in the lab as chemicals can get between the eye and the lens. However, additional PPE or alternative measures can be taken for those required to wear them.
- Be familiar with the location and the use of the following in your laboratory: e.g. broken glass receptacle, bio-hazard waste receptacle, first-aid kit, gas shut-off valves, fire blanket, closest fire alarm, fire extinguisher, eye wash, safety shower, spill kit, telephone, and emergency exits and routes.
- Lab activities have students moving around the lab or involve moving objects. Be alert and aware of what's going on around you at all times.
- Faculty must be notified immediately of any health reaction/issue or accident occurring in the lab. Any spillage or breakage should be cleaned up by the Lab Coordinator, Faculty, or Staff.
- Be organized. Maintain a clean, open work area free of anything except materials directly required for the exercise. Keep laboratory material/equipment away from edges of work surfaces and electrical cords from hanging below the surface of tables or draped across sinks. Use caution when working with electrical equipment to avoid a shock.

- Accidents or injuries must be reported to your instructor as soon as possible. Know the location of the fire blanket, fire extinguisher, shower and eyewash in case of emergency.
- Dispose of wastes as instructed. Hazardous chemicals and microbial cultures must be properly discarded in designated containers and kill areas. We will inform you of the proper disposal procedures. Do not discard any materials that have contacted microbial cultures or hazardous chemicals in sinks or wastebaskets.
- In addition to these rules, faculty may also provide additional safety instructions that are specific to the laboratory course you are taking. Additional safety instructions may be more stringent than the rules outlined here. You must adhere to all safety instructions provided by the laboratory.

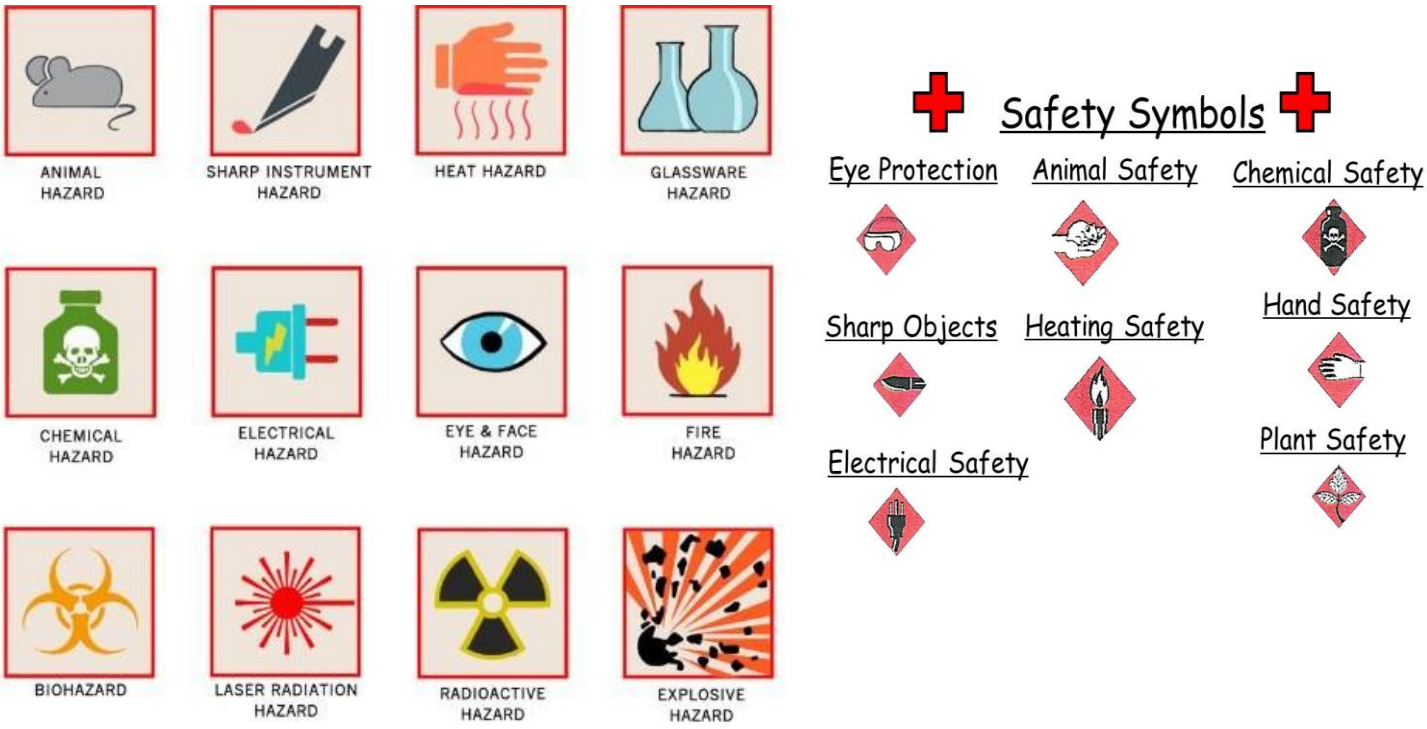


Figure 1.1 Hazards and safety symbols

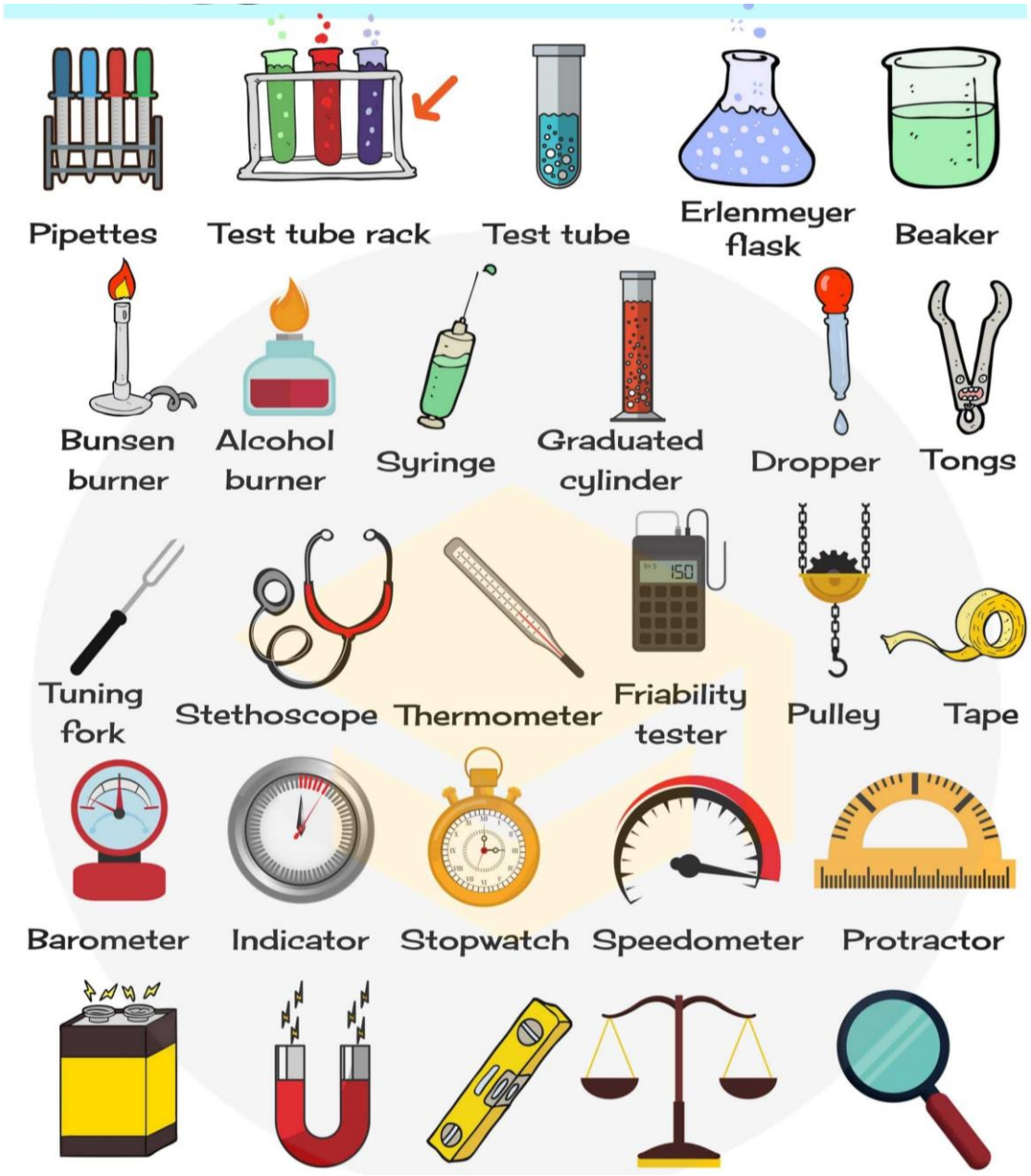


Figure 1.2 Selected laboratory equipment

Chapter 2: The Scientific Method

Learning Outcomes:

- To define science and discuss the characteristics of the science.
- To describe the basic and integrated process skills of science.
- To design and conduct lab experiments.
- To collect data and analysis to compare control and variable group; discuss the influence of independent variable on the dependent variable.
- To discuss scientific method and write scientific theory.

Introduction

The scientific method is the way scientists approach a question. It is an empirical method to the study of biology for acquiring knowledge in systemic order. The scientific method includes careful observation, and applying rigorous skepticism about what is observed, given that cognitive assumptions can distort how one interprets the observation. It involves formulating hypotheses, via induction, based on such observations; the testability of hypotheses, experimental and the measurement-based statistical testing of deductions drawn from the hypotheses; and refinement (or elimination) of the hypotheses based on the experimental findings. These are *principles* of the scientific method, as distinguished from a definitive series of steps applicable to all scientific enterprises The general steps of the scientific method are depicted in the **Figure 2.1** below. The hypothesis, or suggested explanation for the observation, is the basis for setting up experiments. Good experimental design is essential to the scientific method.

The scientific method is central to the study of biology: it is a process of acquiring and verifying information through experimentation. The general steps of the scientific method are depicted in the figure below. The hypothesis, or suggested explanation for the observation, is the basis for setting up experiments. Good experimental design is essential to the scientific method. A few keys to good experimental design include effective use of controls, reproducibility, a large sample size, and multiple trials. In an experiment, in order to determine that any changes that occur are due to investigator manipulation only, there must be some basis for comparison. A control group is necessary to establish this basis of

comparison. In the control group, everything is kept the same as the experimental group except for the independent variable. The experimental group is actually being experimented upon. For example, in a drug trial there will be a group that receives the drug (the experimental group) and a group that receives a placebo (the control group). The drug itself is considered the independent variable and any change(s) that occur because of the drug are considered the dependent variable. In order to ensure that it is only the drug causing changes, all other variables must be tightly controlled (such as diet, exercise, smoking, etc.). These are referred to as controlled variables.

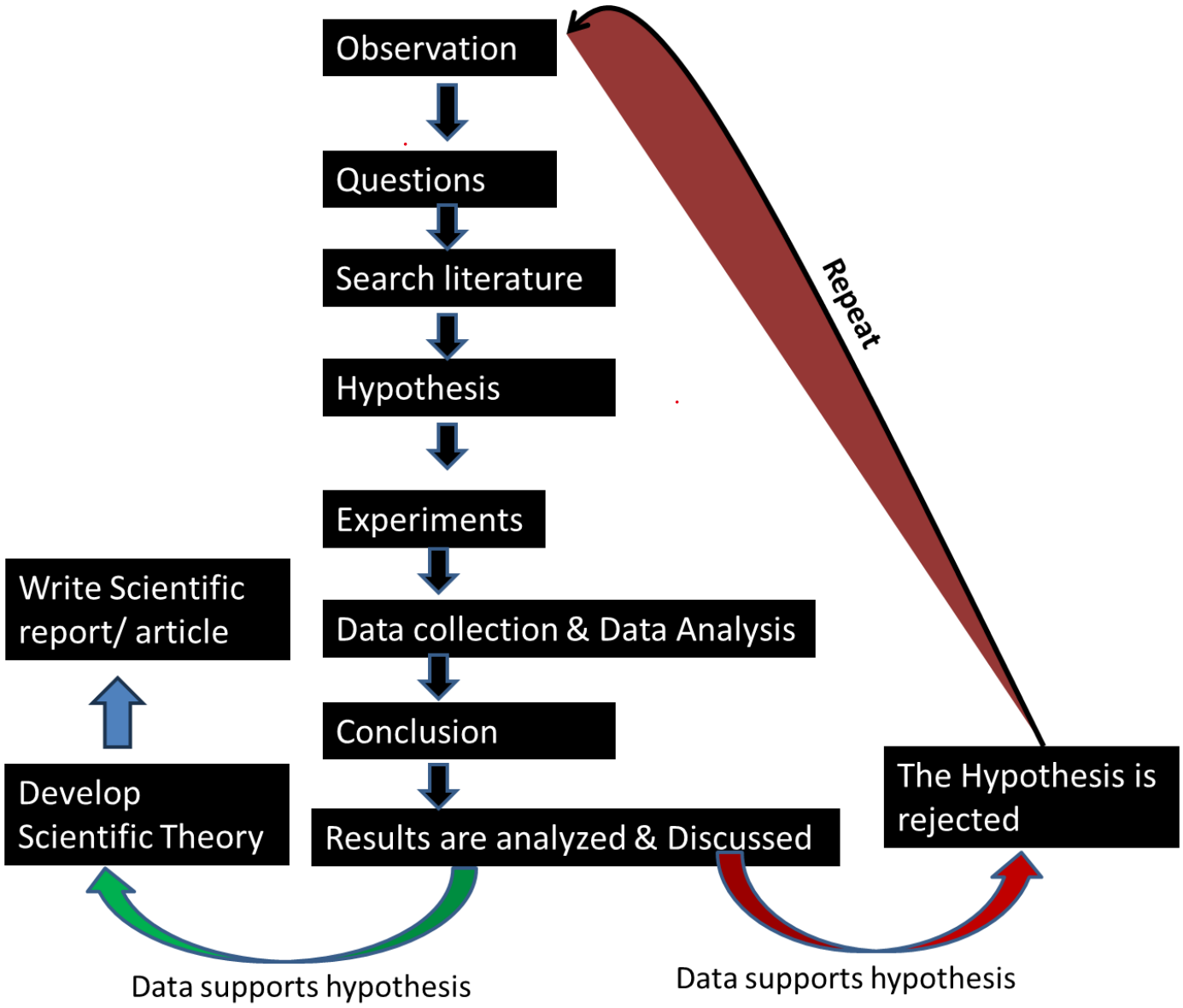


Figure 2.1: Flow char of Scientific method

Experiment on Penicillin Discovery by Sir Alexander Fleming

How Penicillin Was Discovered In 1928, Sir Alexander Fleming was studying

Staphylococcus bacteria in growing in culture dishes. He noticed that a mold called Penicillium was also growing in some of the dishes. A clear area existed around the mold because all the bacteria that had grown in this area had died. In the culture dishes without the mold, no clear areas were present. Fleming hypothesized that the mold must be producing a chemical that killed the bacteria. He decided to isolate this substance and test it to see if it would kill bacteria. Fleming transferred the mold to a nutrient broth solution. This solution contained all the materials the mold needed to grow. After the mold grew, he removed it from the nutrient broth. Fleming then added the nutrient broth in which the mold had grown to a culture of bacteria. He observed that the bacteria died which was later used to develop antibiotics used to treat a variety of diseases.

Test yourself.

1. What experiment was conducted by Sir Alexander Fleming in 2028?

2. What was Fleming's hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. This experiment led to the development of what major medical advancement?

Experiment of The Strange Case of BeriBeri

In 1887 a strange nerve disease attacked the people in the Dutch East Indies. The disease was beriberi. Symptoms of the disease included weakness and loss of appetite, victims often died of heart failure. Scientists thought the disease might be caused by bacteria. They injected chickens with bacteria from the blood of patients with beriberi. The injected chickens became sick. However, so did a group of chickens that were not injected with bacteria. One of the scientists, Dr. Eijkman, noticed something. Before the experiment, all the chickens had eaten whole-grain rice, but during the experiment, the chickens were fed polished rice. Dr. Eijkman researched this interesting case and found that polished rice lacked thiamine, a vitamin necessary for good health.

1. State the Problem.

2. What was the hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. What should be the new hypothesis and how would you test it?

Hypothesis: Antibiotic B is a better treatment for ulcer than Antibiotic A

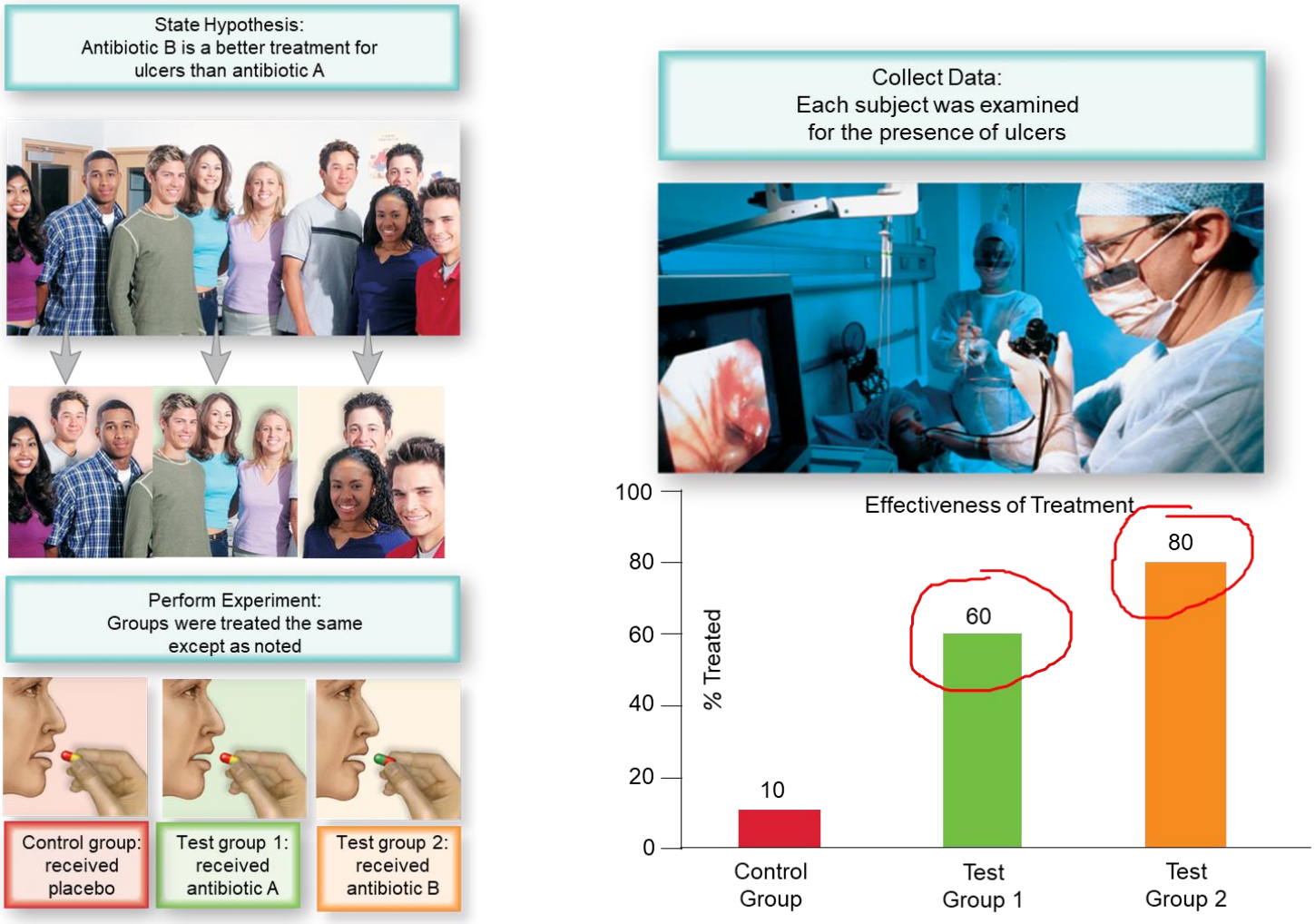


Figure 2.2 Hypothesis states that antibiotic B is better treatment than antibiotic A.

1. What is basis of the formulation of hypothesis in the ulcer treatment?

2. How was the hypothesis tested?

3. Do the results indicate that the hypothesis should be accepted or rejected? Mention the reason why choose option accepted or rejected.

4. What is difference between Control group and test group?

5. Describe the characteristics of science.

6. What can be done to make better scientific attitude and better education in our nation?

Chapter 3: Understanding Scientific Notation for Good Measurement with Metric

Learning outcomes: At the end, after the completion of the current lab, the student should be able to:

- To describe the use of the scientific notation and metric system
- To differentiate and convert between standard notation and scientific notation
- To understand and measure the length, weight, volume and temperature using metric system.
- To understand the basic units and their conversion from one type of unit to another

Introduction

Measurement is a process to find the values of an unknown quantity by using a standard reference whose value is already known. To understand the basic concept of measurement, various types of tools and techniques can be applied to measure particular values of physical quantities such as length, mass, time, amount of substance and temperature.

In the sciences, many of the things measured or calculated involve numbers that are either very large or very small. As a result, it is inconvenient to write out such long numbers or to perform calculations long hand. In addition, most calculators do not have enough window space to be able to show these long numbers. To remedy this, we have available a short hand method of representing numbers called scientific notation. The chart below gives you some examples of powers of 10 and their names and equivalences.

Simple question to ask in any gas station is, what is the price of gas per gallon here in Unites of State, but if visited any gas stations of any other countries like European, Asian, South African, or Australian, you will ask the price of gas in liters not in gallon. If you need small quantity of gas let's say only 0.2 liter, then you can ask price in even in smaller unit of the liter which is milliliters (ml). Therefore, in this lab, you will learn to measure the different physical measurement and their units conversion from one type to another **(Table 3.1-3.6).**

The International system of units (SI) is the standard metric system that has been used in most of the countries. In this regard, we also take advantage to use SI system to understand these basic metric units for length, area, volume, weight and temperature. We can follow the **Table 3.1-3.6** to convert units from one type to another.

Pre-Lab Questions

1. Why Scientific notation is important?

2. What system of measurement is used in science?

Length

As per the SI system, the standard measuring unit of length is meter. However, we can use various units to measure length such as a meter, kilometer, millimeter, feet, inches, and so on.

Pre-Lab Questions

1. What is the basic unit of length?
2. 3.5 meters is an equivalent of _____ cm.
3. What is the conversion of 20 cm into mm_____.
4. _____mm is equivalent to 50 mm.
5. Some college student needs as much as 6 meters length that has 2 meters width to prepare college uniform. These values are equates to length _____ cm and width _____ mm, respectively

Choose the best answer

1. In the Celsius scale the boiling point of water is
   - 182 ºC
   - 0 ºC
   - 100 ºC
   - 75 ºC

2. The SI unit of volume is
   - Square Meter (m^2)
   - Cubic Meter (m^3)
   - Kilogram (kg)
   - Angstrom (Aº)

3. A pipette and a burette are used for measuring
   - Time
   - Acceleration
   - Volume
   - Voltage

4. A thermometer measures
   - Length
   - Density
   - Temperature
   - Magnetic Intensity

5. Which is the odd one of the following:
   - Pipette
   - Burette
   - Measuring Jar
   - Spring Balance

Table 3.1 Metric Conversion Chart for Length

Measurement Units	Unit abbreviations (Symbol)
1 Centimeter = 10 Millimeters	1 cm = 10 mm
1 Decimeter = 10 Centimeters	1 dm = 10 cm
1 Meter = 100 Centimeters	**1 m = 100 cm**
1 Kilometer = 1000 Meters	1 km = 1000 m
1 Meter = 10 Decimeter	1 m = 10 dm
1 Meter= 39.37 inches	1 m =39.37”
1 yard = 0.91 meter	1 yd = 0.91 m
1 Foot = 12 Inches	1 ft = 12 in
1 Foot = 30.35 cm	1 ft = 30.35 cm
1 Inch = 2.54 Centimeters	1 in = 2.54 cm
1 Yard = 3 Feet	1 yd = 3 ft

Area

Area can be defined as the total space taken by a flat in a two-dimensional (2-D) surface or the shape of object which exist certain length and width. The space enclosed by the boundary of a plane figure is called its area. The area of a figure is the number of unit squares that cover the surface of a closed figure. As per the rule of he SI system, unit of area is the square meter (m^2), which is a derived unit. However, we can measure are in smaller or larger square units like centimeter (cm^2), dekameter (dam^2),

two-dimensional shape can cover, measured in square units. The SI unit of area is the square meter (m^2), which is a derived unit.

- Square millimeter (mm^2) can be visualized as a square that is 1 mm on each side (or 1 mm x 1 mm).
- Square centimeter (cm^2) can be visualized as a square that is 1 cm on each side (or 1 cm x 1 cm).
- Square meter (m^2) can be visualized as a square that is 1 m on each side (or 1 m x 1 m). Square meters are used in intermediate applications, such as calculating the area of a room to purchase the right amount of carpet.
- Hectare is a special name for 10,000 square meters (m^2), which can be visualized as a square that is 100 m on each side (or 100 m x 100 m). Larger areas within a city neighborhood or the size of a farm can be measured in hectares (ha).
- Square kilometer (km^2) can be visualized as a square that is 1 km on each side (or 1 km x 1 km). Square kilometers typically measure very large areas.

Make any 5 questions and answer yourselves after you read above text

1. For example: 10 cm is an equivalent of 100 mm
2. _____
3. _____
4. _____
5. _____

Materials and methods

Table 3.2 Metric Conversion Chart for Area

Measurement Units	Unit abbreviations (Symbol)
1 square Centimeter = 100 square Millimeters	1 cm^2 = 100 mm^2
1 square Decimeter = 100 square Centimeters	1 dm^2 = 100 cm^2
1 square Meter = 10, 000 square Centimeters	1 m^2 = 10, 000 cm^2
1 square Kilometer = 1 million square Meters	1 km^2 = 1,000, 000 m^2
1 square Kilometer = 100 hectares	1 km^2 = 100 ha

Activity 1: Measure the length and width of your lab manual in centimeter and convert it into centimeter _____cm_____mm

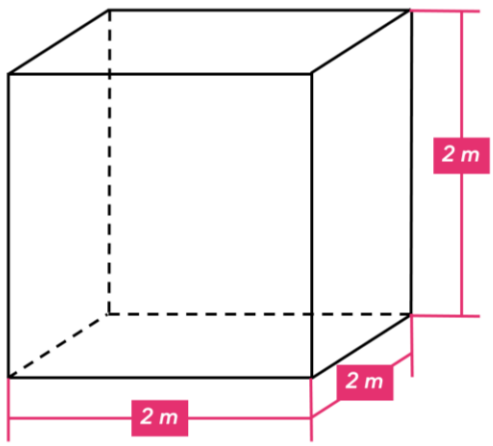
What is the area of your lab manual?

Area (A)= length and width

Area in cm_____ Area in mm_____

Volume

Volume is defined as the space occupied within the boundaries of an object in three-dimensional space. It is also known as the capacity of the object. Volume is the amount of space taken up by an object, while capacity is the measure of an object's ability to hold a substance, like a solid, a liquid or a gas. Volume is measured in cubic units. As per the SI system, the standard measuring unit of volume is liters. However, we can use various units to measure volume in almost every other unit, including milliliters, gallons, pounds, etc. Finding the volume of an object can help us to determine the amount required to fill that object, like the amount of water needed to fill a bottle, an aquarium or a water tank.



$$V = (2m)\times(2m)\times(2m)$$

$$\boxed{V = 8\,m^3}$$

Figure 3.1 Metric Conversion Chart for Volume (Three-dimensional shape cubes)

1. Some diabetes insipidus individual can produce as much as 13 litters to 15 liters of urine in a day. These values are equates to _____ milliliters and _____ milliliters

Table 3.3 Metric Conversion Chart for Volume (Volume units for liquids)

Measurement Units	Unit abbreviations (Symbol)
1 cubic centimeter = 1000 cubic millimeters	1 cm^3 = 1000 mm^3
1 cubic decimeter = 1000 cubic centimeters	1 dm^3 = 1000 cm^3
1 cubic meter = 1, 000, 000 cubic centimeters	1 m^3 = 1,000,000 cm^3
1 cubic meter = 1000 cubic decimeters	1 m^3 = 1000 dm^3
1 cubic kilometer = 1000000000 cubic meters	1 km^3 = 1000,000,000 m^3

2. Lab activity

Drops Count: *How many drops of water will it take to equal 1 milliliter?* _____*drops*

Procedure:

Part A: Count your drops!

Take a guess - How many drops of water will it take to equal 1 milliliter? _____ *drops*

Follow the directions to find the number of drops in 1 milliliter of water, then answer the questions. You will need a small graduated cylinder (25 ml), a beaker of water, and an eyedropper for this section.
(1) Fill a small graduated cylinder with 10 ml of water.
(2) Count the number of drops it takes to raise the water to 11 ml. Record the number in the chart.
(3) Leave the water in the graduated cylinder and count the number of drops it takes to raise the water to 12 ml. Record the number in the chart.
(4) Leave the water in the graduated cylinder and count the number of drops it takes to raise the water to 13 ml. Record the number in the chart.
(5) Calculate your average and round to the nearest tenth.

# of drops to 11 ml	# of drops to 12 ml	# of drops to 13 ml	Average

Based on your average, how close were you to your guess? _____

Based on your average, how many drops would it take to make 1 liter? _____

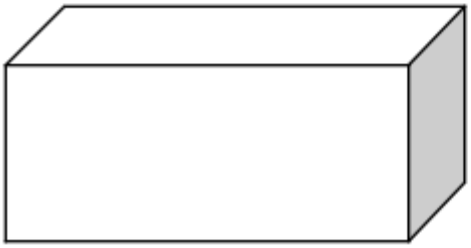
Part B: Water Displacement

Follow the directions to find the volume of three marbles using water displacement.
(1) Add 20 ml of water to a 100 ml graduated cylinder. Record this amount in the chart.
(2) Add three marbles to the cylinder and measure the volume. Record this amount in the chart.
(3) Find the difference between the two measurements and record in the chart. The difference between the two measurements will be the volume of the three marbles.

Volume of water before adding marbles	Volume of water after adding marbles	Difference in volume	Volume of 3 marbles

Part C: Volume by Formula

Use the formula to find the volume of the box. Measure to the nearest centimeter (no decimals) before calculating your answer.



Volume = length x width x height

_____ x _____ x _____ =

Part D: Color Challenge

1. Obtain the following items from your teacher:

   3 beakers with colored water- 25 ml of each color (red, blue, and yellow)
   1 graduated cylinder (25 ml - 50 ml)
   1 eyedropper
   6 test tubes labeled A, B, C, D, E, and F

2. Perform each step outlined below using accurate measurements.

   (1) Measure 17 ml of RED water from the beaker and pour into test tube A.
   (2) Measure 21 ml of YELLOW water from the beaker and pour into test tube C.
   (3) Measure 22 ml of BLUE water from the beaker and pour into test tube E.
   (4) Measure 5 ml of water from test tube A and pour it into test tube B.
   (5) Measure 6 ml of water from test tube C and pour it into test tube D.
   (6) Measure 8 ml of water from test tube E and pour it into test tube F.
   (7) Measure 5 ml of water from test tube C and pour it into test tube B.
   (8) Measure 2 ml of water from test tube A and pour it into test tube F.
   (9) Measure 4 ml of water from test tube E and pour it into test tube D.

3. Complete the chart.

Test Tube	Color	Final Amount (ml)
A		
B		
C		
D		
E		
F		

Table 3.4 Metric Conversion Chart for weight

Measurement Units	Unit abbreviations (Symbol)
1 Kilogram = 1000 Grams	1 Kg = 1000 g
1 Gram = 1000 Milligrams	1 g = 1000 mg
1 Metric Ton = 1000 Kilograms	1 Tonne = 1000 kg
1 Gigagram = 1000 Megagrams	1 Gg = 1000 Mg
1 decagram = 10 Grams	1 dag = 10 g

Lab Activity

Table:3.5 Measure the mass in gram and convert them in kilogram in following table

Object	Weight (gram, gm)	Weight (gram, gm)	calculation
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10			

Mass and Weight Lab

OBJECTIVE

- To distinguish between the mass and the weight of an object.
- To discover the types of units used for mass and weight.
- To verify that the gravitational pull of the earth on any object is 9.8N/kg

INTRODUCTION

There is an interesting relationship between ***mass*** and ***weight***. It depends on what planet you take the weight measurement. There can even be slight differences on earth, depending if you are at sea level or on a mountain-top.

Force $_{\text{gravitational}}$ = **m**ass X **g**ravitational field intensity

MATERIALS

- Dynamometer (spring scale)
- Objects of different masses
- Retort stand
- Clamp

PROCEDURE

1. Select one of the masses at random.
2. Record this mass in grams in Table 1.
3. Determine the weight of the same object, in Newtons, by har object on the hook of the dynamometer. Record this value in Table 1.
4. Repeat steps 1 to 3 using different masses. You should have a total of 10 different masses.
5. Return items as you found them and ask your teacher for more and larger masses.

Table 3.6 Metric Conversion Chart for Temperature

Measurement Units	Unit abbreviations (Symbol)
Celsius = 274.15 Kelvin	1 C = 274.15 K
1 Celsius = 33.8 Fahrenheit	1 C = 33.8 F
1 Kelvin = -2.72.15 Celsius	1 K = -2.72.15 C
1 Kelvin = -457.87 Fahrenheit	1 = -457.87 F
1 Fahrenheit = -17.22 Celsius	1 F = -17.22 C
1 Fahrenheit = 255.93 Kelvin	1 F = 255.93 K

Table 3.Measure the temperatures for hot water, tape water, ice water and -20º C. Plot all temperatures on graph paper.

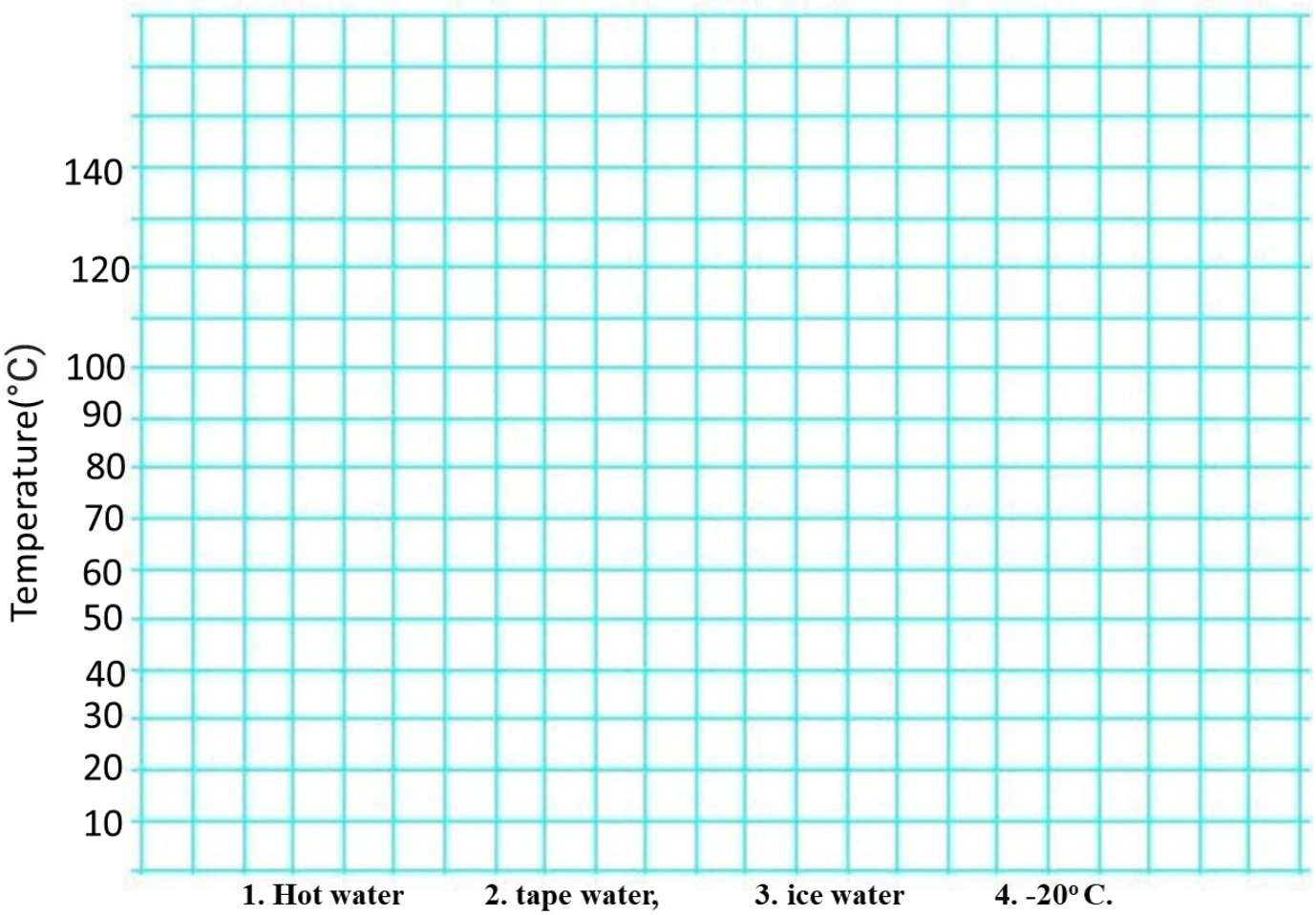


Figure 3.2 Plot the temperature for given items

Chapter 4: Understanding of Microscopy for Invisible Worlds

Learning outcomes: At the end, after the completion of the current lab, the student should be able to:

- To understand the advantage and limitations of both compound light microscope and dissecting microscope
- To utilize the importance of the microscope in biology
- To handle and proper care of microscope and dissecting microscope
- To identify and describe the function of the parts of compound microscope
- To understand the contrast, resolution and determine the total magnification of a microscope using different objectives
- To calculate the field of view at low and high power and to estimate the size of objects under a microscope.
- and differentiate and convert between standard notation and scientific notation
- To understand and measure the length, weight, volume and temperature using metric system.

Introduction

Microscopes are useful tools for visualizing small structures in detail. As we know that many biological organisms and structures such as biological specimens including cells and tissues are difficult or impossible to be seen with the naked eye so they must be magnified to be studied. In the biological science, microscope is the most important and frequently used tools that allows the user to discover the fascinating world of microorganisms. In addition of many biological specimens such as cells and tissues several microorganisms including bacteria are difficult or impossible to be seen with the naked eye so they must be magnified to be studied under microscope **(Figure 4.1)**. Consequently, the basic operation and care of microscopes is an important skill in biology.

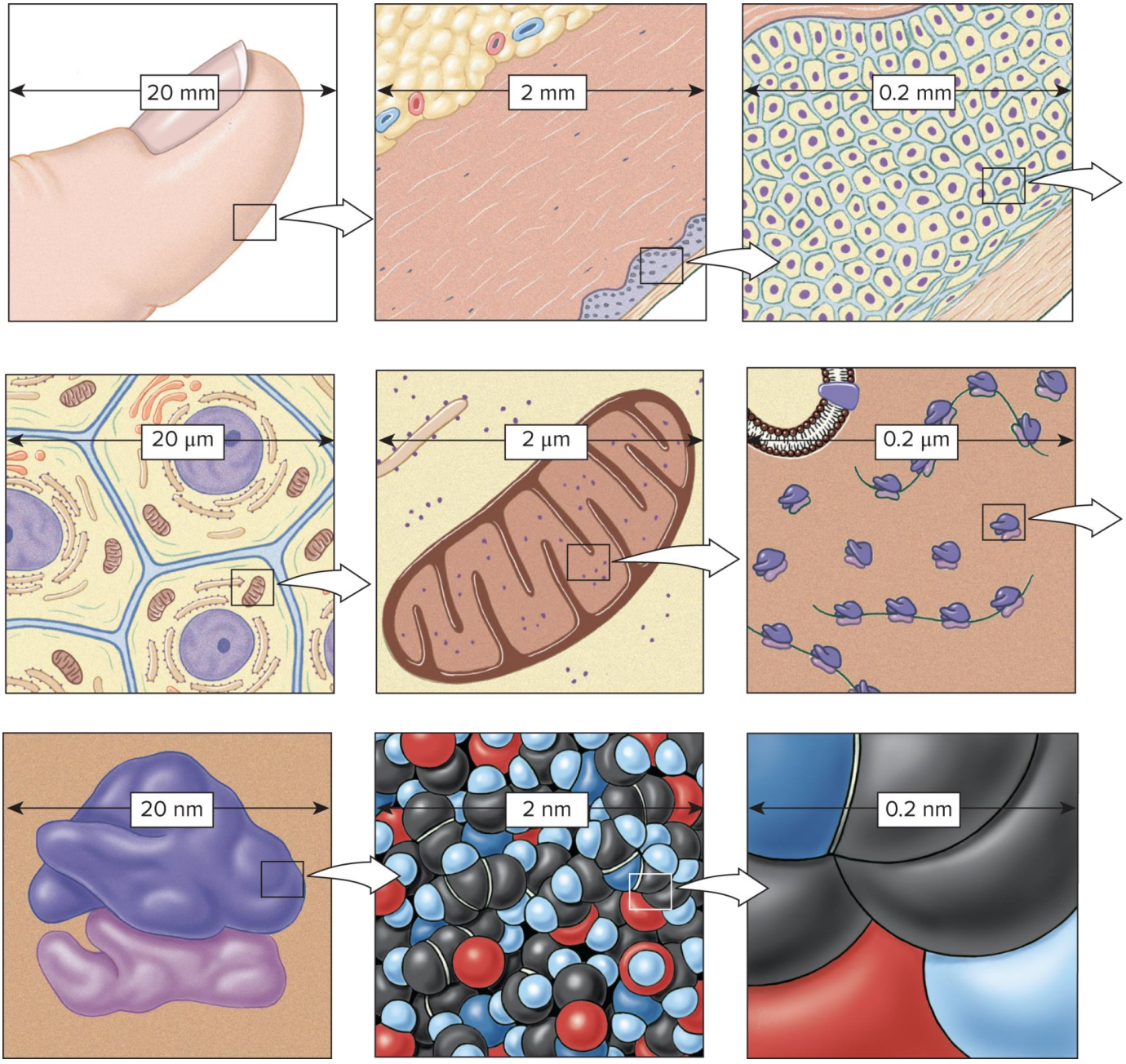


Figure 4.1 Typical human skin cells and their contents. Human skin cells, organelles, and their contents like macromolecules. Diagram shows the diameter of human skin cell is ~ 20 micrometers, mitochondria is ~2 micrometers, ribosome is ~ 20 nanometers, protein molecule is ~2 nanometers and atom is 0.2 nanometers.

There are two frequently used common types of microscopes **(Figure 4.2)** in the biological and microbiological laboratories. 1. The compound light microscope (commonly known as a light microscope) and, 2. the stereo microscope (commonly known as a dissecting microscope). Compound light microscopes pass visible light through two sets of magnifying lenses (the ocular and the objective lenses) to magnify specimens mounted on a glass slide and placed on the flat surface (stage) of the microscope. The specimens must be sliced very thin in order for light coming from the light source to pass up through them so that they can be viewed. Specimens are sometimes stained with dyes to add contrast and to make structures more easily identifiable. Microscopic examination of cells and tissues allows students to observe the principle of complementarity - how cell and tissue structure determines function.

A light microscope is used to visualize objects flattened onto glass slides in great detail. It typically has a magnification power of up to 40x to 1000x. A light microscope might be used when examining individual cells within living tissue. A dissecting microscope is used to view three-dimensional objects and larger specimens, with a maximum magnification of 100x. This type of microscope might be used to study external features on an object or to examine structures not easily mounted onto flat slides.

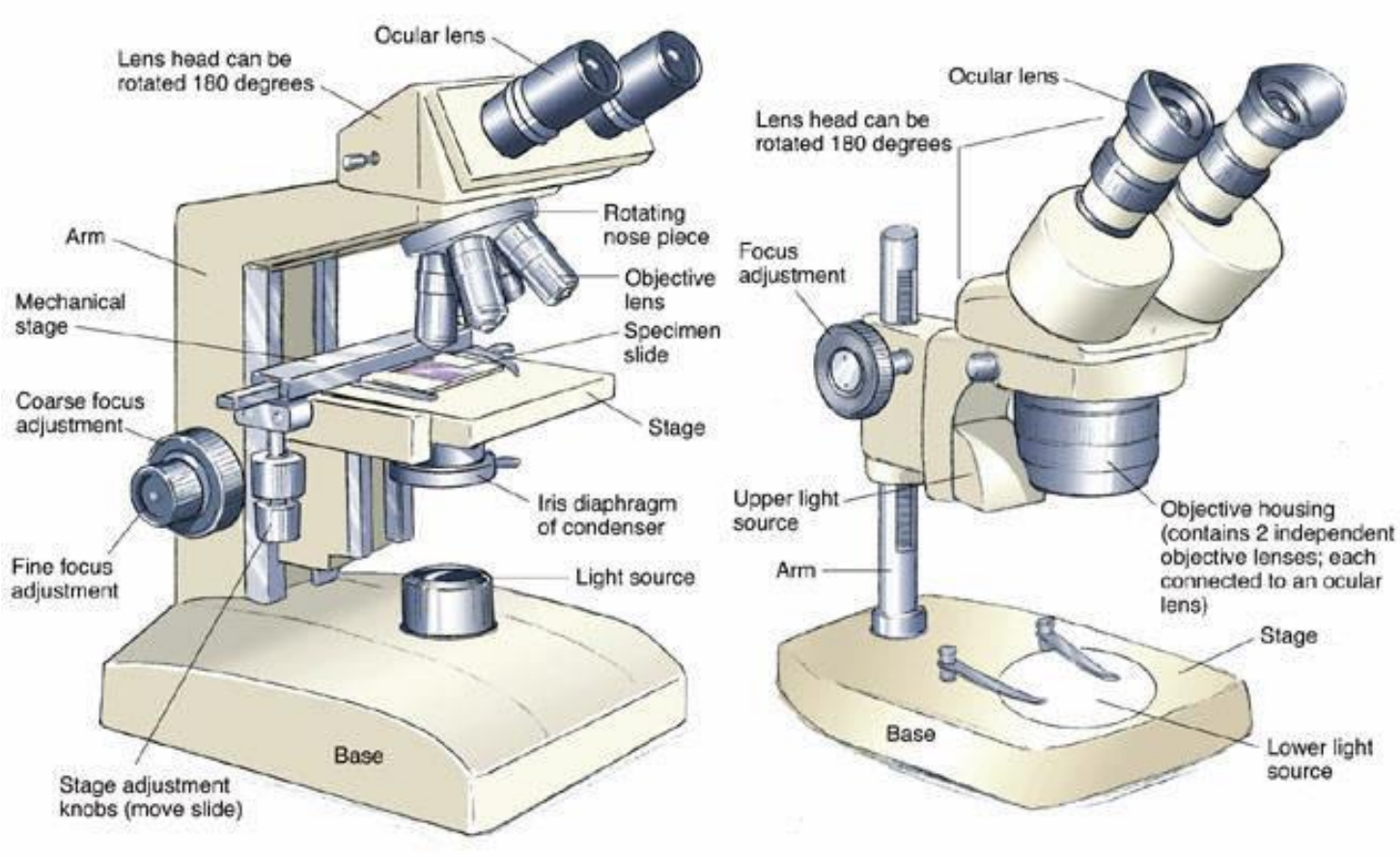
Both microscopes have similar features. Each microscope has an eyepiece as well as a light source. Light microscopes usually have eyepieces that are magnified 10x plus multiple objective lenses that are magnified between 4x and 100x (Fig. 4.2 A). The total magnification is calculated by multiplying the eyepiece magnification (10x) by the objective lens magnification (e.g., 40x), for a total magnification would be 400x. On a light microscope, a sample is placed on a glass slide and light is passed through the sample from underneath the stage. This type of microscope shows great detail of small, thin objects. To view thicker objects, the tissue sample must be thinly sliced and prepared on a glass slide in two-dimensions. With a dissecting microscope whole object can be viewed in three dimensions. Samples do not need to be sliced, and larger, live animals can be observed. Light can be passed through from underneath the sample, but also from the top or side using an external light source. However, the magnification power of the dissecting microscope is less powerful than that of a light microscope**.**

Pre-Lab Questions

3. **Write any two major differences between the compound light microscope and the stereo microscope.**

4. **What happens the size of the cells and number of the cells when you go from 4x to 10 x magnification?**

5. **Total magnification of the cell under 100x objective lens magnification_____**



A **B**

Figure 4.2 Typical structure and major parts of the compound light microscope (A) & the stereo microscope (B).

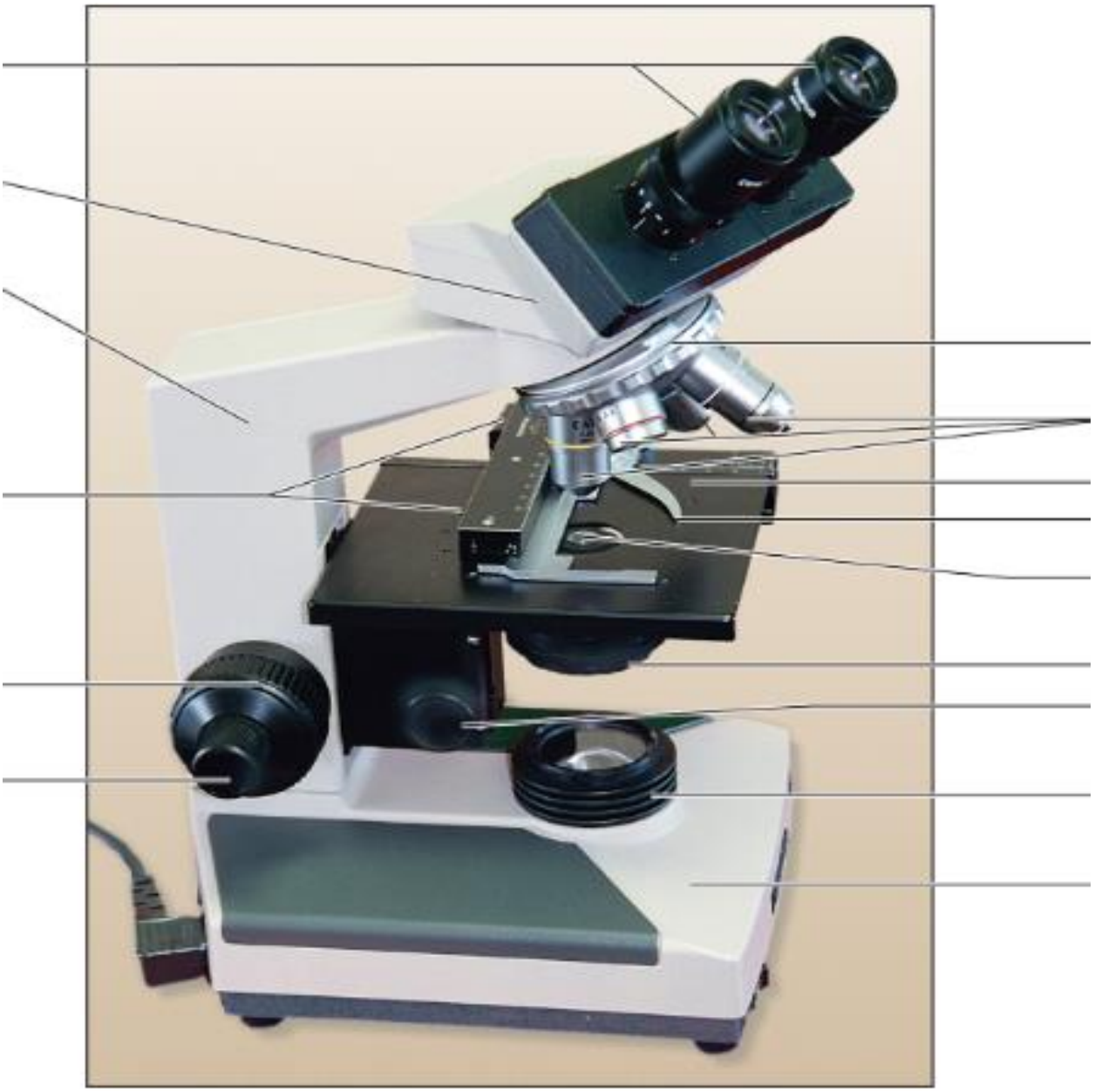


Figure 4.3 Identify and Label the Major Parts of a Compound Light Microscope

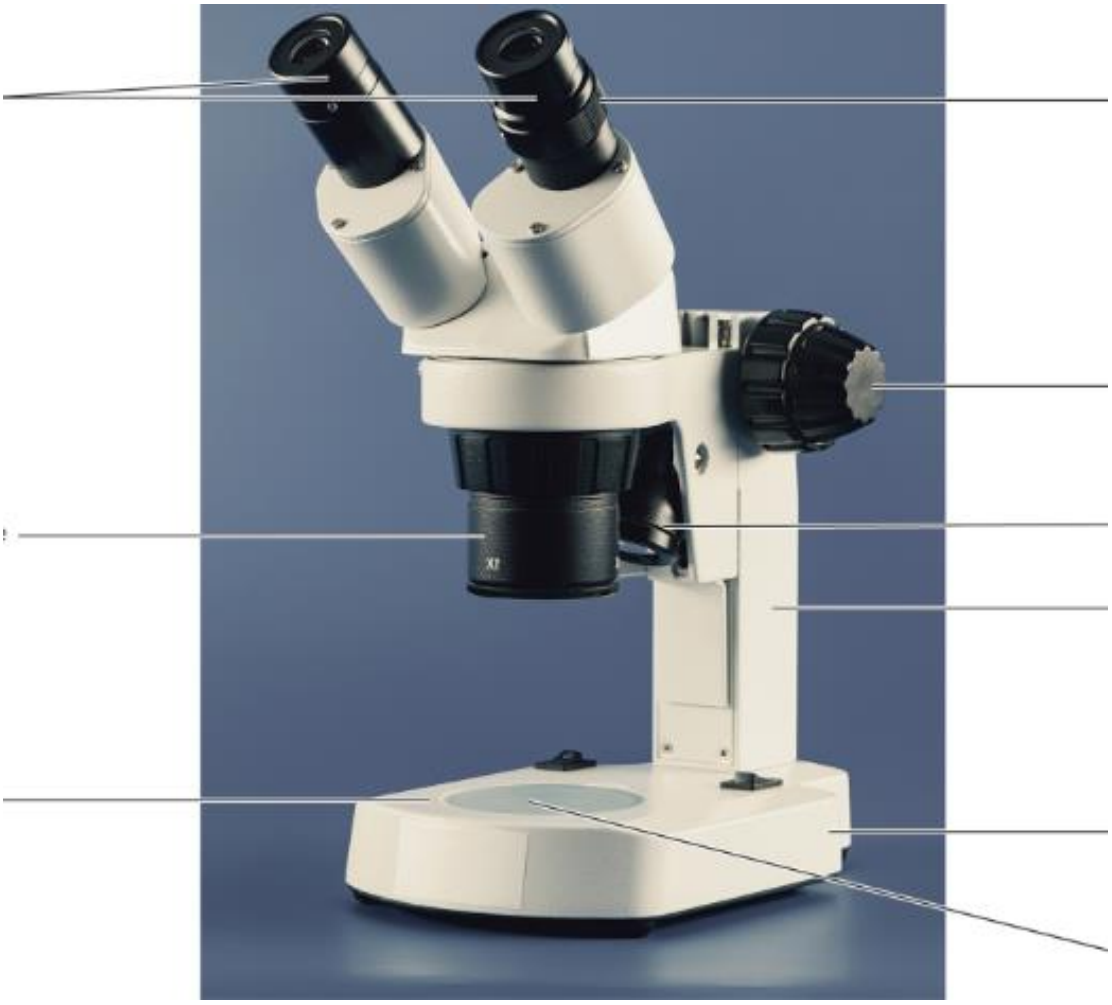


Figure 3.3 Identify and Label the Major Parts of a dissecting (stereoscopic) Microscope

The Dissecting Microscope

The dissecting microscope offer some advantages over a compound microscope. It is extremely easy to operate. Although light microscope can produce high magnification and excellent resolution, it has small distance between the objective lens and specimen. Therefore, it is difficult to manipulate a specimen while observing it with a compound microscope. Specimen that can be observed with compound microscope are limited to those thin enough for light to pass through them. In contrast, a dissecting microscope is used to view objects that are opaque or too large to see with a compound microscope. Most of the dissecting microscopes equipped with 4x to 50x magnification.

Procedure of using Dissecting Microscope

a. Carry the dissecting microscope to the working station by grasping the microscope's arm with one hand and placing other hand under the microscope's base.
b. Follow Figure 3.2 B to familiarize yourself with the parts of your microscope
c. Carefully clean the ocular and objective lens with **lens paper only.** If something wrong, consult the laboratory technician or instructor.
d. You can use a ruler to measure the diameter of the field of view with dissecting microscope at

several levels of magnifications.

Table 4.1 Comparison of Dissecting and Compound Microscope		
Characteristics	**Dissecting Microscope**	**Compound Microscope**
Magnification		
Resolution		

Answer following questions

1. What are the advantage of knowing the diameter of the field of view at a given magnification?

2. What are some examples of biological specimens that would be best examined with dissecting microscope instead of a compound microscope?

3. What is the function of each of the following parts of compound and dissecting microscope?
   a. Oculars
   b. Objective
   c. Condenser
   d. Iris diaphragm
   e. Stage
   f. Course adjustment
   g. Fine adjustment

The Compound Microscope

Compound Microscope Parts

A high power or compound microscope achieves higher levels of magnification than a stereo or low power microscope. It is used to view smaller specimens such as cell structures which cannot be seen at lower levels of magnification. Essentially, a compound microscope consists of structural and optical components. However, within these two basic systems, there are some essential components that every microscopist should know and understand. These key microscope parts are illustrated and explained below. This microscope contains a compound set of two lenses, which magnifies the image and allows attaining a total magnification of over 1000X. The ocular lens typically has a magnification of 10X, our lab microscope has four objectives located on a revolving nosepiece: 4X, 10X, 40X and 100X. The 4X objective is also called the ***scanning lens*** because it allows you to scan or search for the specimen you

want to observe. This lens provides the broadest ***field of view*** (amount of area observed) because it has the largest aperture in the opening of the lens. As you increase the magnification, the aperture decreases and thus, the field of view also decreases; increasing magnification requires more light or ***illumination*** in order to better observe the specimen. Increasing the magnification of an image requires a higher ***resolution*** that is the ability to distinguish any two separate points. The maximum resolution with this type of microscope is reached at about 1o00X. When calculating total magnification, use the following formula:

Total magnification = power of objective X power of ocular

Circle the objective that produces the smallest field of view:

a. 4X

b. 10X

c. 40X

d. 100X

Table 4.2 Total magnification of Compound Microscope

Objective lens	Ocular lens	Total Magnification
4 X (scanning)	**10 X**	
10 X (low power)	**10 X**	
40 X (low power)	**10 X**	
100 X (low power)	**10 X**	

STRUCTURAL COMPONENTS

The three basic, structural components of a compound microscope are the head, base and arm.

- ***Head/Body*** *houses the optical parts in the upper part of the microscope*
- ***Base*** *of the microscope supports the microscope and houses the illuminator*
- ***Arm*** *connects to the base and supports the microscope head. It is also used to carry the microscope.*

When carrying a compound microscope always take care to lift it by both the arm and base, simultaneously.

OPTICAL COMPONENTS

There are two optical systems in a compound microscope: Eyepiece Lenses and Objective Lenses:

Eyepiece or Ocular is what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 10x. Optional eyepieces of varying powers are available, typically from 5x-30x.

Eyepiece Tube holds the eyepieces in place above the objective lens. Binocular microscope heads typically incorporate a diopter adjustment ring that allows for the possible inconsistencies of our eyesight

in one or both eyes. The monocular (single eye usage) microscope does not need a diopter. Binocular microscopes also swivel (Interpupillary Adjustment) to allow for different distances between the eyes of different individuals.

Objective Lenses are the primary optical lenses on a microscope. They range from 4x-100x and typically, include, three, four or five on lens on most microscopes. Objectives can be forward or rear-facing.

Nosepiece houses the objectives. The objectives are exposed and are mounted on a rotating turret so that different objectives can be conveniently selected. Standard objectives include 4x, 10x, 40x and 100x although different power objectives are available.

Coarse and Fine Focus knobs are used to focus the microscope. Increasingly, they are coaxial knobs - that is to say they are built on the same axis with the fine focus knob on the outside. Coaxial focus knobs are more convenient since the viewer does not have to grope for a different knob.

Stage is where the specimen to be viewed is placed. A mechanical stage is used when working at higher magnifications where delicate movements of the specimen slide are required.

Stage Clips are used when there is no mechanical stage. The viewer is required to move the slide manually to view different sections of the specimen.

Aperture is the hole in the stage through which the base (transmitted) light reaches the stage.

Illuminator is the light source for a microscope, typically located in the base of the microscope. Most light microscopes use low voltage, halogen bulbs with continuous variable lighting control located within the base.

Condenser is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.

Iris Diaphragm controls the amount of light reaching the specimen. It is located above the condenser and below the stage. Most high-quality microscopes include an Abbe condenser with an iris diaphragm. Combined, they control both the focus and quantity of light applied to the specimen.

Condenser Focus Knob moves the condenser up or down to control the lighting focus on the specimen

Using A compound Microscope for Lab Activities

Caring and Using the Microscope

Ocular and objective lenses may be wiped clean only with lens paper, never use a paper towel as this may scratch the lens. Do NOT touch the lenses, body oils may also damage the lens.

1. Preparing a Wet Mount:

Materials

- Compound Microscope
- Blank clean slide
- Kimwives
- Pondwater or any other specimens like onion cell or *Elodia* leaf

Procedure

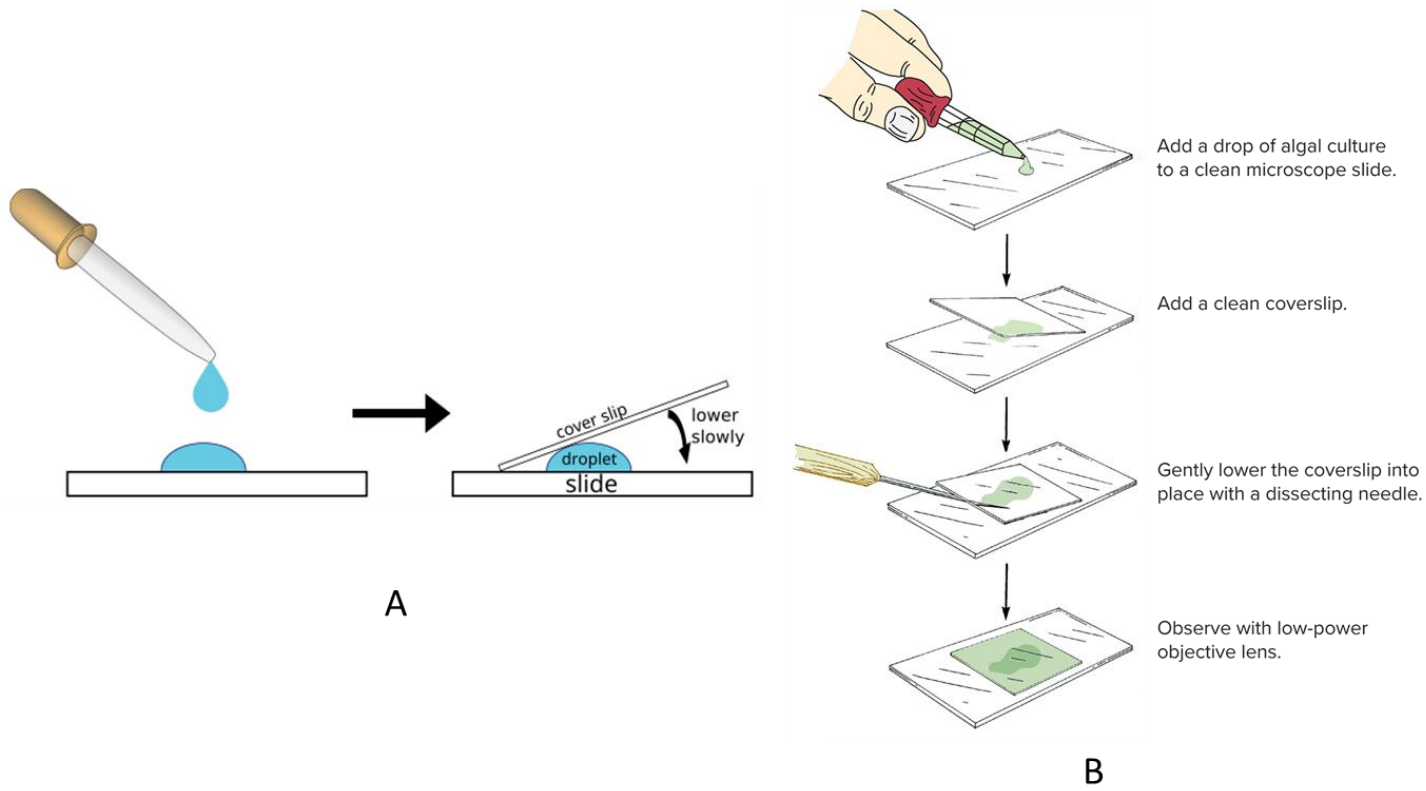


Figure 4.4 Procedure to prepare wet Mount slide.

a. Most specimens must be killed, fixed, sectioned, and stained for microscopy.

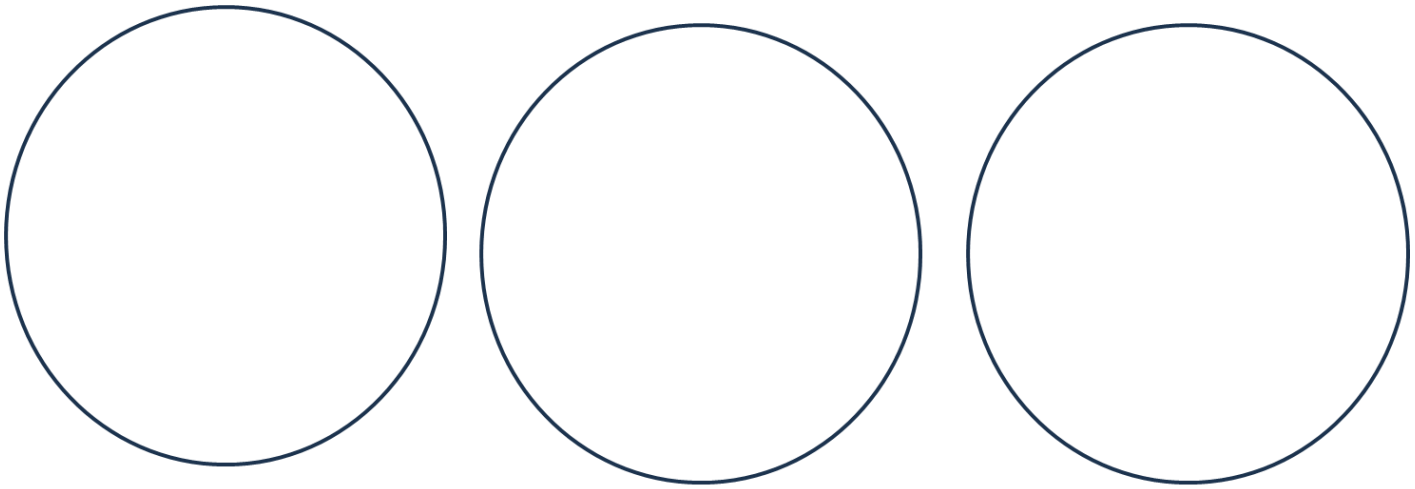
b. Place a drop of the sample in the center of the microscope slide. If the specimen is not in water, place a small, thin section of the specimen on the slide and add a drop of water on top of it.

c. Place a coverslip over the drop of sample by positioning its edge onto the slide to one side of the drop and then lowering the coverslip slowly over the specimen. Do this slowly to avoid excess bubbles; however, if you get a couple of bubbles, be aware not to confuse them with your cells, a bubble looks like a perfect circle with a dark circumference.

d. Follow the steps for scanning your specimen across a side and switch to a higher power as needed

Cheek cells wet mount preparation

- Swab the inside of your cheek and roll the swab across a slide
- Drop some methylene blue onto the slide
- Place a coverslip over the drop
- Visualize and draw your cheek cells
- Document your observations by drawing the cells and by using your phone to snap an image (Figure 4.5).

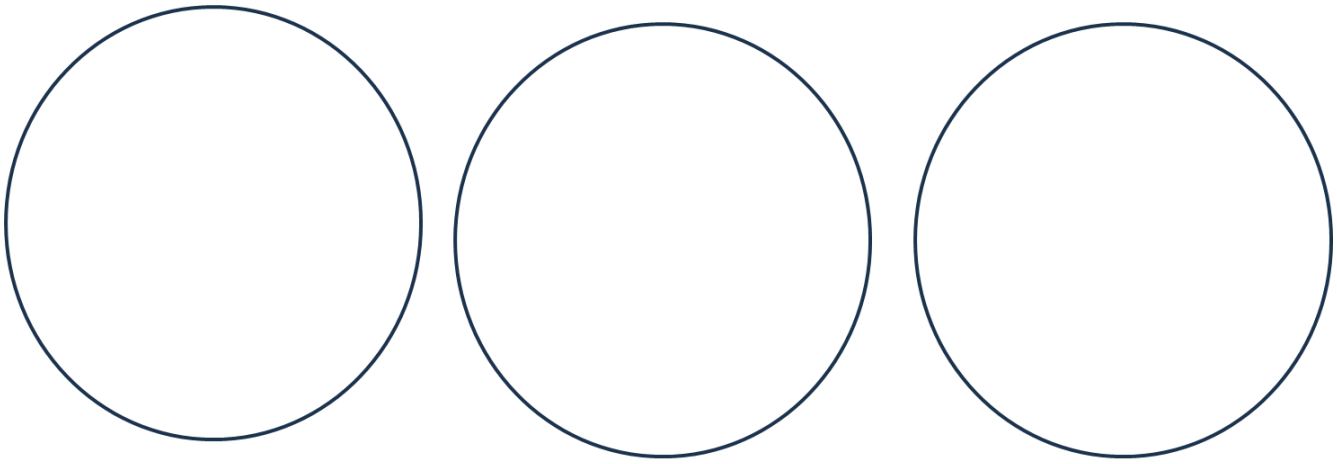


4X 10X 40X

Figure 4.5 Prepare a wet Mount of cheek cells and sketch on the above given field

***Elodia* leaf cells wet mount preparation**

- Cut and take a small piece *Elodia* leaf
- Put a drop of water onto the slide
- Place a coverslip over the leaf
- Visualize and draw your *Elodia* leaf cells
- Document your observations by drawing the cells and by using your phone to snap an image (Figure 4.6).



4X 10X 40X

Figure 4.6 Prepare a wet Mount of *Elodia* leaf and sketch on the above given field

***Spirogyra* cells wet mount preparation**

- Collect spirogyra from pond or obtain from lab tec/ instructor
- Separate single threads by applying pins or proper lab material like pina brush and pins
- Place a coverslip over the ***Spirogyra* thread**
- Visualize and draw your ***Spirogyra* cell**
- Document your observations by drawing the cells and by using your phone to snap an image (Figure 4.7).

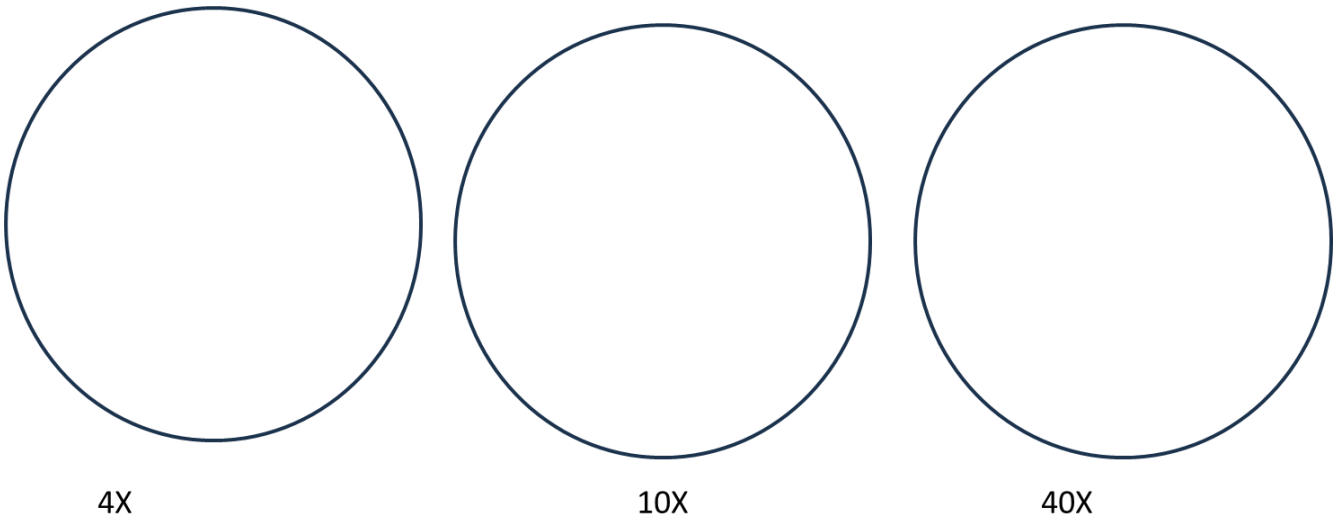


Figure 4.7 Prepare a wet Mount of *Spirogyra* cells and sketch on the above given field

1. **Determining orientation and the depth offocus:**

Materials

- **Microscope**
- **Prepared slides for different specimen (Instructor/ lab technician will provide)**
- **Kimwives and lens paper**
- **Pondwater or any other specimens like onion cell or *Elodia* leaf**

Procedure

i. Plug in the microscope and turn the light switch on.

ii. Add letter "e" slide: Place the slide with the letter "e" on the stage and secure it using the stage clip.

iii. By rotating the revolving nosepiece, allow the smallest objective to click into position for viewing.

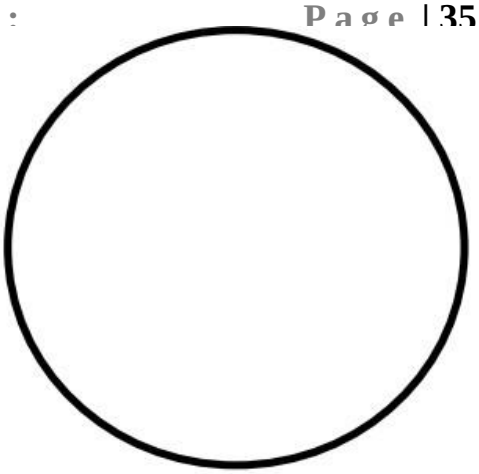
iv. Using the condenser height adjustment knob, make sure the condenser is all the way up under the stage.

v. While looking at the stage, carefully turn the coarse adjustment knob to bring the stage up until it stops.

vi. While viewing down through the oculars, slowly turn the coarse adjustment knob away from you to bring the stage down until you see the letter "e" clearly.

Using the specimen holder knobs, move the iew.

vii. Fine focus your image by until the image is clear to your eyes. Sketch the orientation of the letter "e" as you observe it under the microscope.



Letter "e" (total magnification: _

While looking AT YOUR OBJECTIVE LENSES, rotate the revolving nosepiece slowly while making sure the objective lens is not touching the surface of the cover slip, then, click it into position. NOTE: If it seems like the objective will touch the slide, STOP and return to observing the object under the lower magnification. Use only the fine focus knob to find the clearest image of yourspecimen.

1. Move the slide to the left and to the right. In which Direction did the image move?

2. How is the orientation of letter "e" when you view it? Upside down or right side up?

3. Move the slide toward you or away from you. . In which Direction did the image move?

4. What happens to the brightness of the view when you go from 4X to 10 X

Letter "e" observed under 40X

Document your observations and draw an image under 40X for prepared slides and by using your phone to snap an image (Figure 4.6).

Bacteria:

_____

(total magnification: _____)

Cyanobacterial:

_____

(total magnification: _____)

Protist:

_____

(total magnification: _____)

Field of view (FOV)

Field of view for a microscope is the extent of the observable area in distance units. The optics provide a clear and undistorted view in a field around the optical axis, and the field of view is selected from this. The rays that produce the image in this view are generally aberration-free and do not create a significant falloff in image intensity.

Field of view microscope definition in simple terms it is the area you see under the microscope for a particular magnification. Say, for example, you are viewing a cell or specimen under an optical microscope. The diameter of the circle that you see is the field of view of the microscope **(Figure 4.7).**

As you increase the magnification, the field of vision is reduced. Depending upon the lens system, this can vary. A crude way of measuring the field of view is by using a ruler under the microscope for a particular magnification.

To calculate the field of view of microscope you need to know the eyepiece magnification, field number and objective lens. Once you have this information you can calculate the field of view of the microscope by dividing the field number by the magnification number. Following formula can be used to measure field of view (FOV)

FOV = Field Number (FN*) ÷ Objective Magnification, *FN* = the Eyepiece F.N.*

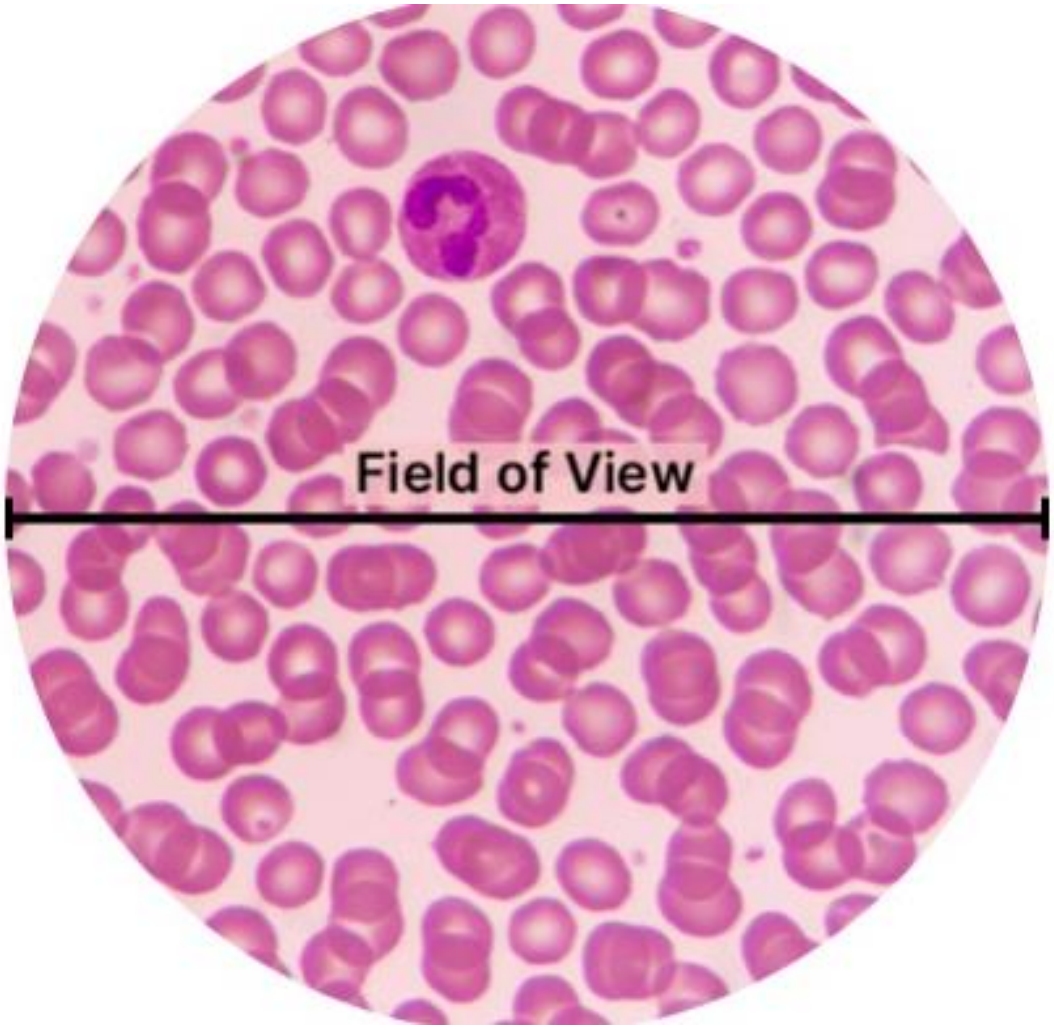


Figure 4.8 The diameter of the circle that you see is the field of view of the microscope

As you increase the magnification, the field of vision is reduced. Depending upon the lens system, this can vary. A crude way of measuring the field of view is by using a ruler under the microscope for a particular magnification. When you switch eyepieces or objective lenses, ensure to repeat the FOV calculations with the new inputs on field number and magnifications. For objects that require higher magnifications, convert your measurements from millimeters to micrometers. Higher power lenses will allow you to view tiny objects, so the angle of view will be small; low power lenses will do the opposite and let you view bigger (wider) objects.

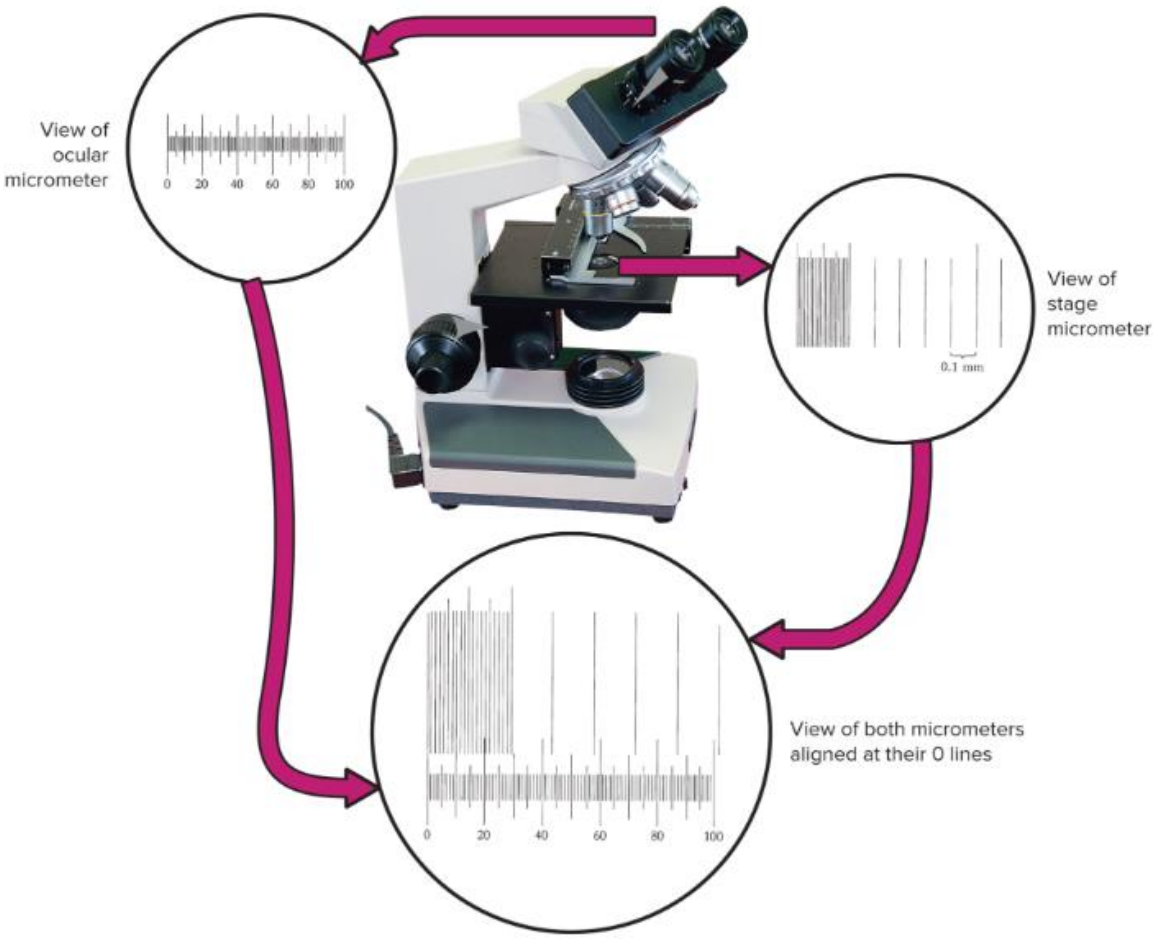


Figure 4.7 Measurement of the size of specimen unis compound microscope. Stage and ocular micrometers. The FOV can be measured with ruled micrometers.

If using a stereo microscope with an auxiliary lens, the magnification factor of this lens should also be employed in the equation by multiplication with the objective magnification. You will have to multiply the eyepiece magnification by the objective magnification to find the total magnification before dividing the field number. **Field of View = FN ÷ (Objective Magnification x Auxiliary**

Lens Magnification) For instance, if your eyepiece reads 10X/22, and the magnification of your objective lens is 40. First, multiply 10 and 40 to get 400. Then divide 22 by 400 to get a FOV diameter of 0.055 millimeters.

The Field of View on a microscope determines the size of the imaged area. It gains importance when you want to measure specifics such as densities -for example, an

experiment to estimate the density of cells in a solution. To get the answer, you have to acquire an image of a Field of View, count the number of cells and divide it by the imaged area. The result would be an estimate of cells/micron.

Note that the extent of the Field of Vision depends on the magnification. Objectives with higher magnifications have smaller Fields of Vision. Thus, the size of the Field of Vision should be selected as a function of the size of the features that are to be studied. Bacteria are smaller (around 5 microns) than most human cells (a brain's astrocyte can have 90 microns in diameter). Therefore, to study astrocytes larger Fields of Vision are necessary.

Calculate the FOV specimens that you observed under microscope for **Figure 4.6** and **Figure** 4.7

Focus and depth

Materials: *Compound Light Microscope, Prepared slide of three different colored threads*

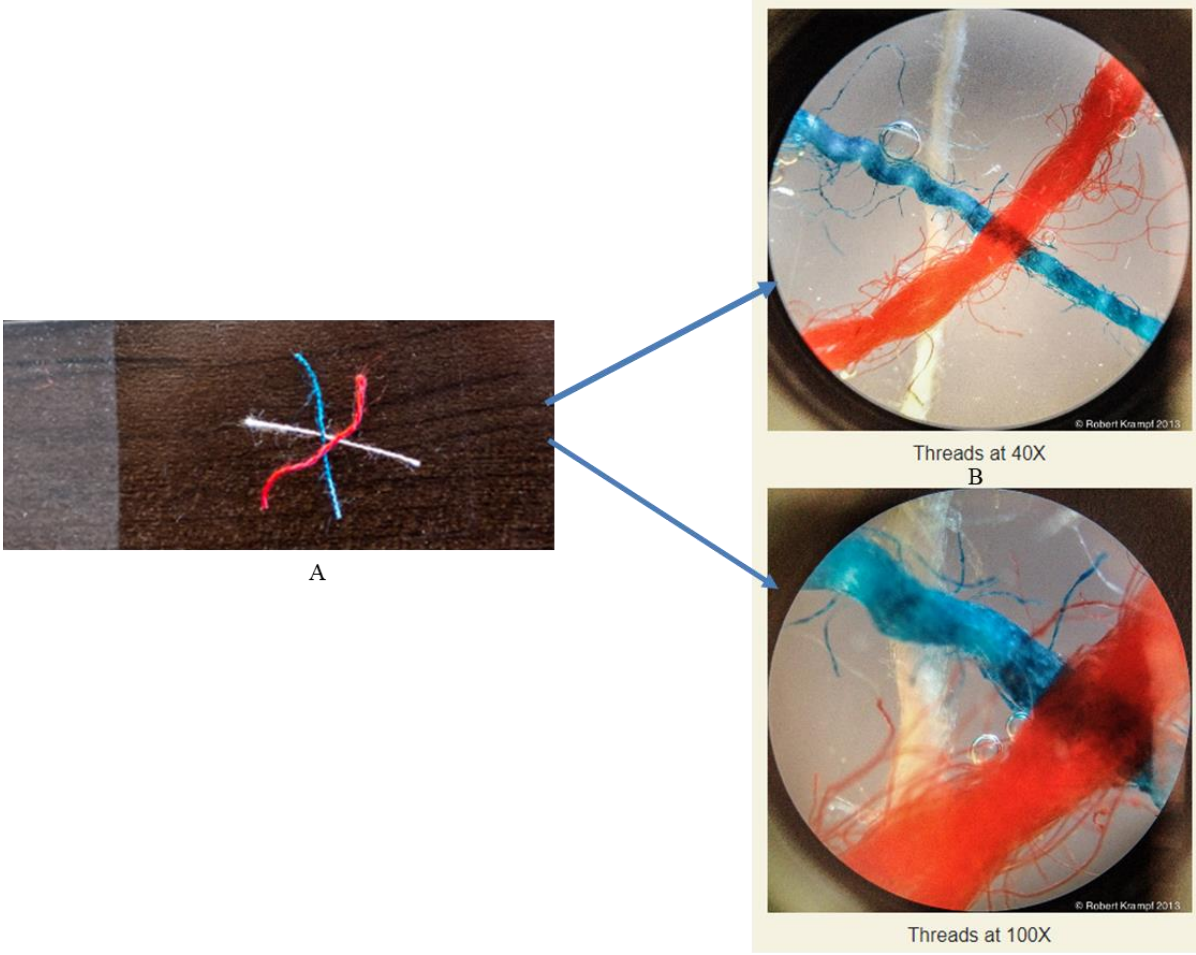
Microscope lenses have a restrictive plant of focus, a specific distance from the lens where the specimen can sharply focused. It is also possible to focus through different planes of the specimen by changing the plane of focus. The plane of focus has some depth called **depth of field**, o the thickness of the specimen in focus at any one time.

A common exercise to demonstrate depth of focus involves laying three different colored threads one on top of the other **(Figure 4.8 A)**. As the observer focuses down, first the top thread comes into focus, then the middle one, and finally the bottom one. On higher power objectives one may go out of focus as another comes into focus.

Set your microscope with the lowest power objective. Place the slide on the stage, and looking from the side, bring the objective as close to the slide as possible. Looking through the microscope, focus away from the specimen until the image comes into focus.

The photo on the right top **(Figure 4.8 B)** was taken at 40X (10X eyepiece and 4X objective) and the right bottom**(Figure 4.8 C)** was taken under 100X (10X eyepiece and 10X objective).

You can take photo under both magnification using your own mobile phone.



A B C

Figure 4.8 Depth of focus of three different colored threads under compound light microscope

Notice that all three threads are fairly well in focus, although the white thread on the bottom is a bit blurry. At low magnification, things appear bright, and it is easy to bring them into focus. Examine with various intensities.

Let's start with the focus. Every arrangement of lenses has a range of things that are clearly in focus. That is called the depth of field. Things that are outside the depth of field are blurry.

You can see the same thing when you take photos with your camera. In a wide angle photo, you have a very deep depth of field, and pretty much everything looks in focus. If you magnify things with a telephoto lens, your depth of field narrows, and you have to choose what will be in focus and what will be blurry.

The same is true when you use a microscope. The higher the magnification, the narrower the depth of field. At even higher magnification, you can't even get a single fiber from the thread in focus. This greatly limits what you can see at higher magnifications. The other thing that changes as you increase magnification is the amount of light. Why does magnifying something make it darker? To help answer that, take a look at the photo on the right.

The large circle is the image at 40X. When you magnify the image by 100X, you are taking the light from the 100X circle, and spreading it out to cover the same area as the 40X circle.

That is like taking the icing from a cupcake and spreading it over a full-sized cake. The sweet icing gets spread a lot thinner, and the cake would not be as sweet.

Now apply that model to the images from the microscope. The more you magnify an image, the thinner the light gets spread, and you reach the point where even with a very bright light, the image is too dark to see anything.

We get around the problems of brightness and depth of field by using something besides light for the microscope. Images like the one below are taken with a scanning electron microscope, using a beam of electrons instead of light. One drawback is that since there is no light, there is no color. These images are always black and white, unless they have been artificially colored.

1. Using low-power objective, slowly focus until the first threads comes into focus. What is the color of first threads?

2. Continue focusing through the threads, nothing the order of colored threads. What is the color of second threads.

3. What is the color of third threads?

4. Is the image always best with highest illumination? Explain Why?

5. Which magnification require the most illumination for the best clarity and contrast

Oil Immersion

Microscopic images of biological samples at high magnification are equally beautiful as they are informative, but getting great images is challenging. Are you getting your best possible images? Oil is frequently used with high magnification objectives. You should use an oil immersion lens when you have a specimen that is no thicker than a few tens to one hundred micrometres. Specifically, you should use it when the structures you wish to observe are quite small, perhaps one or two micrometres in dimension or smaller.

When light passes from a material of one refractive index to another (for example: from glass to air), it bends. In the space between the microscope objective lens and the slide (where air is), light is refracted, the light scatters and it is lost. The refractive index of air is approximately 1.0, while the refractive index of glass is approximately 1.5. When light passes through both glass and air it is refracted. Light of different wavelengths bends at different angles, so as objects are magnified more, images become less distinct.

Basically, when using lower magnification microscope objective lenses (4x, 10x, 40x) the light refraction is not usually noticeable. However, once you use the 100x objective lens, the light refraction when using a dry lens is noticeable. If you can reduce the amount of light refraction, more light passing through the microscope slide will be directed through the very narrow diameter of a higher power objective lens. In microscopy, more light = clear and crisp images. By placing a substance such as immersion oil with a refractive index equal to that of the glass slide in the space filled with air, more light is directed through the objective and a clearer image is observed.

Take a look at the images captured below. The images were captured using the UX1 microscope with the 100x Achromat objective lens and the 100x Plan Achromat objective

lens. Observation was performed dry and with immersion oil. Notice the difference in image quality between the images captured dry versus those captured with immersion oil. For example, oil immersion dramatically enhances the visualization of specimen like duodenum image under 100X with (Figure 4.9 A) and without (Figure 4.9 B) immersion oil.

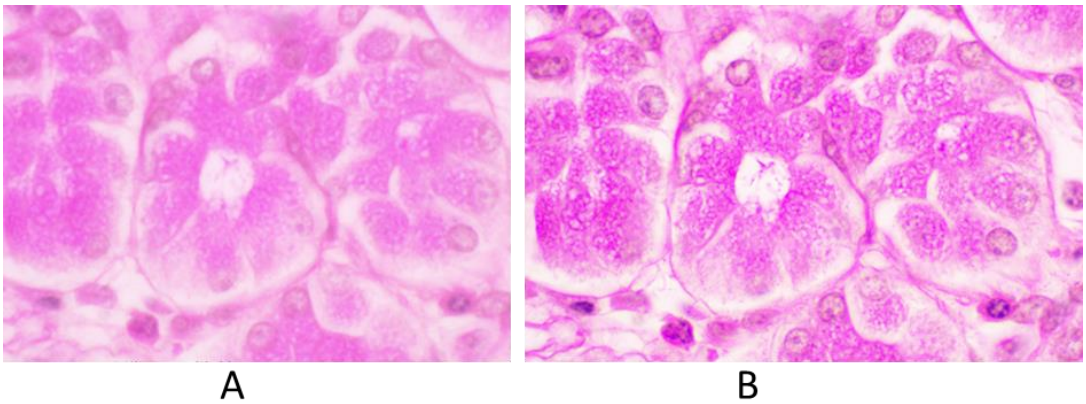


Figure 4.9 Microscopy image of duodenum captured using Plan Achromat 100x objective lens without immersion oil (A) and with immersion oil (B)

How to Use Microscope Immersion Oil

Before using immersion oil, make sure that your 100x objective lens is made for use with immersion oil.

1. Begin by focusing your sample using the 40x objective lens. Rotate the objective lens part way between the 40x and 100x lens so you can reach the cover slip on your slide.
2. Place a drop of immersion oil on the top of your cover slip and another drop directly on your 100x oil objective lens.
3. Slowly rotate your 100x oil objective lens into place and adjust the fine focus until you get a crisp and clear image.
4. When finished viewing with your 100x oil immersion lens, carefully wipe the oil from all glass surfaces using a piece of lens cleaning paper.
5. Use a second piece of lens paper moistened with a small amount of alcohol (ethyl or isopropyl) or lens cleaning solution, wiping all glass surfaces again to remove any remaining oil. Failing to remove immersion oil from lenses will result in hardened oil on lenses that will affect future clarity.

6. If you need to remove immersion oil that has been left on a lens and hardened, moisten lens paper with a small amount of xylene or microscope lens cleaning solution. Gently wipe lens surfaces, allowing enough time for the solution to soften the hardened oil. Once oil is removed wipe surfaces again.

When To Use Microscope Immersion Oil?

- Immersion oil should only be used if you have an immersion oil lens. The lens will actually have "oil", "immersion" or "HI" (homogeneous immersion) printed on it.
- Immersion oil is best for viewing samples that are dead or are not moving and no thicker than a few micrometers. Very small moving protists can occasionally be viewed with immersion oil, but the best specimens for oil immersion do not move.
- Oil immersion is required when viewing individual bacteria strands or details of striations in skeletal muscle.
- Immersion oil should be used anytime you want to view a clearer image at 1000x.
- There are several disadvantages to using immersion oil. For example, a wet mount slide must be incredibly secure in order to use immersion oil with it. If switching between a dry 40x lens and a 100x oil lens, care must be taken not to get oil on the 40x lens, which could damage it.

1. Examine and sketch the given specimen under microscope with and without immersion oil. Also take a snap using your mobile for both conditions.

Without immersion oil

With immersion oil

1. Examine and sketch the given specimen under microscope with and without immersion oil. Also take a snap using your mobile for both conditions.

Specimens: Coin, Fossil, Feather, Leaf

Specimen_____

Specimen_____

Magnification_____

Magnification_____

Specimen_____

Specimen_____

Magnification_____

Magnification_____

Chapter 5: Understanding of Cell Structure and Function

Learning Outcomes: At the end, after the completion of the current lab, the student should be able to:

- To state the components of the cell theory
- To describe the primary difference between prokaryotic cells and eukaryotic cells
- To describe the primary difference between animal cells and plant cells
- To identify whether the given cell like an animal cell, plant cell and bacterial cell are prokaryotic cell or a eukaryotic cell
- To identify structures, present in bacterial cells on a model or picture (use both your lab manual and your textbook as a reference)
- To list which of the 3 domains (Bacteria, Archaea or Eukarya) have prokaryotic organisms
- To identify the structures, present in either plant or animal cells on a model or picture
- To list two features or cellular components that all cells have in common

Introduction

Cells are the fundamental unit of life. All living things are composed of cells. While there are several characteristics that are common to all cells, such as the presence of a cell membrane, cytoplasm, DNA and ribosomes, not all cells are the same. There are two types of cells, prokaryotic (Greek "before" and "kernel", which means before true nucleus) and eukaryotic (Greek "good" and "kernel" which means true nucleus) cells. Organisms belonging to the domains Bacteria and Archaea are composed of prokaryotic cells, whereas organisms belonging to the domain Eukarya (protists, fungi, plants and animals) are composed of eukaryotic cells.

Prokaryotic cells are generally smaller than eukaryotic cells (about ten times smaller) and lack a nucleus and other membrane-bound organelles, whereas eukaryotic cells are compartmentalized by membrane-bound organelles with

specialized functions, and the DNA is contained inside the nucleus. The cytoplasm is the region of the cell outside the nucleus. It contains fluid, ribosomes, the cytoskeleton, and, in eukaryotes, other membrane-bound organelles. The minute “powerplants" in the cytoplasm of cells are called mitochondria

These organelles are roughly the size of many bacteria and can only be seen at higher magnifications in specially prepared slides. Plastids are organelles found in plants and algae. Some organic compounds are produced and stored in plastids. The green pigment, chlorophyll, is located in special photosynthetic plastids called chloroplasts. Other kinds of plastids include chromoplasts, which contain pigments other than chlorophyll, and amyloplasts which store starch.

The central vacuole often occupies a large space within the cytoplasm of plant cells, but may be small or absent in other types of cells. Often, other organelles found in plant cells are located adjacent to the plasma membrane because the central vacuole takes up so much space within the cell. Under healthy conditions for plant cells, the central vacuole is large and produces turgor pressure against the cell wall, which is located outside the cell membrane. The cell wall keeps plant cells from bursting. Some other cells also have cell walls, but they are generally made of different materials. Plant cell walls are made of cellulose, while bacteria have cell walls made of peptidoglycan and fungi have cell walls made of chitin. Archaea and algae also have cell walls made of various compounds.

Discovery of the cell

All organisms are made up of cells, but reaching this conclusion was a long way. The size of most cells is less than the resolving power of the human eye, which is approximately 200 micrometers (0.2 mm). The resolving power is the ability to distinguish two close points. For visualizing cells, it was therefore necessary the invention of devices that allowed higher resolving power than the human eye. This device was the

light microscope (manual chapter 4).

The history of the cell discovery started at the beginning of the 17th century, when the first lenses and the structures to use them for visualizing biological samples were crafted. That is, the light microscope was invented. The modern idea of the cell has been built over the years since, a process strongly connected to the crafting and refinement of light microscope, and therefore to the technology. In this manual you can find some of the most outstanding advances and concepts in the history of the discovery of the cell:

17th Century

1590-1600. A. H. Lippershey, Z. Janssen and H. Janssen (father and son). They are credited with the invention of the first compound microscopes. Two magnifying lenses were placed at each end of a tube. The development of this device and the lens quality improvement would later allow the visualization of cells.

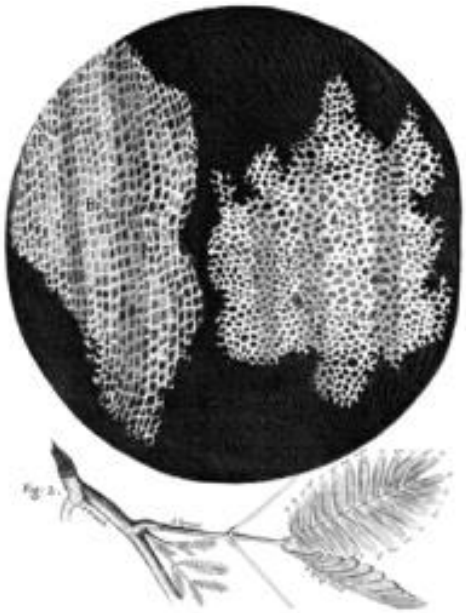


Figure 5.1 Drawing by R. Hooke depicting cork sheets as observed under the light compound microscopy. He named "cell" to each of the small chambers. The whole image looks like a bee honeycomb. Published in *Micrographia*. 1664.

1610. Galileo Galilei described the insect cuticle. He transformed a telescope into a microscope by changing the positions of the lenses. So, he independently invented the compound microscope (he did not know about Janssen's work).

1664. R. Hooke (physicist, meteorologist, biologist, engineer, and architect) published a book entitled *Micrographia*, which describes the first evidence of cells. He studied the cork and observed an arrangement of plant tissue resembling a bee honeycomb (Figure 1). Each of those little compartments was named "cell" (from the Latin “cella”: small room), but he did not guess that these "cells" were the functional units of living beings. However, the word "cell" was used to name first the interior of those little chambers, and thereafter

to name the units in animal tissues.

1670-1680. N. Grew and M. Malpighi studied many species of plants and described a similar microscopic organization for all of them. In the same way as R. Hooke, N. Grew described plant tissues, but the little chambers were regarded as fermentation bubbles (as in bread). He introduced the name parenchyma in plant and made many drawings about the organization of plant tissues. M. Malpighi gave names to many plant structures such as trachea (due to its similarity to the trachea of insects). These authors revealed the detailed microscopic organization of plants.

By that time, the lenses were of very poor quality, with large chromatic aberrations, and microscopists used to put a lot of imagination in their descriptions. In this way, G. d'Agoty saw a fully formed child in the head of a sperm cell, the homunculus.

1670. A. van Leeuwenhoek crafted simple microscopes, with a single lens, but with a perfection that allowed him to reach magnifications higher than 270 times, more than what compound microscopes could offer at that time. He can be regarded as the founder of microbiology because he was the first to report on descriptions of bacteria and unicellular eukaryotes. He observed drops of water, blood, semen, hairs, and many more. Moreover, he thought that all animals are made up of multiple units, but failed to associate these units with the cells of plants. Leeuwenhoek had named cells in liquid samples as "animalcules". Even after his detailed microscopic descriptions, many years passed before animal and plant cells were considered entities similar to the "animalcules".

18th Century

Many improvements in the polishing of microscope lenses during the 18th century allowed much sharper images. The technology of better lens polishing begins in the 18th century and continued during the 19th century. C. M. Hall (1729) is credited with the discovery of a method to minimize the chromatic aberrations of lenses, that is, the dispersion of the light going through the lens. These improvements were first applied to telescopes. F. Beeldsnijder y H. Van Deyl (1791 to 1806) made the first microscope lenses

without chromatic aberrations.

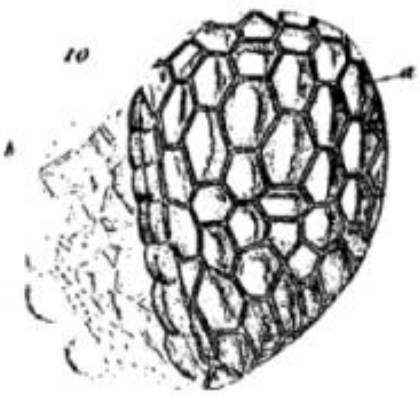
1759. The first attempt to put animals and plants at the same level was made by C. F. Wolf, who said that there is a fundamental unit with globular shape in all living organisms. In his book, *Theoria generationis*, he claimed that living organisms are formed as a result of progressive development and body structures appear by growth and differentiation of less developed structures. This theory was contrary to another very popular theory at that time: the preformationist theory, which suggested that a complete organism is already present inside each gamete, i.e. a fully developed tiny body that would get to the adult stage just by increasing the size of each part.

1792. L. Galvani discovered the electric nature of muscle contraction.

19th Century

In 1812, D. Brewester looks through immersion objectives for the first time. In 1820-1837, G. B. Amici further refined the lenses, removing more chromatic aberrations. He designed objectives with a resolving power and allowing a sharpness non seen before. His designs are currently used in the modern objectives.

1820-1830. The development of the cell theory started in France with H. Milne-Edwards and F. V. Raspail. They observed a large variety of tissues from different animals and concluded that tissues were composed of globular and unevenly distributed units. Plants were also regarded as having these units. In addition, they claimed that these vesicles perform a physiological role. R. J. H. Dutrochet, French too, wrote *if one compares the extreme simplicity of this striking structure, the cell, with the extreme diversity of its content, it is clear that it is the basic unit of an organized entity. Actually, everything is ultimately derived from the cell*. He studied many animals and plants, and came to the conclusion that the plant cells and the globules of animals were the same thing, but with different morphology. He was the first to suggest some physiological functions for these units. F. V. Raspail, a chemist, proposed that each cell is like a laboratory that influences the organization of tissues



and organisms. Finally, he said, previously to R. Virchow, "Omnis cellula e cellula", that is, every cell comes from another cell.

Figure 5.2. Drawing of adipose tissue. *Chemie organique fondé sur des méthodes nouvelles d'observation* by F. V. Raspail (1833).

1831. R. Brown described the cell nucleus. This is rather controversial because in a letter reported by Leeuwenhoek in 1682, he described a rounded structure inside red blood cells of fish. This structure could not be anything else but the nucleus. However, he did not name it. Furthermore, in 1802, F. Bauer, from the Czech Republic, described a cell structure very similar to the cell nucleus.

1832. B. Dumortier described the binary division in plant cells. He reported the synthesis of new cell wall between the two new cells, and proposed that this mechanism was the way cells proliferate. **1835**. R. Wagner described the nucleolus.

1837. J. Purkinje, from Czech Republic, one of the best histologist at that time, suggested the basic tenets of the cell biology theory, claimed that the animal tissues were made up of cells, and that animal tissues can be compared to plant tissues.

1838. M. J. Schleiden, a German botanist, wrote the first tenet of the cell theory for plants (he did not study animal tissues). That is, all plants are made up of units named cells. The German physiologist T. Schwann, in his book *Mikroscopische Untersuchungen*, extended this concept to animals, and later to all living organisms. He went beyond by saying that animals and plants follow the same basic principles.

Although the German school, T. Schwann and M.J. Schleiden, has been credited for the development of the cell theory tenets, there are at least four researchers who wrote similar statements years before: L. Oken (1805), Dutrochet (1824), Purkinje (1834) and G. G. Valentin (1834). R. J. H. Dutrochet stood out in the biology research field (see above).

1839-1843. F. J. F. Meyen, F. Dujardin and M. Barry connected and unified different

fields of biology , and show that individual protozoa are nucleated cells, similar to those that make up animals and plants. In addition, they proposed that continuous cell lineages are the base of life.

1839-1846. 1839-1846. J. E. Purkinge and H. van Mohl independently proposed the name protoplasm for the content (excluding the nucleus and the cell wall) of plant cells. Plant cells showed a "cell membrane" (remember that this "membrane" was the cell wall plus cortical cytoplasm) that could not be observed in animal cells. Comparing plant and animals at the same level was not common in microscopy studies at that time. Then, when plant cells were observed in detail, it was proposed that the living part of the cell was the content of the tiny chambers, that is, the protoplasm. N. Pringsheim (1854) claimed that the protoplasm was the living matter of plant cells. Then, the living force was thought to be contained in the protoplasm in both plant and animal cells.

1856. R. Virchow wrote "The cell, as the simplest form of life-manifestation that nevertheless fully represents the idea of life, is the organic unity, the indivisible living one". By mid-nineteenth century, this theory was widely accepted. **1858**. The use of dyes was a outstanding progress to discern and study the structures in biological samples, tissues and cells, at light microcopy. J. von Gerlach is credited with the first attempts by using carmin dye to study the nervous tissue. **1879**. W. Flemming described the chromosome segregation and proposed the name mitosis. **1899**. C. E. Overton suggested that the interface between the protoplasm and the extracellular space was lipidic. Based on osmosis and lipid diffusion experiments, he proposed the existence of a thin layer of lipids lining the protoplasm.

20th Century

1932. The electron microscope was invented, and it was possible to study cell structures as small as several nanometers (10^{-3} micrometers) (Figure 5). The presence of the plasma membrane, and other membranes within the cell, was confirmed. It was the first time that cell membranes were observed. With the help of the electron microscope, it was shown that the eukaryotic cell were complex and rich in compartments. By 1960, the cell

ultrastructure had already been studied and described.

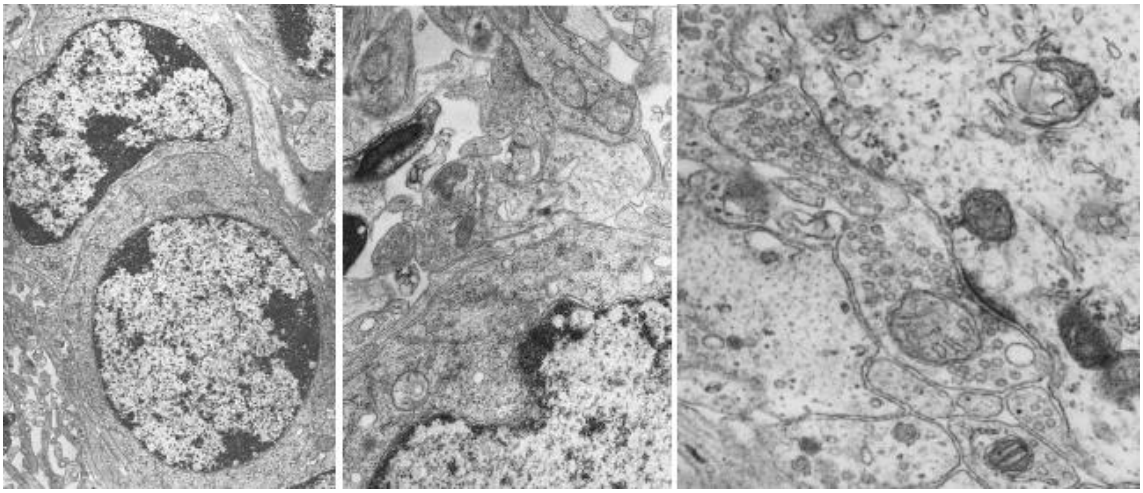


Figure 5.3 Transmission electron microscopy images. The magnification power of these microscopes is shown from the image on the left to the image on the right. The dark gray and black lines, more easily visible in the image on the right, correspond to cell membranes.

Diversity of Cell: Two basic types of cells are classified on the basis of the presence or absence of membrane bound nucleus and cell organelles.

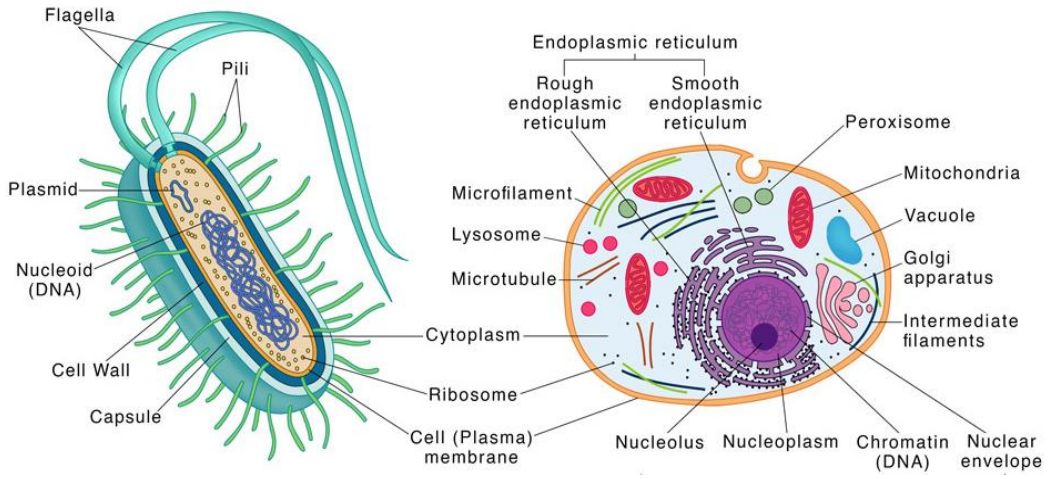


Figure 5.4 Typical prokaryotic cell (A) and Eukaryotic cell (B).

Those cells which lacks membrane bound nucleus and cell organelles such as endoplasmic reticulum, mitochondria. Prokarya (*pro – before, karya – nucleus*) is the superdomain containing Bacteria and Archaea. Prokaryotes do not possess nuclei, however chromosomal DNA is present in a nucleoid region of the cytoplasm. In addition to the genomic DNA, small mobile DNA elements called plasmids may exist in some bacteria that provide additional genetic diversity.

1_____2. _____3_____

4._____5_____ 6_____

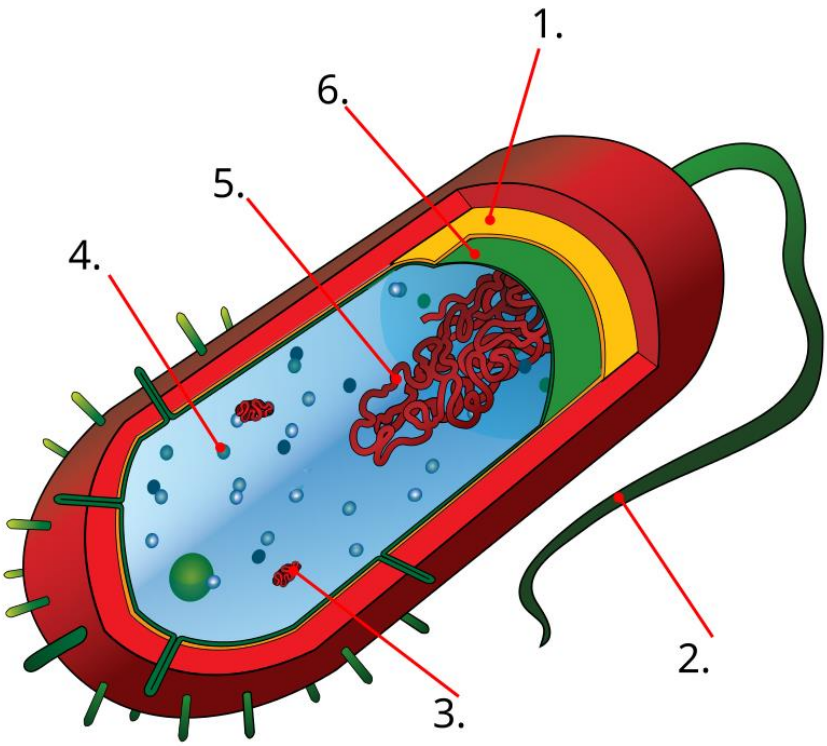


Figure 5.5 Identify the different parts of the bacterial cell and list them on given numbers

Prokaryotic Cells: Bacteria, cyanobacteria and archaea are prokaryotic cells, and which generally lack membrane-bound organelles and nucleus.

Bacteria: Bacteria are microscopic tiny living organisms that have only one cell (**Figure 5.6)**. The word for just one is "bacterium." Millions (if not billions) of different types of bacteria can be found all over the world, including in your body. They're on your skin and in your airways and mouth. They're also in your digestive system, reproductive system and urinary tract. Scientists estimate you have 10 times more bacterial cells than human cells in your body.

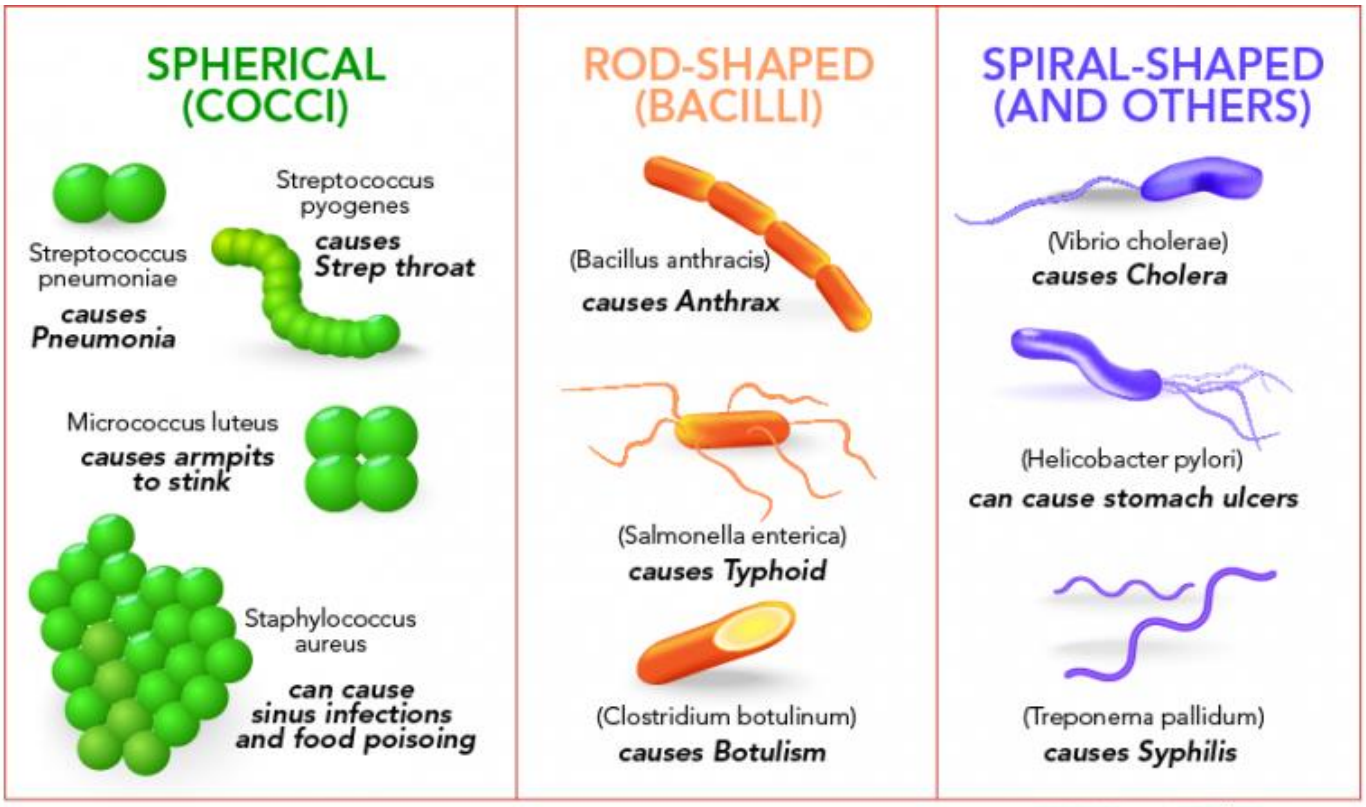


Figure 5.6 Shapes of Bacteria

Materials: prepared Gram stain slides, lens paper, compound microscope

Procedure

1. Using the compound microscope, getting the stained bacteria cells in focus at high power.
2. Examine and draw a few of the bacteria cells.

Specimen_____

Specimen_____

Magnification_____

Magnification_____

Specimen_____

Specimen_____

Magnification_____

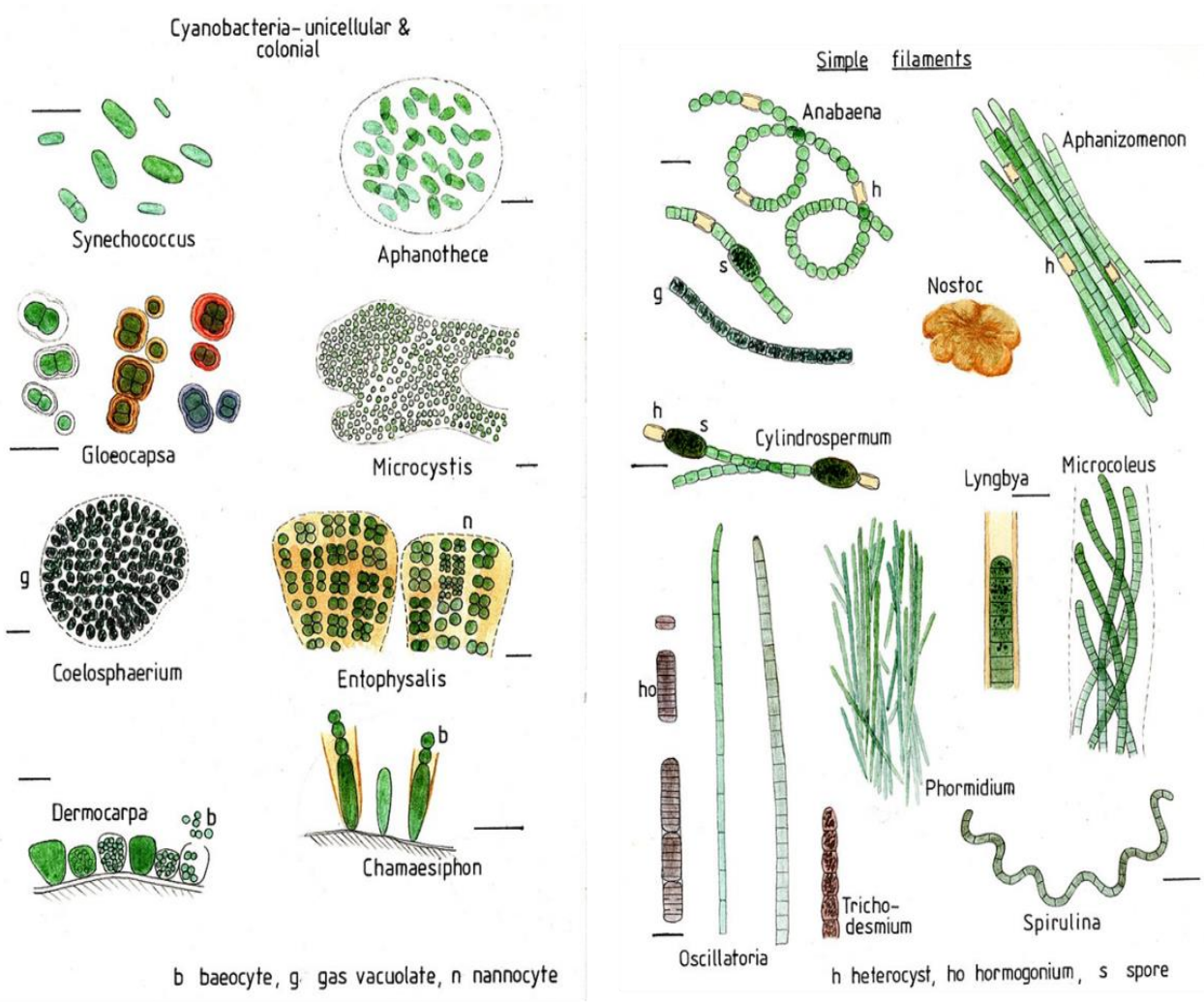
Magnification_____

3. Do you see any details in the cytoplasm of individual bacteria cells?

4. What is the difference in size between the prokaryotic cells and the eukaryotic cells? Which ones in general are bigger and which ones are smaller?

Cyanobacteria: The Cyanobacteria, also referred as blue-green algae, is commonly confused with algae because it shares traits with algae and bacteria. Since cyanobacteria perform photosynthesis through their green chlorophyll-a pigment and contain the bluish pigment, phycocyanin (used for photosynthesis), they are commonly referred to as blue-green algae. However, cyanobacteria is technically a bacteria and not algae because they are toxin producers But the presence of *cyanobacteria* doesn't always pose a risk because not all blooms produce toxins at significant levels. Laboratory testing is the only way to ensure that water is safe. cyanobacteria can grow at increasing rates that results in a bloom. Though they are quite small, they often grow in large colonies which can become the scums and green water that indicate a bloom is present. *Cyanobacteria* are not the only phototrophic bacteria, but they are generally the dominant bacterial phototrophs in the oxygenated portions.

Many *Cyanobacteria* are capable of nitrogen fixation, and thus they can be key players in both the carbon and nitrogen cycles in some systems. Some *Cyanobacteria* are known to produce toxins and form nuisance blooms in eutrophic systems. Freshwater clusters include the *Planktothrix agardhii*, *Aphanizonmenon flos aquae*, and *Microcystis* clusters, and the cluster *Synechococcus* 6b contains both marine and freshwater representatives.



A. Unicellular and colonial cyanobacteria.

B. Simple cyanobacterial filaments

Figure 5.6 Different types of *Cyanobacteria*.

Materials: prepared Gram stain slides, lens paper, compound microscope

Procedure

5. Using the compound microscope, getting the stained *Cyanobacteria* in focus at high power.
6. Draw a few of the *Cyanobacteria* cells (*Nostoc*, *Anabaena* and *Oscillatoria* any two).

Specimen_____

Specimen_____

Magnification_____

Magnification_____

7. Do you see any details in the cytoplasm of individual *Cyanobacteria* cells?

8. What is the difference in size between the prokaryotic cells and the eukaryotic cells? Which ones in general are bigger and which ones are smaller?

Eukaryotic Cell: All organism including animals, plants, fungi and protozoans which possesses a clearly defined nucleus. The eukaryotic cell has a nuclear membrane that surrounds the nucleus, in which the well-defined chromosomes, containing the hereditary material are located. Each eukaryotic cell has a plasma membrane, cytoplasm, a nucleus, ribosomes, mitochondria, peroxisomes, and in some, vacuoles; however, there are some striking differences between animal and plant cells. While both animal and plant cells have microtubule organizing centers

(MTOCs), animal cells also have centrioles associated with the MTOC: a complex called the centrosome. Animal cells each have a centrosome and lysosomes, whereas plant cells do not. Plant cells have a cell wall, chloroplasts and other specialized plastids, and a large central vacuole, whereas animal cells do not.

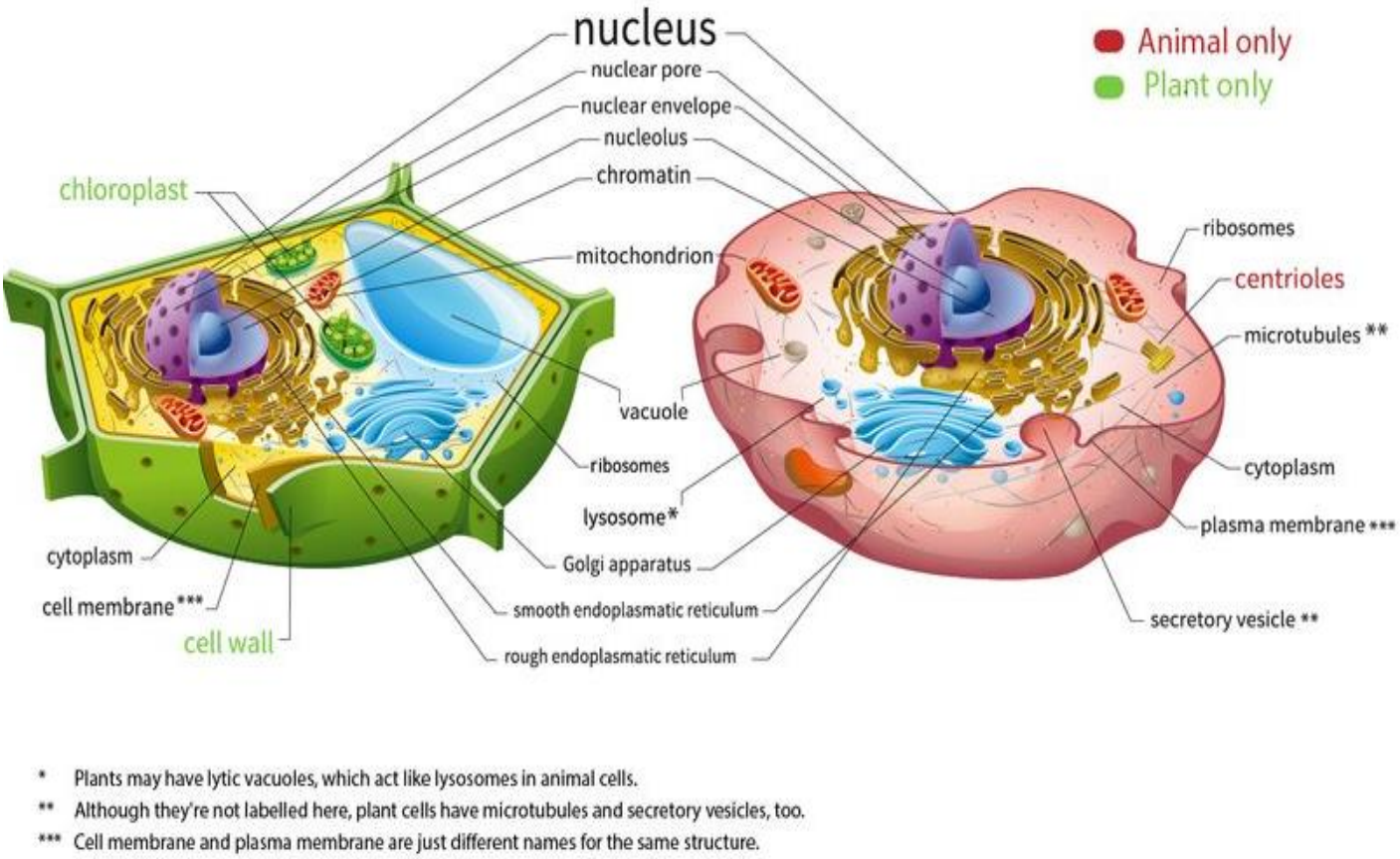


Figure 5.7 Typical structure of plant cell and animal cell

Animal Cells: Animals are eukaryotic, multicellular organisms which obtain their energy from feeding on other organisms or organic materials. Some animals are mobile (move) whereas others are sessile (don't move). They do not photosynthesize and therefore do not possess chloroplasts. They also lack cell wall and central vacuole that plant cells have.

Materials: Any Gram-stained prepared animal cell's slides, lens paper, compound microscope

Procedure

1. Using the compound microscope, getting the stained *Cyanobacteria* in focus at high power.

2. Draw a few of the *Cyanobacteria* cells (*Nostoc*, *Anabaena* and *Oscillatoria* any two).

Specimen_____ Specimen_____

Magnification_____ Magnification_____

Specimen_____ Specimen_____

Magnification_____ Magnification_____

Animal cell

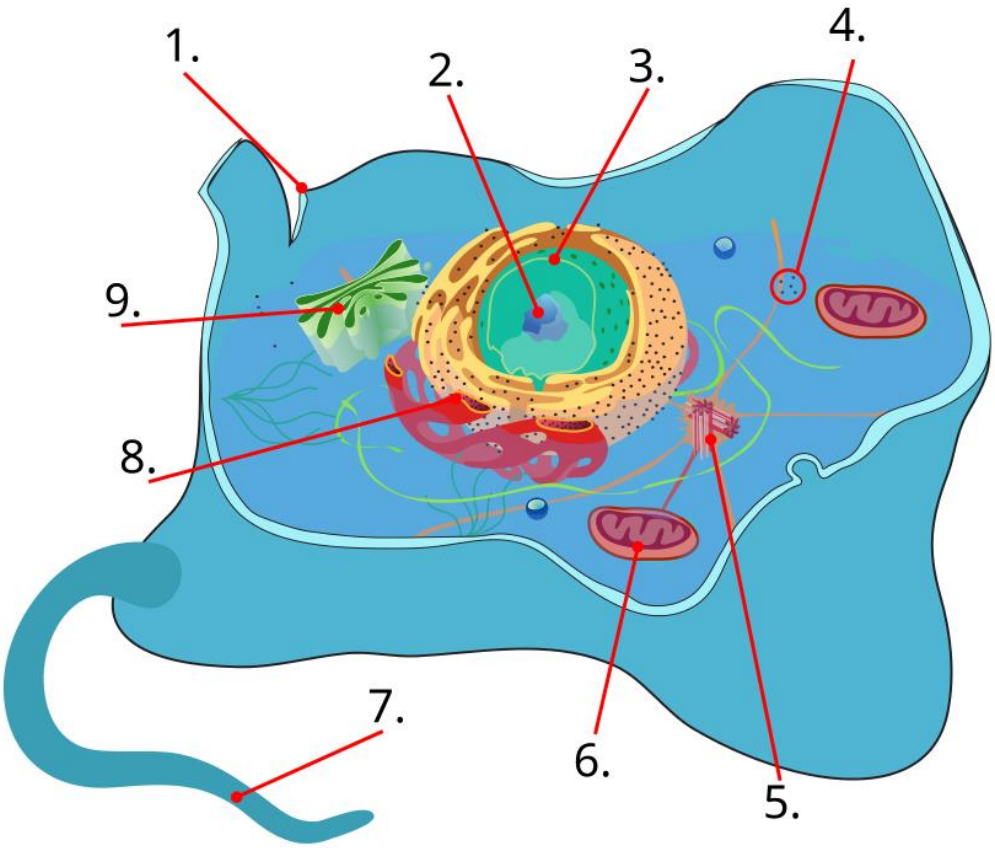


Figure 5.7 Identify the different parts of the animal cell and list them on given numbers.

Plant Cells: Most plants (except parasitic plants) use light as an energy source in a process called photosynthesis. Plants cells contain three unique organelles not found in animal cells: a **cell wall** provides a rigid structure (often cube or rectangular shape), **chloroplasts** performed photosynthesis (transformation of sunlight to glucose), and **central vacuole**, which stores water,

starch, pigments or toxins.

Elodea cells

Make a wet mount of an Elodea leaf with the upper side of the leaf facing up and view under 100X and 400X total magnification and record your results below.

Elodea (total magnification: _____) ***Elodea*** (total magnification: _____)

a. What color are the cells?_____

b. Do you see any chloroplasts?_____.

c. If you do, do you see any movement of the chloroplasts?_____.

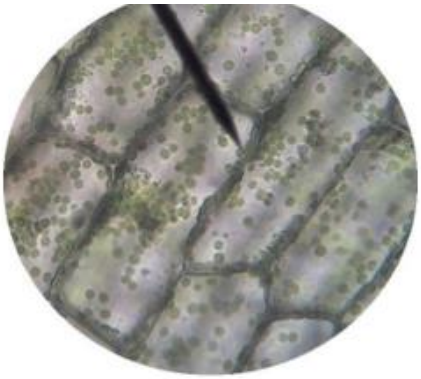
d. Why do you think this might occur?_____

e. Can you determine where the central vacuole is? (Think about the location of the other organelles if a central vacuole is present)

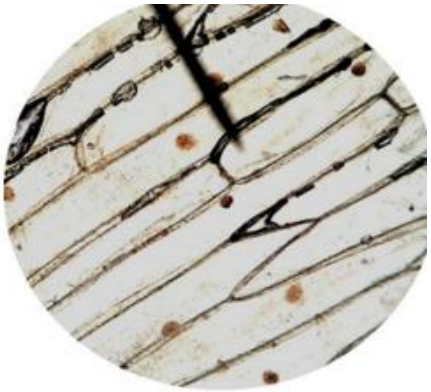
_____

f. Where do you think the pigments are located in the cells? _____

Elodea cells at low power
"Anacharis 40x" by biologycorner, via Flickr



Elodea cells at high power
"Anacharis 400x" by biologycorner, via Flickr



Onion cells at high power
"Onion 100x" by biologycorner, via Flickr

Onion cells

Make a wet mount of an onion and view under 100X and 400X total magnification (see "B. Procedure: Scan your specimen; Move to a higher power" above) and record your results below.

(total magnification: _____)

(total magnification: _____)

a. What color are the cells?_____

b. Do you see any chloroplasts?_____.

c. If you do, do you see any movement of the chloroplasts?_____.

d. Why do you think this might occur?_____

e. Can you determine where the central vacuole is? (Think about the location of the other organelles if a central vacuole is present)

_____

f. Where do you think the pigments are located in the cells? _____

Plant cell

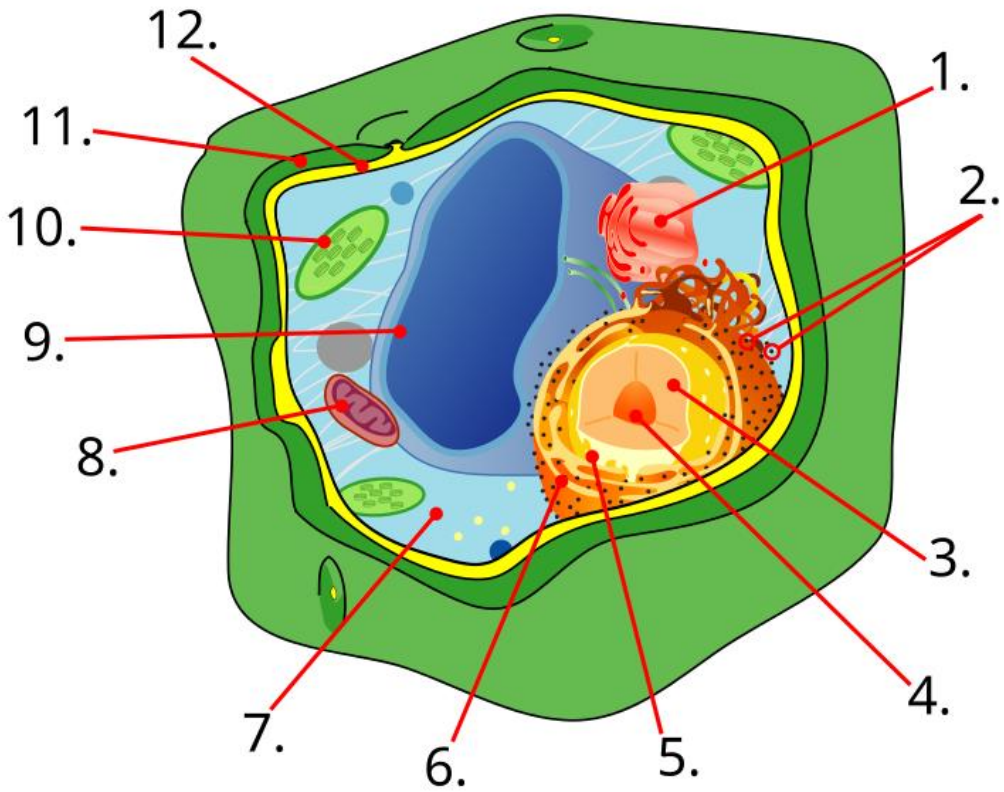


Figure 5.7 Identify the different parts of the plant cell and list them on given numbers

Storage of the Microscope

1. Turn off the light switch and remove the slide and reposition the slide mount arm so that it does not extend out past the stage.
2. Turn the revolving nosepiece to the lowest objective or to no objective.
3. Bring the stage down using the coarse adjustment knob.
4. Carefully unplug the cord, wrap it around itself and place it next to the base of the microscope. Do not wrap the cord around the base of the microscope or insert the wrapped cord under the stage.
5. Cover the microscope with the bag and place the microscope in its correct, numbered spot in the cabinet with the arm of the microscope facing out.

Summary Table:

Organism	Prokaryotic or Eukaryotic	Single-celled, colonial, or multicellular	Autotrophic or Heterotrohic
Bacteria			
Protist			
Plant			
Animal			

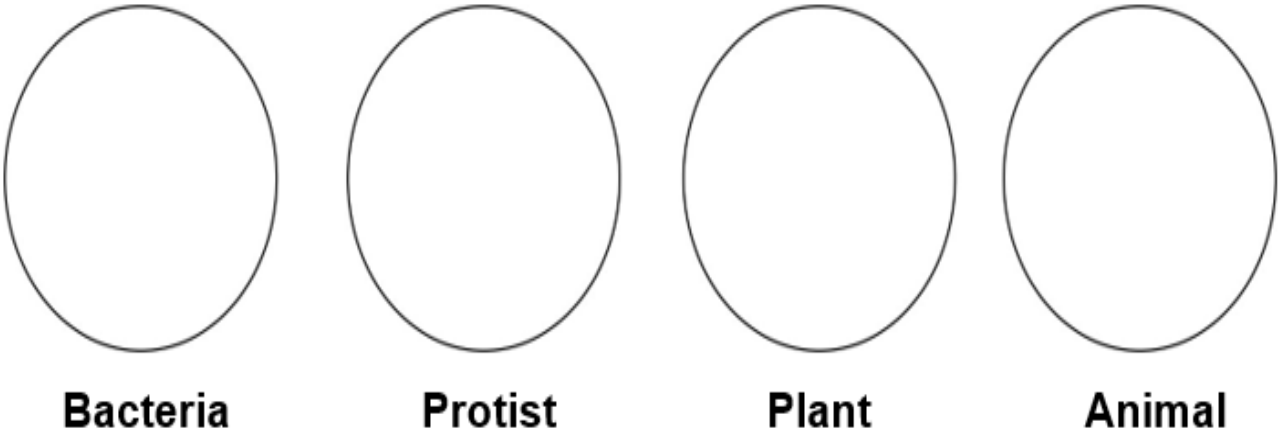


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Figure 5.7 Draw the given specimen that you observed under microscope at 40 x Compare the sizes of bacterial, protist, plant, and animal cells by drawing a simple sketch in the fields of view provided SPECIMEN

Chapter 6: Understanding of Physiological Phenomenon: Diffusion and Osmosis

Learning Outcomes: At the end, after the completion of the current lab, the student should be able to:

- To define the following terms: diffusion, osmosis, equilibrium, tonicity, turgor pressure, plasmolysis
- To understand Brownian motion and its relationship to molecular movement
- list the factors that may affect the speed of simple diffusion
- To list which molecules, in general, can freely diffuse across the plasma membrane of a cell
- To describe what drives osmosis (why do water molecules move?)
- To explain why water moves out of a cell when the cell is placed in a hypertonic solution
- To explain why water moves into a cell when the cell is placed in a hypotonic solution
- To describe what physically happens to a cell if water leaves the cell
- To describe what physically happens to a cell if water enters the cell

Introduction

Diffusion and osmosis are somewhat difficult-to-understand scientific concepts that are often best explained through lab activities. In diffusion, matter is dispersed in a way that makes it achieve equal concentration throughout the environment, moving from a higher concentration to a lower concentration. In any of the biological system, substances often move through solution and across membranes in a predictable direction. The passive directional movement of molecules is **diffusion** (**Figure 6. 1** A and B, before and after diffusion, respectively). In osmosis, liquid is diffused through a semi-permeable membrane.

Understanding the concepts of diffusion and osmosis is critical for conceptualizing how substances move across cell membranes. **Diffusion** can occur across a semipermeable membrane; however diffusion also occurs where no barrier (or membrane) is present. A number of factors can affect the rate of diffusion, including temperature, molecular weight, concentration gradient, electrical charge, and distance. Water can also move by the same mechanism. This diffusion of solvent (water) is called **osmosis** (**Figure 6. 1** C and D, before and after osmosis, respectively).

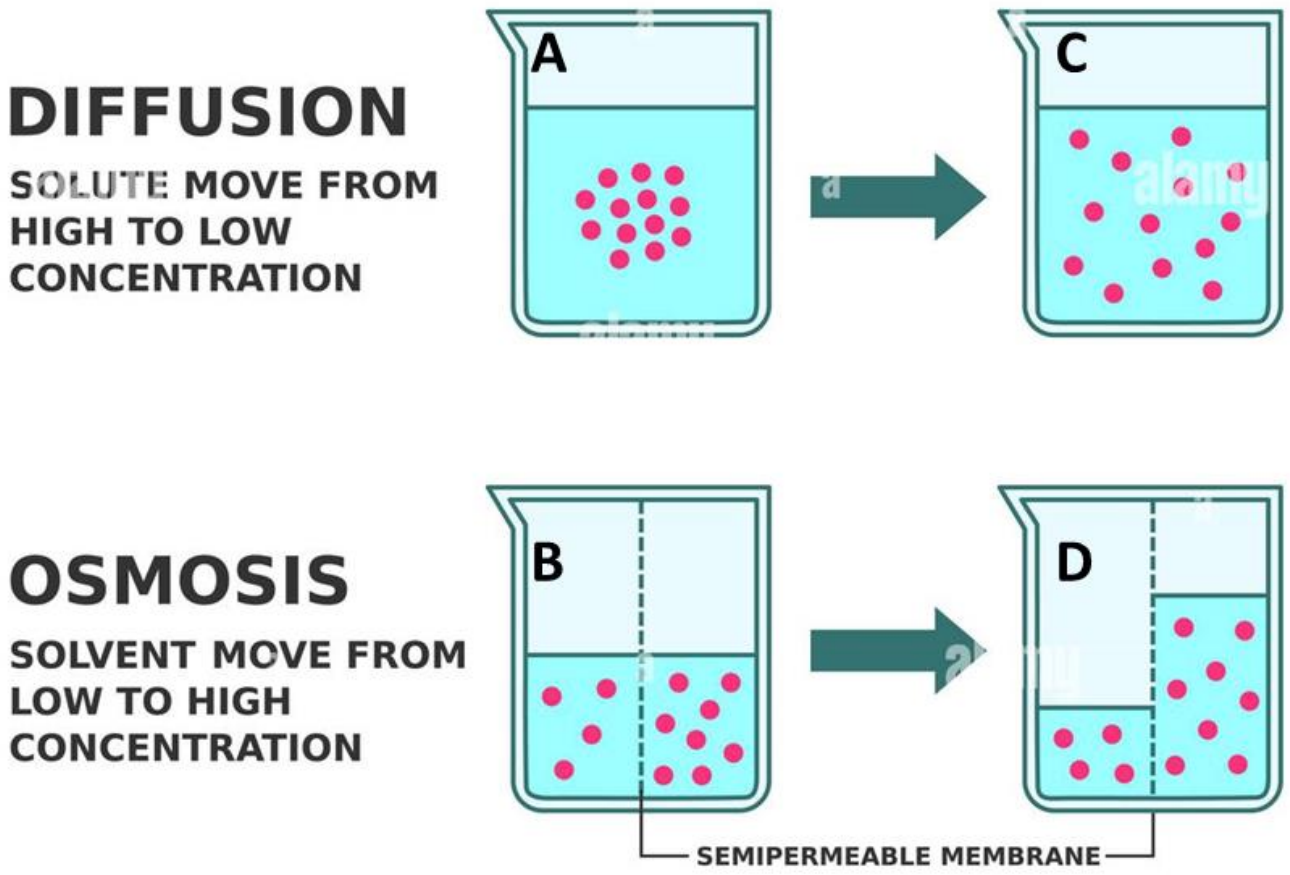


Figure 6. 1 Diffusion vs osmosis

The cell membrane plays the dual roles of protecting the living cell by acting as a barrier to the outsideorld, yet at the same time it must allow the passage of food and waste products into and

out of the cell for metabolism to proceed. How does the cell carry out these seemingly paradoxical roles? To understand this process you need to understand the makeup of the cell membrane and an important phenomenon known as diffusion.

Diffusion is the movement of a substance from an area of high concentration to an area of low concentration due to random molecular motion. All atoms and molecules possess kinetic energy, which is the energy of movement. It is this kinetic energy that makes each atom or molecule vibrate and move around. (In fact, you can quantify the kinetic energy of the atoms/molecules in a substance by measuring its temperature.) The moving atoms bounce off each other, like bumper cars in a carnival ride. The movement of particles due to this energy is called Brownian motion. As these atoms/molecules bounce off each other, the result is the movement of these particles from an area of high concentration to an area of low concentration. *This* is diffusion. The rate of diffusion is influenced by both temperature (how fast the particles move) and size (how big they are).

In the present lab, we will explore the processes of diffusion and osmosis. We will examine the effects of movement across membranes in dialysis tubing, by definition, a semi-permeable membrane made of cellulose. We will also examine these principles in living plant cells like potato tissue and *Elodia* leaf cell under different concentration of salt solutions (Instructor will provide different concentrations solution.

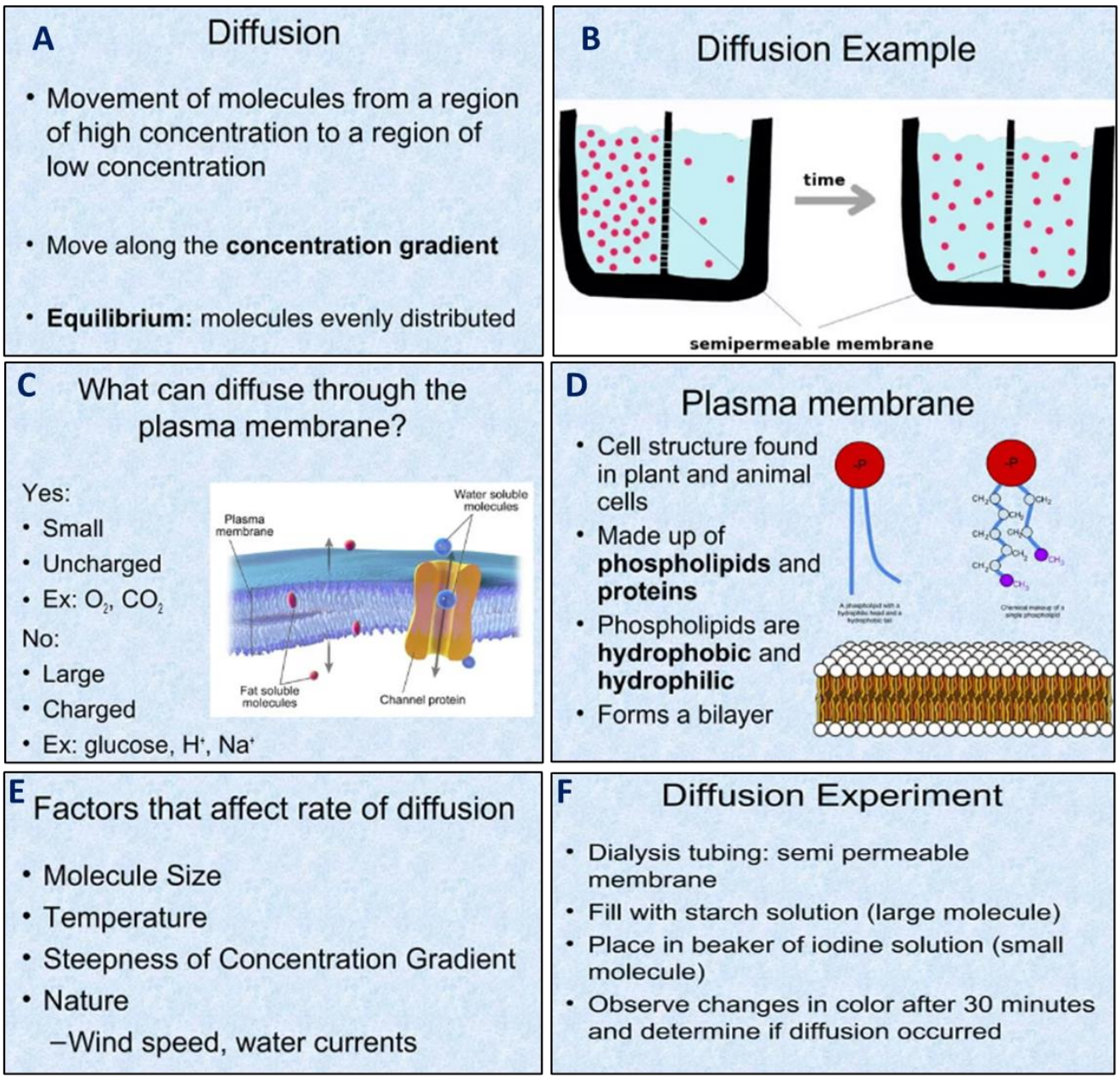


Figure 6.2 Summary of Diffusion and factors that affect the rate of diffusion. Osmosis and diffusion are chemical phenomena occurring due to differences in concentration. The particles move from a higher concentration region to a low concentration region in diffusion. In contrast, in osmosis, the particles move from a lower concentration region to a high concentration region.

Diffusion:

Figure 6.3 Diffusion of dye in water from region of greater concentration to region of leaser concentration.

How long did it take for dye to diffuse equally in water inside the beaker?

_____

_____

Diffusion Across a Semi-Permeable Membrane: Dialysis

Materials: 250 ml beaker, 25 ml graduated cylinder, cornstarch solution, iodine solution, tap water, dialysis tubing bag, string, timing device (your mobile), ruler, paper towels

Procedure

1. Cut a piece of dialysis tubing, approximately 10 cm.
2. Soak the dialysis tubing for about 5 minutes prior to using.
3. Tie off one end of the tubing with dental floss or rope
4. Use a pipette and fill the bag with a 1% starch solution leaving enough room to tie the other end of the tubing.
5. Tie the other end of the tubing closed with dental floss or rope
6. Fill a 250 mL beaker with distilled water.
7. Add Lugol's iodine to the distilled water in the beaker until the water is a uniform pale yellow color.
8. Place the dialysis tubing bag in the beaker.

9. The molecular formula for Lugol's solution is I2KI (atomic mass = 127). Starch consists of long chains of glucose (atomic mass of each glucose = 180). Iodine turns a deep blue in the presence of starch***. Formulate a hypothesis for each of the following. Remember to provide a reasonable explanation for your predictions.***

10. The movement of starch

11. The movement of iodine

12. The color of the solution in the bag after 30 minutes

13. The color of the solution in the beaker after 30 minutes

14. Add the dialysis bag to the beaker and allow the experiment to run for 30 minutes. Record the colors of both the dialysis bag and the beaker.

Lab Activities:

Tea Bag Activity: An uncomplicated diffusion and osmosis activity can be completed using a tea bag, warm water and a preferably clear container. Simply place the tea bag and warm water in the container and observe what happens. Ask students to explain what happens using the proper vocabulary. Essentially, water flows (osmosis) through the tea bag (the permeable membrane) and the tea leaves dissolve (diffuse) throughout the water, turning the water brown. The water also flows into the tea bag, seeking equal concentration.

Kitchen food smell: The smell of food cooked in the kitchen diffuses in the entire house is an example of diffusion that we encounter in everyday life. Go and feel

Perfume Activity: Moreover, perfume fragrance also spreads throughout the room. It is also an example of diffusion. Spread in the classroom and not the time to get fragrance in your classroom seat.

How long did it take for tea to diffuse from tea bag in cold water and Hot water?

Cold Water_____

Hot Water_____

Process of Plasmolysis—Observing Osmosis in a Living System, *Elodea* leaf

If a plant cell is immersed in a solution that has a higher solute concentration than that of the cell, water will leave/enter (circle one) the cell. The loss of water from the cell will cause the cell to lose the pressure exerted by the fluid in the plant cell's vacuole, which is called turgor pressure. Macroscopically, you can see the effects of loss of turgor in wilted houseplants or limp lettuce. Microscopically, increased loss of water and loss of turgor become visible as a withdrawal of the protoplast from the cell wall **(plasmolysis)** and as a decrease in the size of the vacuole (**Figure 6.4**).

Procedure

1. Obtain a leaf from the tip of an *Elodea* Place it in a drop of water on a slide, cover it with a coverslip, and examine the material first at scanning, then low power objective and then at high power objective.

2. Locate a region of health. Note the location of the chloroplasts. **Sketch a few cells.** For the next step, **DO NOT move the slide**.

3. While touching one corner of the coverslip with a piece of Kimwipe to draw off the water, add a drop of 40% salt solution to the opposite corner of the coverslip. **Do this simultaneously.** Be sure that the salt solution moves under the coverslip. Wait about 5 minutes, then examine as before. *Sketch these cells next to your sketch of cells in step two, note the location of the chloroplasts. Label it **40% salt solution**.*

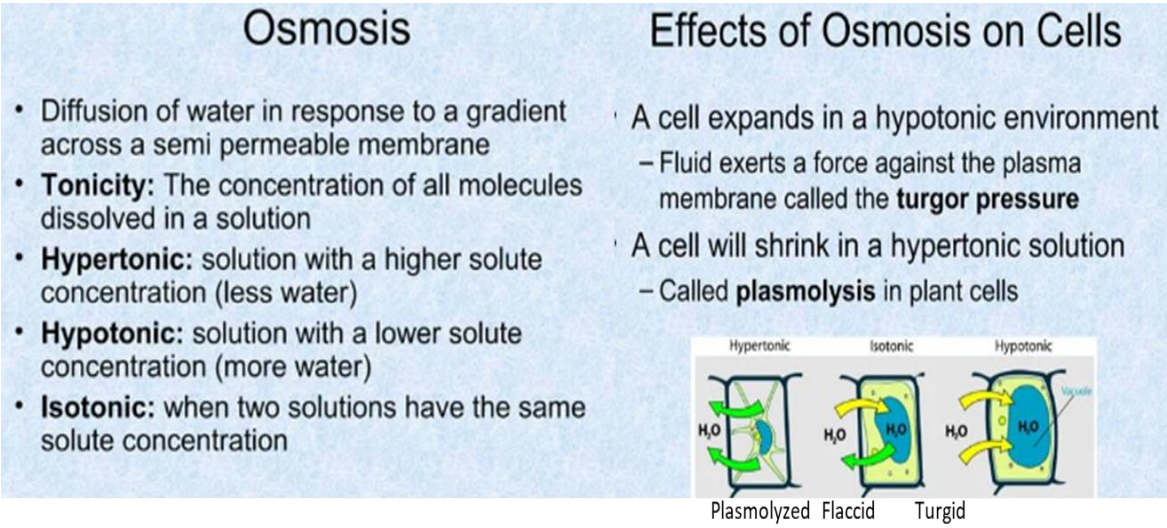


Figure 6. 4 Summary of Osmosis and factors that affect the rate of osmosis.

Lab Questions

1. What happened to the cells in the salt solution?
2. Assuming that the cells have not been killed, what should happen if the salt solution were to be replaced by water?
3. Are plant cells normally hypertonic, hypotonic, or isotonic to their environment? Why?
4. Can plant cells burst? Explain.

Overall Conclusions

1. Review your hypothesis for each experiment. Was your original hypothesis supported or rejected for each experiment. Explain why or why not. This should be based on the best information collected from the experiment. Explain how you arrived at this conclusion.
2. If it was incorrect, give the correct answer, again based on the best information collected from the experiment.

1. Identify and explain two things that people may have done incorrectly that would have caused them to get different answers from the rest of the class. Be **specific**.

Q1. The passage of water across a selectively permeable membrane is known as

(a) Osmosis (b) Diffusion (c) Facilitated diffusion (d) None of the above

Q2. Which of the following solutions contains a low solute concentration relative to another solution?

(a) Hypotonic solution (b) Isotonic solution (c) Hypertonic solution (d) None of them

Q3. What is osmosis?

_____

Q4. What is Diffusion?

_____

Q5. What are isotonic, hypertonic and hypotonic solutions?

Isotonic
Solution:_____
__

Hypertonic
Solution:_____

Hypotonic
Solutio:_____

Q6. Give some examples of osmosis that we encounter in everyday life?

Example 1

Example 2

Example 3

Match the followings:

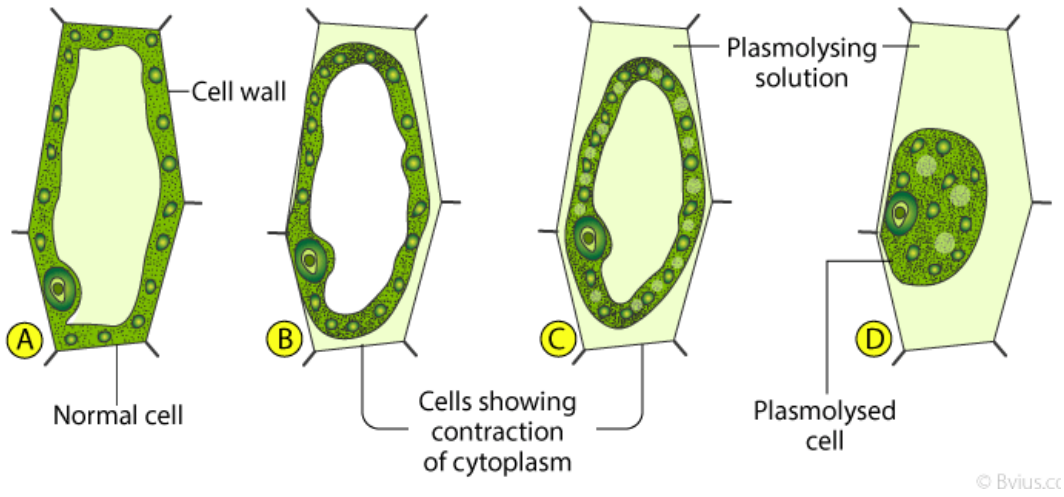
Column I	Column II
Diffusion	Hydrophite substances.
Osmosis	Shrinkage protoplasm.
Imbibition	Semipermeable membrane.
Plasmolysis	Free movement of ions and gases.

Plasmolysis

Plasmolysis is the process during, which cells lose water when placed in a hypertonic solution, that is greater in the concentration of solutes compared with the inside of a cell. During plasmolysis, the organelles inside the cell shrink away from the cell wall, which results in severe water loss and leads to the collapse of the cell wall and finally results in cell death.

Osmosis is primarily responsible for the occurrence of plasmolysis. There are three stages in plasmolysis. Based on certain criteria, plasmolysis can be classified into two different types:

- Concave plasmolysis – It is a reversible process.
- Convex plasmolysis- It is an irreversible process.\



Which of the following of the plant cell has hypotonic solution.

A

B

C

D

Give your explanation why do you choose option as correct answer.

Chapter 7: Understanding of Photosynthesis: Leaf structure, Stomata and Guard cells, plant pigments, Oxygen production

Learning Outcomes: At the end, after the completion of the current lab, the student should be able to:

- To describe photosynthesis, including the substrates and products of the light dependent and light independent reactions (also called the dark reaction).
- To understand the morphology and structure of the leaf
- To understand the stomatal activities under dark condition and light condition
- To understand the basic principle of the chromatography and to describe the plant pigments role during photosynthesis.
- To design an experiment to determine factors that affect photosynthesis in terms of oxygen production under light and dark condition

In the present lab, we will study the structure of leaf, stomatal activities under dark condition and illumination condition; and the measure of the photosynthesis in terms of oxygen released during photosynthesis. Also, plant pigments analysis will be performed by paper chromatography.

Photosynthesis is a process used by plants and other organisms to convert light energy into chemical energy that can later be released to fuel the organisms' activities (energy transformation). This chemical energy is stored in carbohydrate molecules, such as sugars, which are synthesized from carbon dioxide and water - hence the name photosynthesis, from the Greek phōs, "light", and synthesis, "putting together". In most cases, oxygen is also released as a waste product. Most plants, most algae, and cyanobacteria perform photosynthesis; such organisms are called photoautotrophs. Photosynthesis is largely responsible for producing and maintaining the

oxygen content of the Earth's atmosphere, and supplies all of the organic compounds and most of the energy necessary for life on Earth.

Although photosynthesis is performed differently by different species, the process always begins when energy from light is absorbed by proteins called reaction centers that contain green chlorophyll pigments. In plants, these proteins are held inside organelles called chloroplasts, which are most abundant in leaf cells, while in bacteria they are embedded in the plasma membrane. In these light-dependent reactions, some energy is used to strip electrons from suitable substances, such as water, producing oxygen gas. The hydrogen freed by the splitting of water is used in the creation of two further compounds that act as an immediate energy storage means: reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP), the "energy currency" of cells.

In plants, algae and cyanobacteria, long-term energy storage in the form of sugars is produced by a subsequent sequence of light-independent reactions called the Calvin cycle; some bacteria use different mechanisms, such as the reverse Krebs cycle, to achieve the same end. In the Calvin cycle, atmospheric carbon dioxide is incorporated into already existing organic carbon compounds, such as ribulose bisphosphate (RuBP). Using the ATP and NADPH produced by the light-dependent reactions, the resulting compounds are then reduced and removed to form further carbohydrates, such as glucose.

The first photosynthetic organisms probably evolved early in the evolutionary history of life and most likely used reducing agents such as hydrogen or hydrogen sulfide, rather than water, as sources of electrons. Cyanobacteria appeared later; the excess oxygen they produced contributed directly to the oxygenation of the Earth, which rendered the evolution of complex life possible. Today, the average rate of energy capture by photosynthesis globally is approximately 130 terawatts which is about three times the current power consumption of human civilization. Photosynthetic organisms also convert around 100-115 thousand million metric tons of carbon into biomass per year.

The main source of light on Earth is the Sun. Sunlight provides the energy that green plants use to create sugars mostly in the form of starches, which release energy into the living things that

digest them. This process of photosynthesis provides virtually all the energy used by living things. The primary properties of visible light are intensity, propagation direction, frequency or wavelength spectrum, and polarization, while its speed in a vacuum, 299,792,458 meters per second, is one of the fundamental constants of nature. Visible light, as with all types of electromagnetic radiation (EMR), is experimentally found to always move at this speed in a vacuum.

Intensity of light

Light is electromagnetic radiation within a certain portion of the electromagnetic spectrum (Figure). The word usually refers to visible light, which is visible to the human eye and is responsible for the sense of sight. Visible light is usually defined as having wavelengths in the range of 400-700 nanometres (nm), or 400×10^{-9} to 700×10^{-9} m, between the infrared (with longer wavelengths) and the ultraviolet (with shorter wavelengths). This wavelength means a frequency range of roughly 430-750 terahertz (THz).

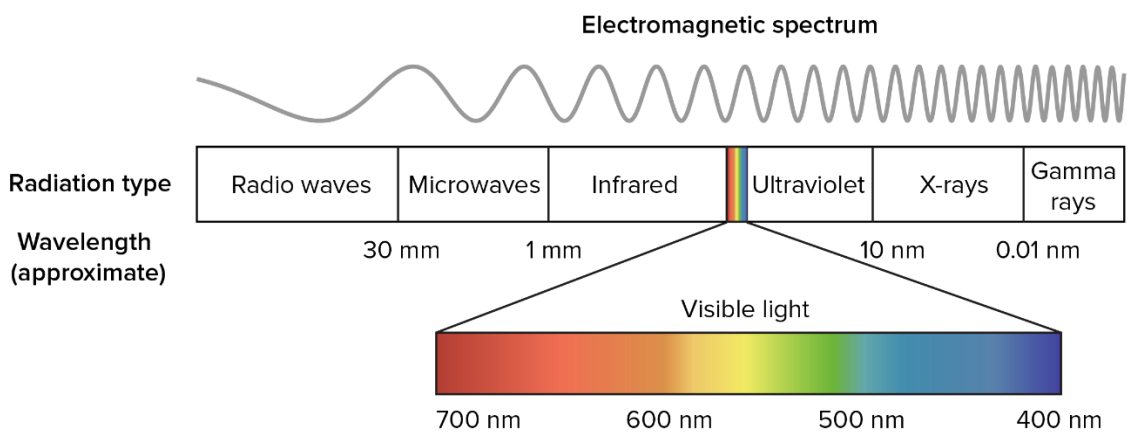


Figure 7.1: **Electromagnetic spectrum.**

Every electromagnetic wave has a particular **wavelength**, or distance from one crest to the next, and different types of radiation have different characteristic ranges of wavelengths (as shown in the diagram below). Types of radiation with long wavelengths, such as radio waves, carry less energy than types of radiation with short wavelengths, such as X-rays.

The **visible spectrum** is the only part of the electromagnetic spectrum that can be seen by the human eye. It includes electromagnetic radiation whose wavelength is between about 400 nm and 700 nm. Visible light from the sun appears white, but it's actually made up of multiple wavelengths (colors) of light. You can see these different colors when white light passes through a prism: because the different wavelengths of light are bent at different angles as they pass through the prism, they spread out and form what we see as a rainbow. Red light has the longest wavelength and the least energy, while violet light has the shortest wavelength and the most energy.

In this experiment (Figure), we will study the effect of light intensity on the photosynthetic activity of *Elodea* will vary the light intensity by changing the distance between the light source and the plant. We will count the emerging oxygen bubbles as an indicator of the photosynthetic activity of the plant or oxygen evolution under sun light (high light) and low light intensity (Shate)

Pigments absorb light used in photosynthesis

In photosynthesis, the sun's energy is converted to chemical energy by photosynthetic organisms. However, the various wavelengths in sunlight are not all used equally in photosynthesis. Instead, photosynthetic organisms contain light-absorbing molecules called **pigments** that absorb only specific wavelengths of visible light, while reflecting others.

The set of wavelengths absorbed by a pigment is its **absorption spectrum**. In the diagram below, you can see the absorption spectra of three key pigments in photosynthesis: chlorophyll *a*, chlorophyll *b*, and β-carotene. The set of wavelengths that a pigment doesn't absorb are reflected, and the reflected light is what we see as color. For instance, plants appear green to us because they contain many chlorophyll *a* and *b* molecules, which reflect green light.

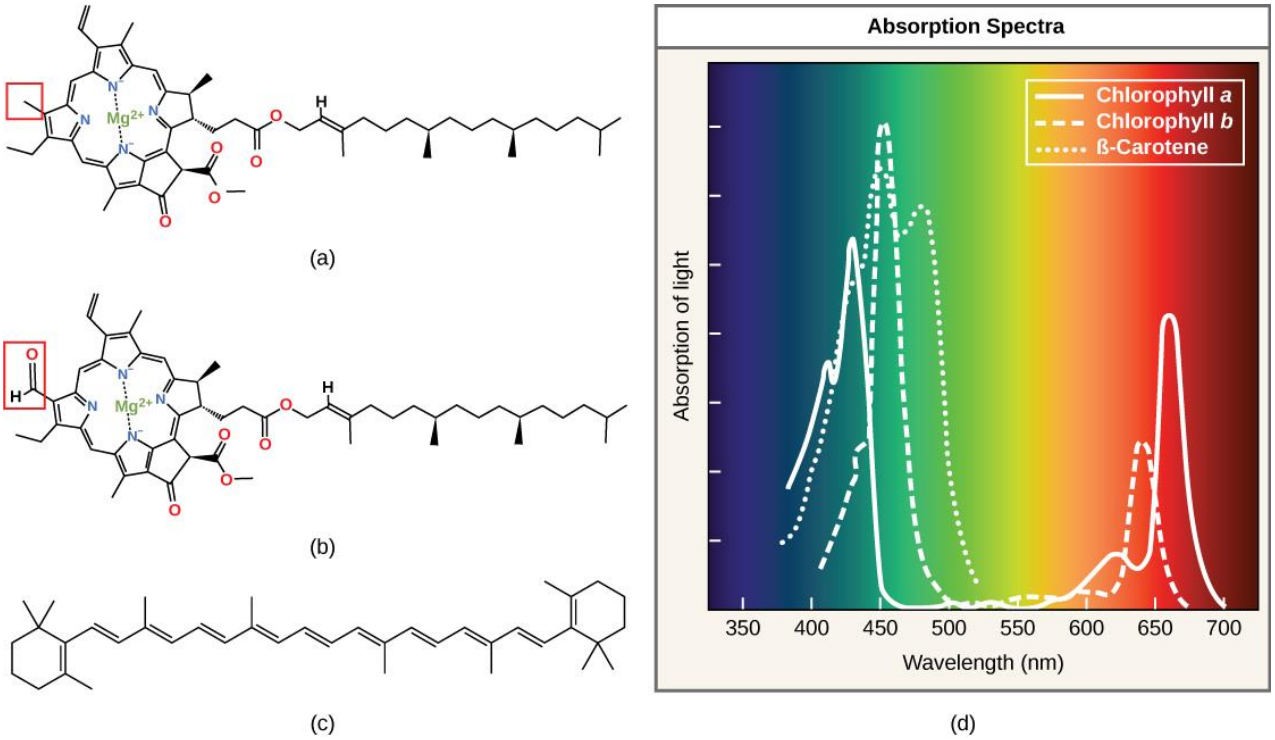


Figure 7. 2: Wavelengths of light and photosynthetic pigment light absorption zones. (a) Chlorophyll *a*, (b) chlorophyll *b*, and (c) *β*-carotene are hydrophobic organic pigments found in the thylakoid membrane. Chlorophyll *a* and *b*, which are identical except for the part indicated in the red box, are responsible for the green color of leaves. *β*-carotene is responsible for the orange color in carrots. Each pigment has (d) a unique absorbance spectrum.

Leaf Structure: Leaves are the most conspicuous part of a plant and come in many different size., shapes, and even color. There are over a thousand different types of leaves. We can sort these leaves into groups based on their shape and structure. Some monocot and dicot plant leaves and stick them on the empty page of the manual. You are going to use science skills to observe the leaves and compare to put them into groups based on simple properties. Many leaves are differentiated into a blade (flattened, expanded portion) and petiole (i.e., "stalk"; a petiolate leaf has a petiole, a sessile leaf lacks one). Stipules (a small pair of appendages) may also be present at the base of a leaf or seemingly on the stem at the node – the stipules are always considered part of the leaf, however (the leaf is stipulate when present, exstipulate when lacking). Whereas most generally attach to the stem on one side, the leaves of many monocot angiosperms (e.g., grasses, lilies, orchids, aloes) lack a true petiole and have a sheathing leaf base (i.e., the leaf base

sheathes the stem for some distance) that is attached to the stem in a circular manner, completely around the node. In this latter case of circular leaf bases, one would find that when such leaves fall off, they leaf circular leaf scars.

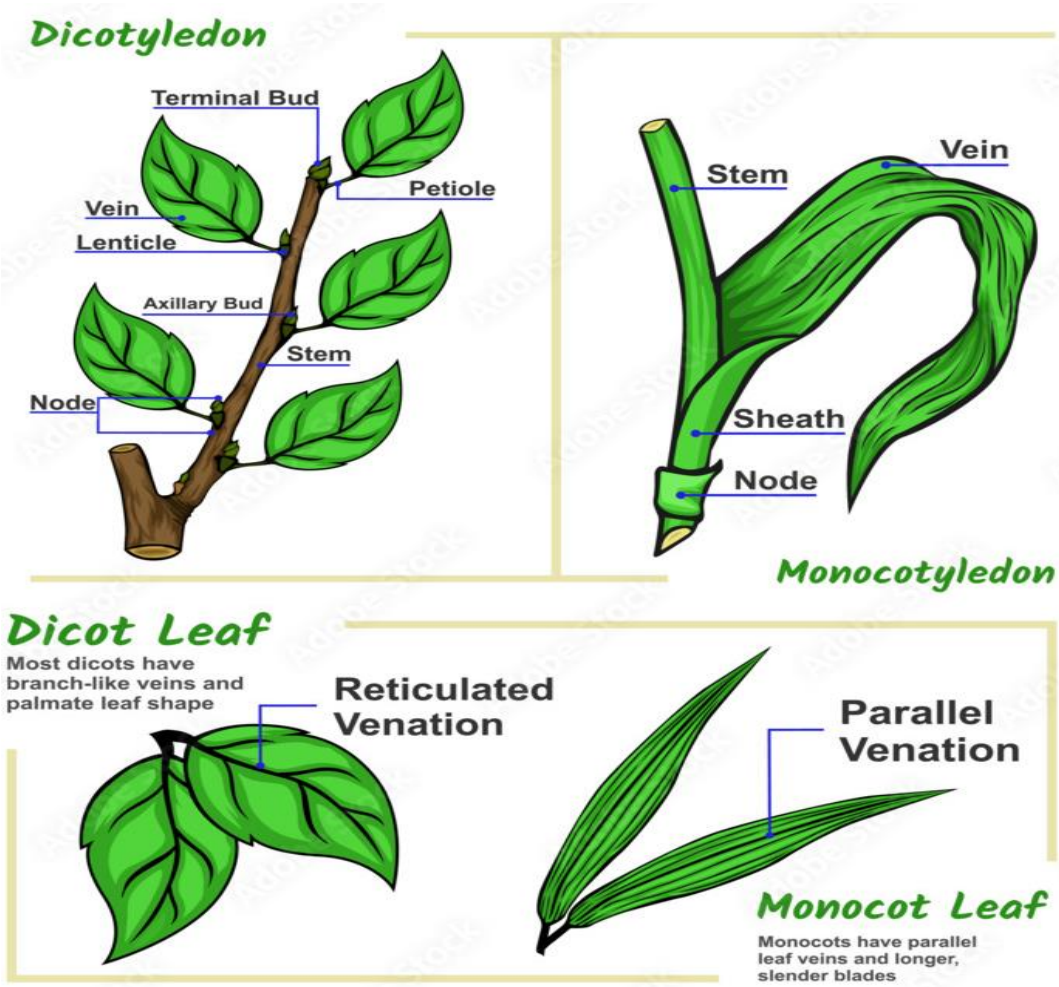


Figure 7.3 Parts of leaf and representation of simple and compound leaves

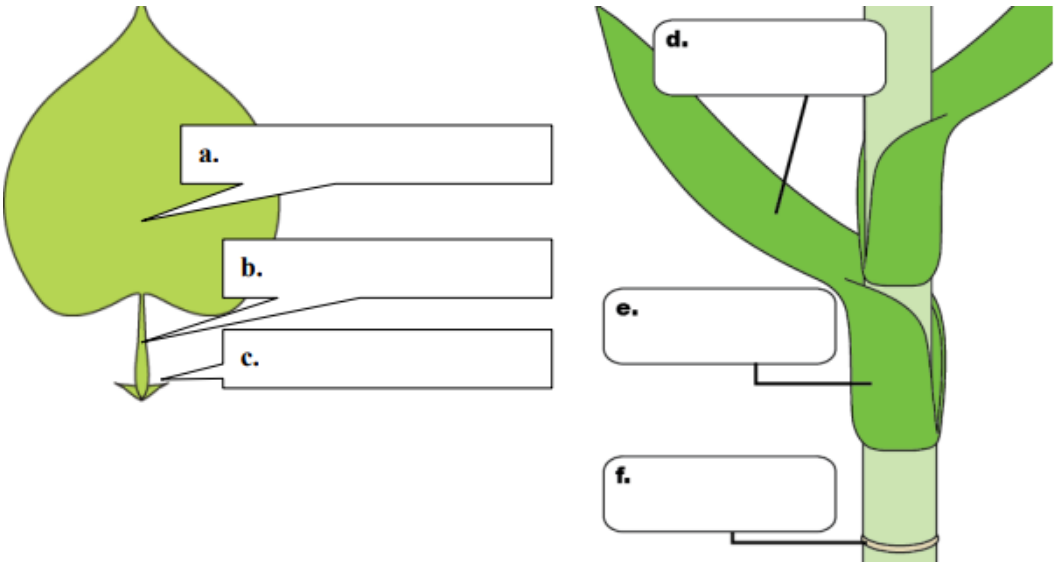


Figure 7.4 Parts of leaf and representation of simple and compound leaves

Q1: Palm trees have parallel veins, long leaves, vascular tissue, and flowers with groups of three petals. Based on this information, palm trees can best be classified as:

a. Angiosperm

b. Gymnosperm

c. Monocot

d. Both “b” and “c”

Q2: Write any three differences between monocot and dicot plants.

Activities:

Collect two monocots and two dicot plants and draw both types below.

Draw monocot 1

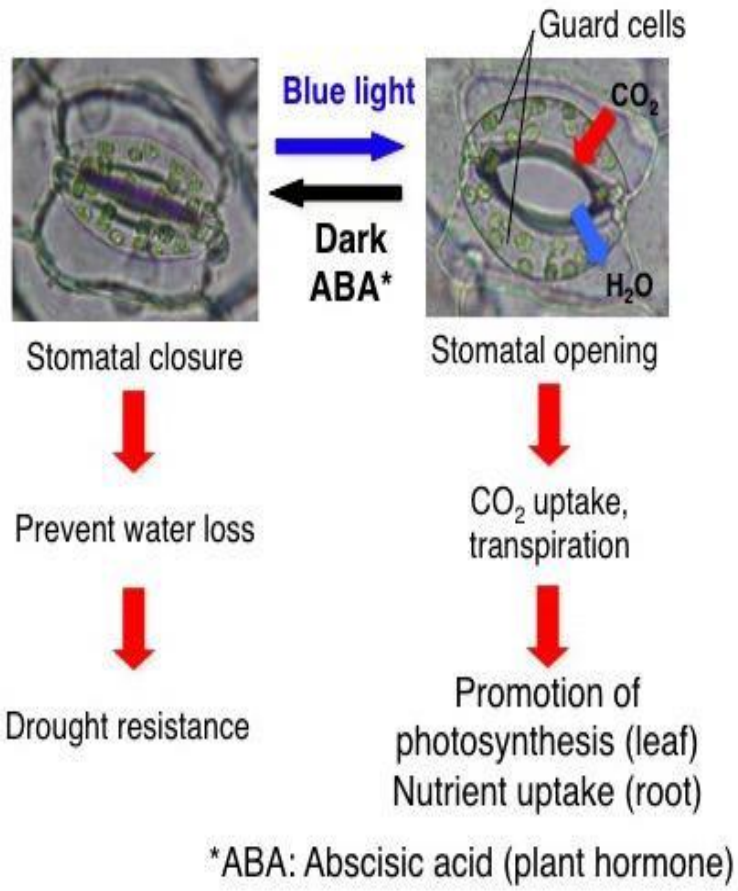
Draw monocot 2

Draw dicot 1

Draw dicot 2

Stomatal activities:

In order to provide the cells of the leaf with sufficient CO2 and to allow for the escape of O2, the bottom side of the leaves contain countless openings, the stomata. Each stoma can be opened and closed by the expanding and contracting action of two guard cells. Leaf stomata are the principal means of gas exchange in vascular plants. *Stomata* are small pores, typically on the undersides of leaves, that are opened or closed under the control of a pair of banana-shaped cells called *guard cells*. When open, stomata allow CO2 to enter the leaf, and allow for water and oxygen to escape. In addition to opening and closing the stomata, plants may exert control over their gas exchange rates by varying stomata density in new leaves when they are produced (such as in the spring or summer). The more stomata per unit area is the *stomata density*.Why might it be adaptive for a plant to control its rates of water loss and CO2 uptake? One answer can be found in the sun. Increases in heat could denature proteins of membranes of chloroplasts. When overheated, plants may open the stomata to evaporate water to lower the temperature. Based on this, you could hypothesize that leaves in the sun would have a higher stomata density than leaves in the shade. On the other hand, if water is not available, such as under drought conditions, excessive evaporation might lead to desiccation (drying out.) Plants in hot areas may benefit from fewer stomata so that they can conserve water. The discussion illustrates an important concept in experimental biology, that there are often alternative hypotheses to explain variation in nature. In this case, the stomata density may increase or decrease in response to environmental variation in sunlight and water availability. Stomata open in response to blue light, and close in the presence of abscisic acid (ABA), which is biosynthesized when the plant is under dark conditions and/or drought stress. Stomata are



essential for the uptake of carbon dioxide (CO_2) in order for the plant to carry out photosynthesis.

Materials: Compound microscope, Slide, Clear fingernail, polish, clear tape, scissors, colored pencils, fresh leaf (geranium, tradescantia or any fresh leaf) both in light and dark condition for several hours. Mobile to take picture under different magnification

Procedure:

Leaf ex[posed to light

Magnification:_____

Leaf in darkness

Magnification:_____

Write procedure here:

_____

_____

_____

_____

_____

_____

_____

_____

_____

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_____

_____

Paper Chromatography: https://www.youtube.com/watch?v=ej2zXOwASVI

Materials: Scissors, Chromatography paper strip, pencil, capillary tube, spinach leaves to extract pigment, test tube, cork stopper, solvent (Petroleum ether and Acetone mix 9:1 ratio), metric ruler, tape, hook or paper clip, paper towels, test-tube rack, mortar and pestle, sand (to make easier to grind)

Chromatography -is a technique used to separate molecules from a mixture. It uses two phases for separation –A stationary phase (paper) and a mobile phase (solvent).• Molecules in a mixture will move at different rates based upon their affinity for either the stationary phase or mobile phase.

• In the present lab, pigments extracted from a spinach leaf will separate via *Paper Chromatography* into the four distinct pigments discussed above.• In paper chromatography the paper is the stationary phase while the solvent is the mobile phase.• There are a variety of solvents that can be used. Here we used a solvent with 9 parts petroleum ether: 1 part acetone.• If we used a different solvent the pigments would separate, but would come out in a different order since they separate based upon their affinity for the solvent.

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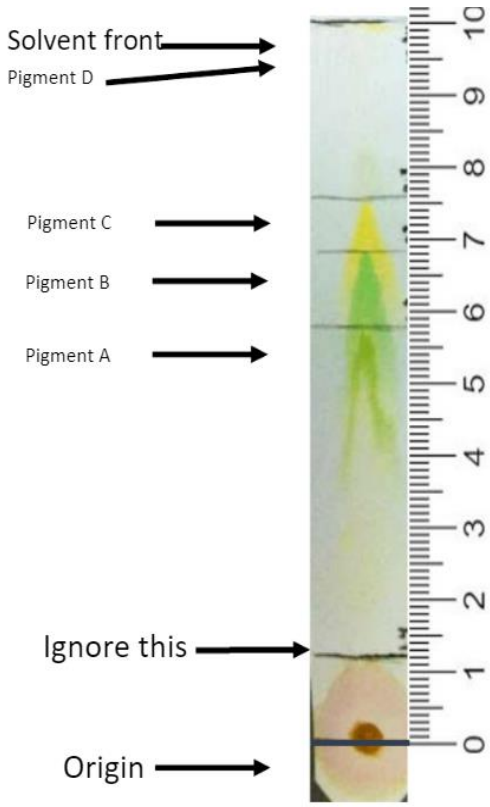


Figure 7.5: Separation of photosynthetic pigment form spinach leaf extraction by paper chromatography. The Relative front (Rf) is the distance traveled by each pigment (sample) from its original location relative to t

he distance the solvent traveled.

$$R_f = \frac{\text{Distance moved by pigment}}{\text{Distance from pigment origin to solvent front}}$$

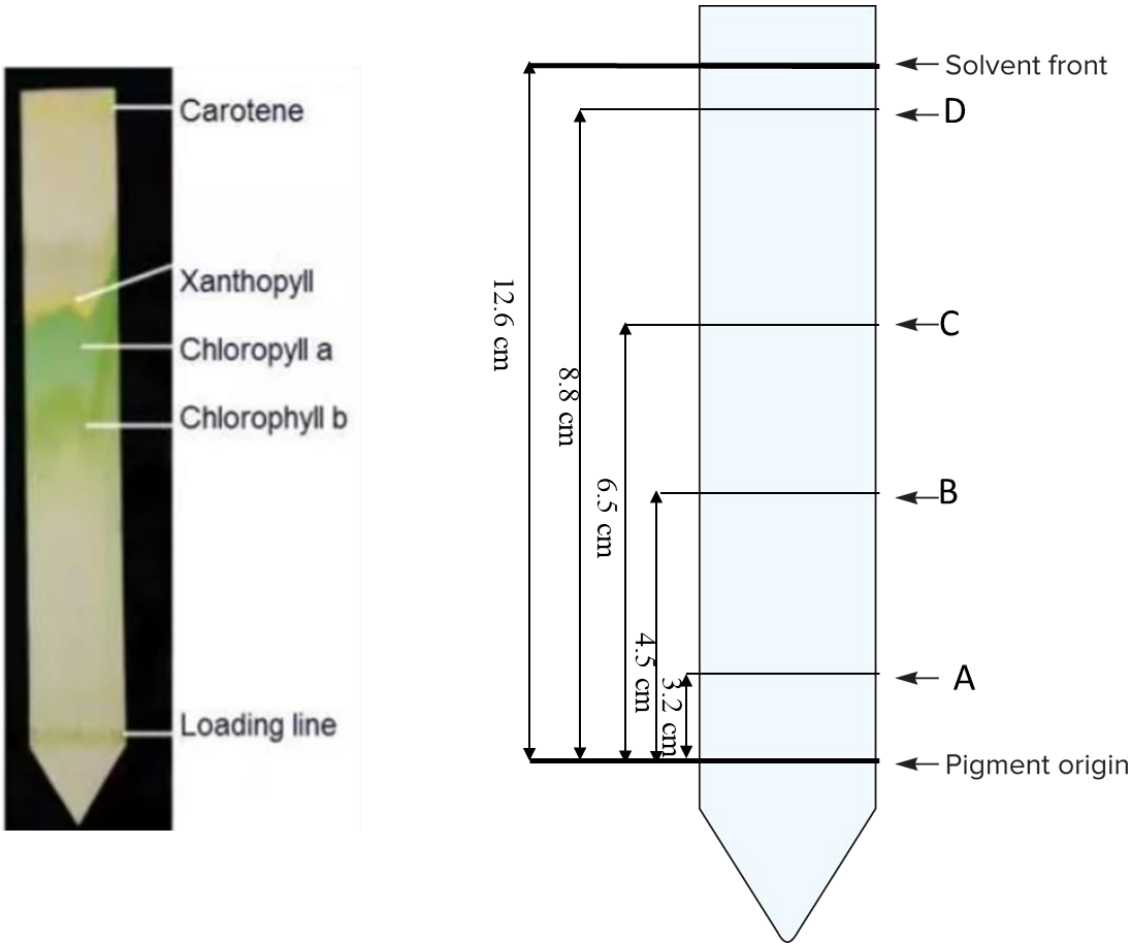


Figure 7.6: Separation of photosynthetic pigment form spinach leaf (left), calculate the Rf value for latter A, B, C and D.

Calculate Rf for followings by applying given Figure

Rf of A =

Rf of B =

Rf of C =

Rf of D =

Measuring photosynthesis rate (Oxygen evolution)

• You can determine the rate of photosynthesis by measuring either the amount of oxygen produced, or the amount of carbon dioxide consumed.
• In this week's lab you will measure the rate of photosynthesis by measuring the amount of oxygen produced in the photosynthetically active aquatic plant called *Elodea*.
• It is easy to measure oxygen production in an aquatic plant – you just count the number of oxygen bubbles produced!

Write the brief report on the separation of plant pigment by highlighting the procedure you use to separate the plant pigment by paper chromatograph.

Experimental procedures

1. Obtain a small beaker.
2. Add NaCl running buffer to the beaker until it reaches a height of about 5 mm.
3. Obtain a strip of chromatography paper and put it down on the bench.
4. Obtain the bottle containing the dark green food dye mixture.
5. Obtain a glass capillary and insert the tip of the capillary into the food dye mixture liquid. A little bit of dye will ascend into the capillary.
6. Remove the capillary and apply.
7. Touch the left side of the chromatography paper about 1 cm above its lower end with the tip of the capillary. A little bit of green liquid will spread out on the paper. Lift the capillary and touch the paper again just to the right of the dye you just applied. Repeat this until you have a horizontal line of dye from the left to the right side of the paper.
8. Place the chromatography paper into the beaker as shown below.
9. Observe how the running buffer moves up the paper and separates the dye mixture into three components (red, yellow and blue.

Chapter 8: Understanding of Cellular Respiration: Alcoholic fermentation (Yeast Fermentation and Measurement), Lactic Acid Formation (Making Kimchi)

Learning Outcomes: At the end, after the completion of the current lab, the student should be able to:

- To describe the cellular respiration
- Explain the differences between anaerobic fermentation and aerobic cell respiration.
- Describe the role of enzymes on the rate of cellular respiration.
- Measure the effect of temperature and other factors on cellular respiration.
- identify the presence of CO_2 as a product in the cellular respiration and fermentation.
- Identify using of sugar as fermentation
- substrate in fermentation.
- Identify the presence of alcohol as a product of yeast fermentation process,
- Evaluate the correlation between the amount of released CO_2 and decreasing pH.
- Interpret the effect of the rate of the cellular respiration on global climate chang

Introduction

Cellular respiration is a chemical reaction that occurs in your cells to create energy and when you are exercising your muscles are creating ATP to contract. Cellular respiration requires oxygen which is breathed in and creates carbon dioxide which is breathed out. In the

Glucose is the preferred carbohydrate of cells. Glycolysis (*glyco* – sugar; *lysis* – splitting) is a universal process of all cells that occurs in the cytosol whereby the glucose (a 6-carbon sugar) is split into two pyruvate (a 3-carbon molecule) molecules to generate ATP and reduced NADH. ATP (adenosine triphosphate) is the energy currency of the cell that stores chemical energy in 3 high energy phosphate bonds. NADH (reduced nicotinamide adenine dinucleotide) is

a high energy electron carrier that acts as a coenzyme in reactions and as a rechargeable battery of sorts. The uncharged state that is not carrying high energy electrons is called NAD^+.

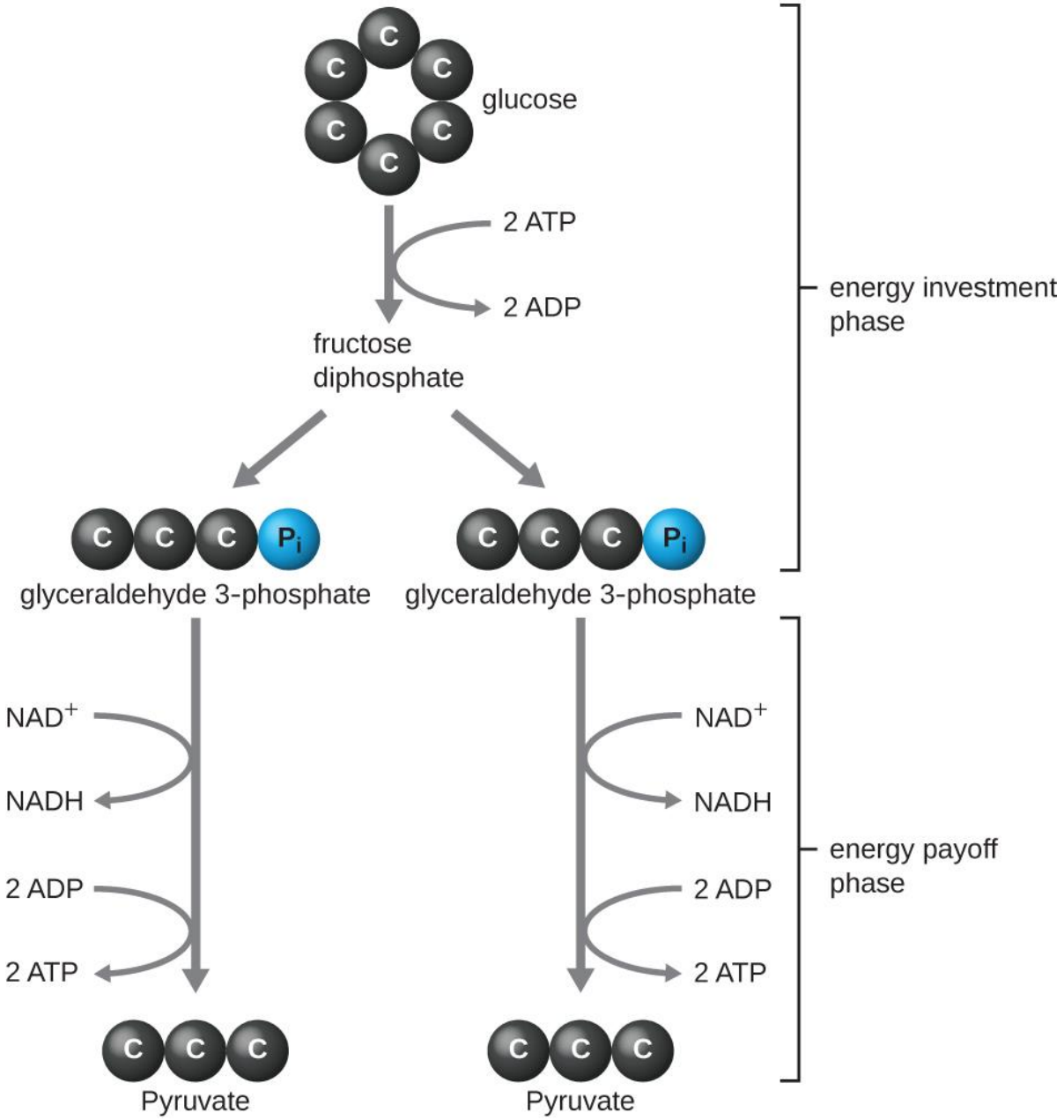


Figure 8.1 Glycolysis

Fermentation

In the absence of oxygen, cells may decide to utilize the pyruvate from glycolysis to rapidly generate additional ATP molecules in a process called fermentation. **Fermentation** is the anaerobic process of reducing pyruvate to generate ATP. This process uses the NADH generated from glycolysis as the reducing agents. Fermentation is a familiar process that occurs in yeast to generate ethanol. In other organisms, like humans, fermentation results in the production of lactic acid. Both lactic acid and ethanol are toxic, but this aids the cells in generating ATP when energy is required rapidly. Fermentation also generates CO_2 as a waste molecule as pyruvate is broken down into a 2-carbon compound.

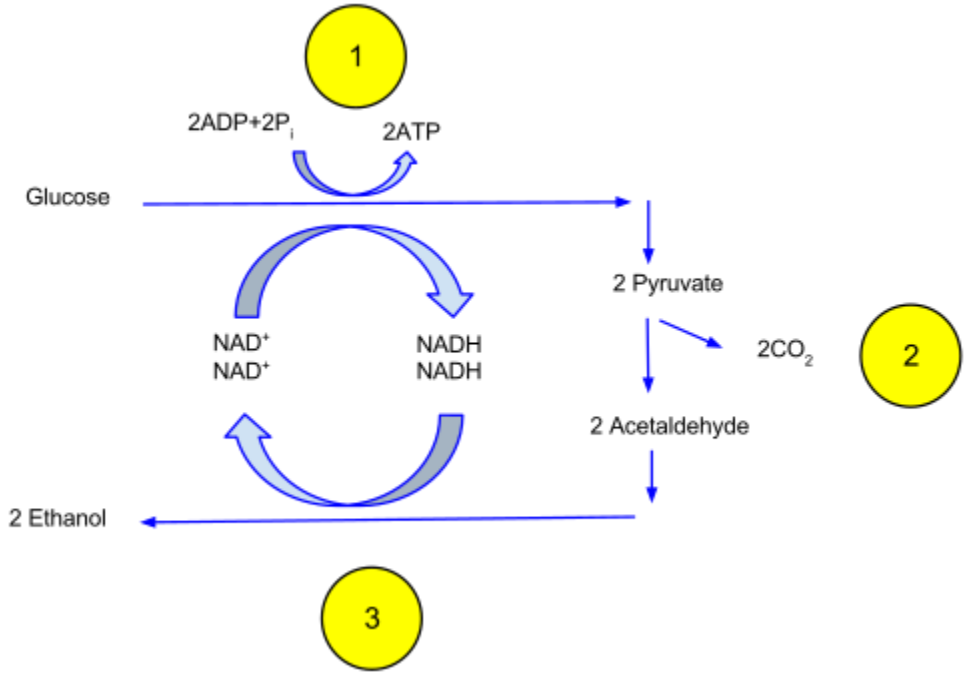


Figure 8.2 Fermentation Fermentation in yeast generates ATP in the absence of oxygen but yields little ATP at the cost of the reduced NADH. Credit: Davidcarmack (CC-BY-SA)

The Preparatory Reaction: In the presence of O_2, aerobic organisms will use a reaction of pyruvate decarboxylation in the cytosol. This reaction generates a molecule of **Acetyl-CoA** from the Coenzyme A which can enter the mitochondria.

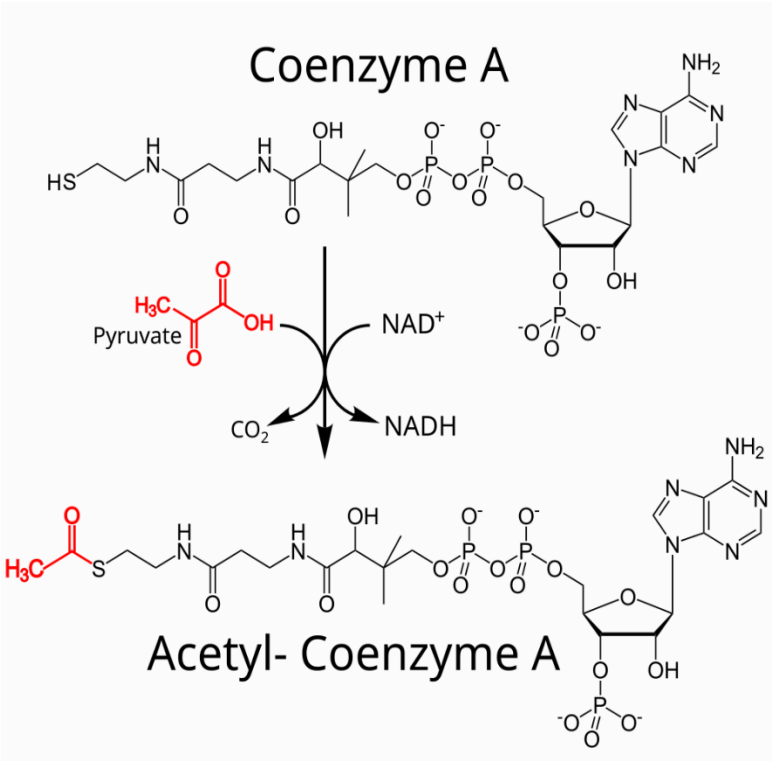


Figure 8.3 The Preparatory Reaction. Coenzyme A (CoA) is charged with an Acetyl group (2 carbon compound) to generate Acetyl-CoA and a CO_2. When there is an excess of carbohydrates, the Acetyl-CoA is used as a starting point for long-term energy storage in lipid synthesis.

Mitochondria

Mitochondria are the power station of eukaryotic cells. They are derived from a process described by the **endosymbiotic theory** whereby aerobic prokaryotes were engulfed by a protoeukaryote. In this mutualistic arrangement, the prokaryote detoxified the deadly O_2 gas in the environment and used it to fully break down glucose to yield many ATP molecules. Evidence for this theory comes from the independent replication of the mitochondria, the bacterial-like mitochondrial DNA, the bacterial-like mitochondrial ribosomes, the bacterial lipids found in the inner membrane and the eukaryotic nature of the outer membrane. Mitochondria are genomically similar to bacteria of the order Rickettsiales. Some bacteria of this order are still free-living and some are intracellular pathogens.

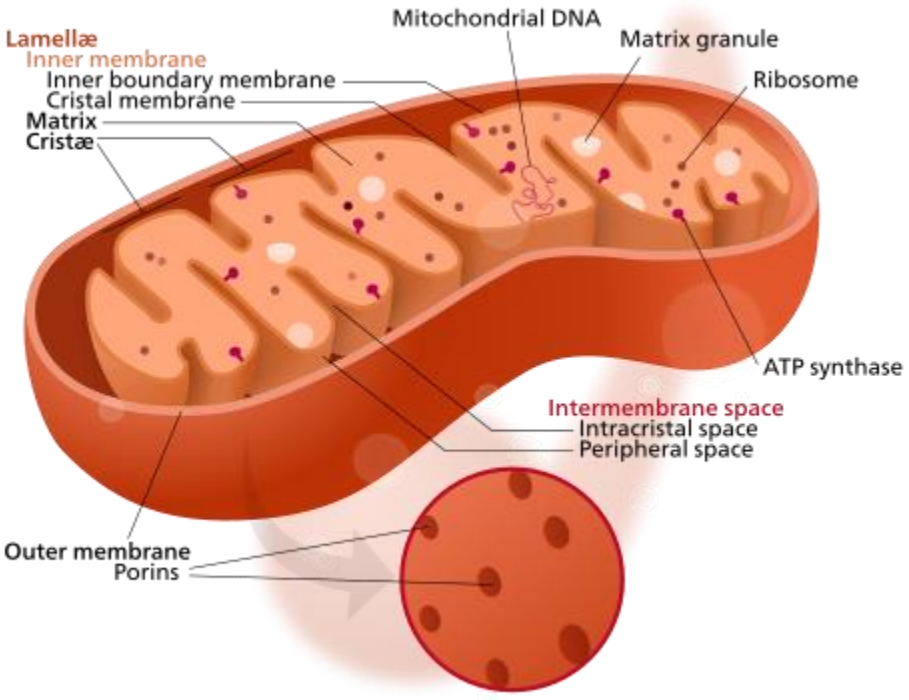


Figure 8.4 The Preparatory Reaction. Coenzyme A (CoA) is charged with an Acetyl group (2 carbon compound) to generate Acetyl-CoA and a CO_2. When there is an excess of carbohydrates, the Acetyl-CoA is used as a starting point for long-term energy storage in lipid synthesis.

Aerobic Respiration

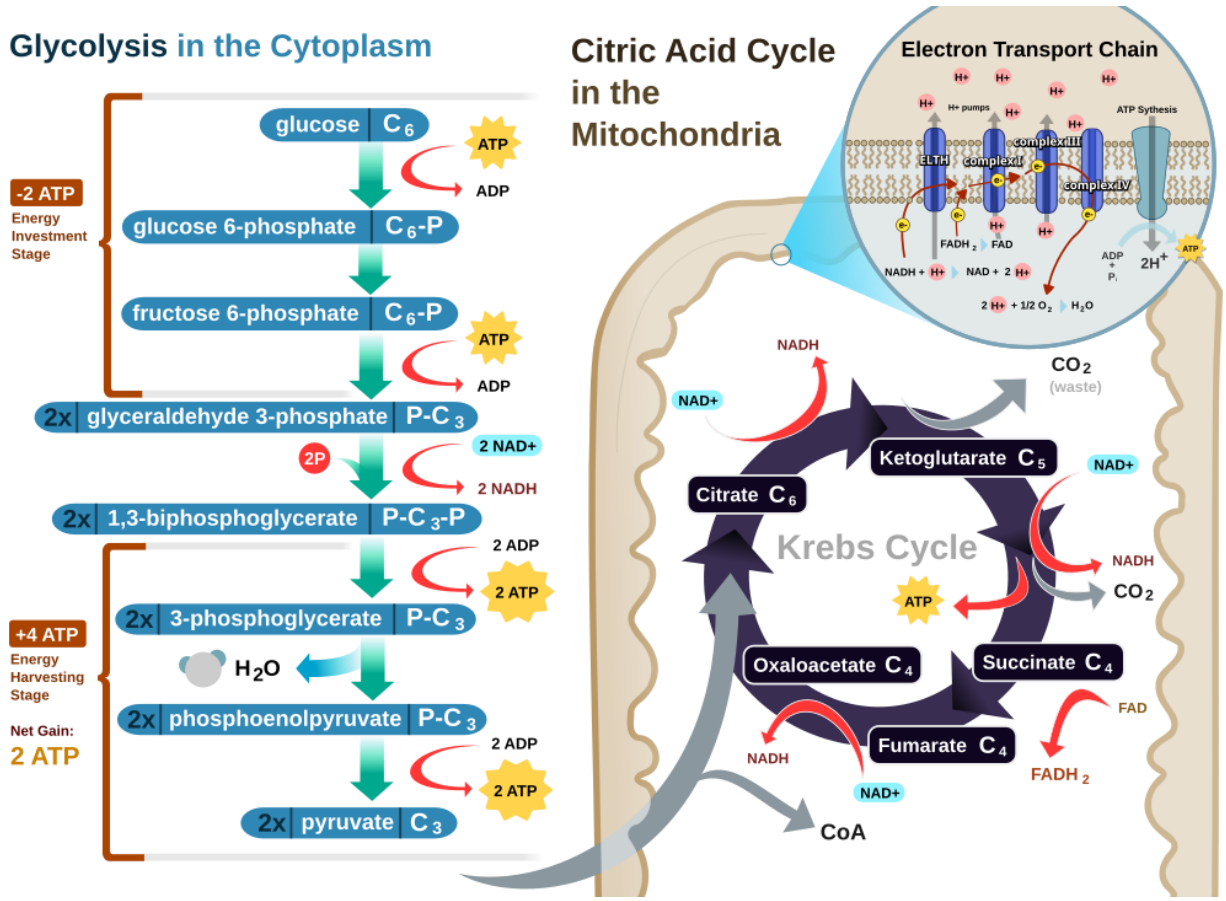


Figure 8.45 Cellular Respiration. Left side is glycolysis (anaerobic). The Right side is what occurs in the presence of oxygen in eukaryotes. The aerobic reactions occur inside the mitochondria after being fed Acetyl-CoA molecules from the cytoplasmic preparatory reaction.

Acetyl-CoA enters the mitochondrial matrix where it is used in the **Krebs Cycle** (aka Tricarboxylic acid cycle (TCA), aka Citric acid cycle). For each pyruvate, there are 2 turns of the cycle where additional NADH and another high energy electron carrier **$FADH_2$** (flavin adenine dinucleotide) are generated. The electrons stored by NADH and $FADH_2$ are transferred to proteins called **cytochromes** that have metal centers for conducting these electrons. In the process of moving these electrons, the cytochromes in this **Electron Transport Chains** (ETC) power the movement of protons into the intermembrane space. The terminus of these electrons is an O_2 molecule that is reduced into 1/2 H_2O molecules. This apparent movement of water molecules from the chemical synthesis is termed **chemiosmosis**. A channel in the membrane

called **ATP synthase** acts as a gateway for the H^+ back into the matrix, but use this motion to convert ADP into ATP.

Introduction

What happens when you exercise? As you know, increasing your activity level will cause an increase in your respiration rate. What you may not know is that this is closely tied to cellular respiration in your cells. As you increase your activity, mitochondria in your cells use oxygen to break down sugars to charge up ATP molecules; ATP is then used for energy directly elsewhere in your cells, including muscle work. A waste product of the breakdown of sugars is carbon dioxide gas, which you exhale. When your body breaks down glucose, carbon dioxide is produced. Too much carbon dioxide can result in damage to muscles or other body parts, so there must be some way to get rid of this carbon dioxide. When you inhale, this brings fresh air with high oxygen levels into your lungs. When you exhale, this moves air with high carbon dioxide levels out of your lungs.

For quick energy, the body uses already-stored ATP and new ATP made by **lactic acid fermentation** also called **anaerobic respiration** because it does not require oxygen. Aerobic cellular respiration releases energy more slowly than fermentation (anaerobic respiration), which is why you have to pace yourself during long sessions of exercise. Glucose is a common fuel for the process of cellular respiration. The complex sugar, starch, is broken down into a simple sugar, glucose, by the digestive system. Although in the lab we cannot physically see this breakdown, we will examine how exercise and physical exertion affects cellular respiration.

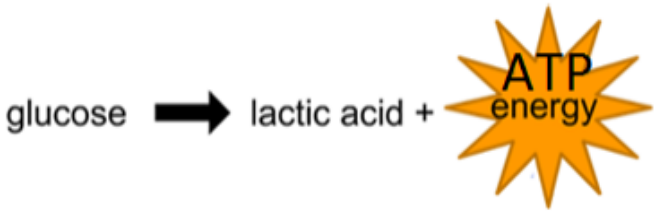
All parts of your body need energy to do their work. For example, muscles need energy to contract, and your body requires energy to synthesize needed molecules. Your body gets the energy it needs by combining food molecules with oxygen in a process called cellular respiration. The sugar glucose is combined with oxygen to release carbon dioxide, water, and ATP. ATP is the energy your body can use. This is shown in the following chemical reaction, which is called **aerobic cellular respiration**.

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + ATP$$

(Glucose) (Oxygen) (Carbon Dioxide) (Water) (Energy)

You know that a fire needs fuel and oxygen from the air to keep burning. Similarly, your muscles and other parts of your body need to have a continuous supply of glucose (or other high-energy molecules) and oxygen to make ATP, or energy, for muscle contractions and other body functions. Since the chemical reaction above requires oxygen, it is considered aerobic respiration.

During intense or long periods of exercise you may not be able to inhale enough oxygen to produce enough energy (ATP) to continue. The tissue without enough oxygen may still make ATP by anaerobic respiration (fermentation) but this chemical reaction is produces less energy. Additionally anaerobic respiration results in the production of lactic acid, a waste product that builds up in muscles and causes cramps. The body will only use the anaerobic respiration reaction when oxygen is not available because of it is much less efficient at making ATP and produces lactic acid which can inhibit physical activity. The chemical reaction for anaerobic respiration is shown to the right.



Develop a model to describe how food is rearranged through chemical reactions forming new molecules that support growth and/or release energy as this matter moves through an organism.

Cellular respiration (see chemical reaction below) is a chemical reaction that occurs in your cells to create energy; when you are exercising your muscle cells are creating ATP to contract. Cellular respiration requires oxygen (which is breathed in) and creates carbon dioxide (which is breathed out). This lab will address how exercise (increased muscle activity) affects the rate of cellular respiration. You will measure 3 different indicators of cellular respiration: breathing rate, heart rate, and carbon dioxide production. You will measure these indicators at rest (with no exercise) and after 1 and 2 minutes of exercise. Breathing rate is measured in breaths per minute, heart rate in beats per minute, and carbon dioxide in the time it takes bromothymol blue to change color. Carbon dioxide production can be measured by breathing through a straw into a solution of bromothymol blue (BTB). BTB is an acid indicator; when it reacts with acid it turns from blue to yellow. When carbon dioxide reacts with water, a weak acid (carbonic acid) is formed (see chemical reaction below). The more carbon dioxide you breathe into the BTB solution, the faster it will change color to yellow.

$$6CO_2 + 6H_2O \;\; ======\rightarrow \;\; 6HCO_3 + 6H^+$$

I. Purpose.

☐ To observe the effects of exercise on cellular respiration.

☐ To identify the role of carbon dioxide production, breathing rate, and heart rate in determining the rate of cellular respiration.

II. Pre-Lab. Use your background information AND your Cellular Respiration notes to answer the following

1. What is the equation for cellular respiration? Label the reactants and the Products.

2. In what part of the cell does cellular respiration occur?

3. Write a prediction/hypothesis of how exercise will affect your body's production of carbon dioxide (i.e. do you think your body will produce more or less carbon dioxide as you exercise). Make sure you EXPLAIN WHY.

Materials:

- Beaker/Test Tube/Cup
- bromothymol blue solution (BTB)
- straw
- stop watch

Safety:

- Always have an adult with you to help you during your experiment.
- Always wear eye protection and gloves when doing chemistry experiments
- Rubbing alcohol is flammable, so it must be kept away from any open flames or heat.
- Conduct this experiment in a well-ventilated area.

Procedure:

PART A: Resting (no exercise)

Measuring Carbon Dioxide Production:

1. Use a graduated cylinder to measure out 20 mL of tap water and pour it into a small beaker.

2. Use a dropper to add 8 drops of bromothymol blue to make a BTB solution.

3. Using a straw, exhale into the BTB solution. (CAUTION: Do not inhale the solution!)

4. Time how long it takes for the blue solution to turn yellow. Record the time in Table 1.

5. Wash out the beaker repeat steps 1-4 twice more.

6. Average the results of the 3 trials. Record this in Table 1.

Measuring Breathing Rate:

1. Count the number of breaths (1 breath = inhale + exhale) you take in 1 minute. Record this in Table 2.

2. Repeat this 2 more times.

3. Average the 3 trials to get your average breathing rate. Record this in Table 2. Measuring Heart Rate:

1. While you calculate your breathing rate, have your partner take your pulse.

2. Count the number of beats in 30 seconds and multiply that number by 2. Record this in Table 3.

3. Repeat this 2 more times.

4. Average the 3 trials to get your average heart rate. Record this in Table 3.

PART B: Increased Muscle Activity (Exercise)

1. Exercise for exactly 1 minute by doing jumping jacks.

2. While you are exercising, your partner should get the BTB solution ready as in Part A.

3. After 1 minute of exercise, immediately exhale through the straw into the BTB solution. Time how long it takes for the BTB to turn yellow. Record this in Table 1.

4. Then quickly calculate your breathing and heart rates as you did before. You only need to do this once.

5. Record these values in Tables 2 & 3. Remake your BTB solution.

6. Exercise as you did before, but for 2 continuous minutes.

7. Immediately exhale through the straw into the BTB solution. Time how long it takes for the BTB to turn yellow. Record this in Table 1.

8. Then quickly calculate your breathing and heart rates as you did before. You only need to do this once.

9. Record these values in Tables 2 & 3.

10. If there is time, repeat the entire procedure for your lab partner. Record data from 2 OR 3 other subjects in the class to get more data depending on if you partner was able to go or not.

Data and Observations:

Table 1. Carbon Dioxide Production (time it takes BTB to change color)

		Student 1	Student 2	Student 3	Student 4	Average
RESTING	Trial 1					
	Trial 2					
	Trial 3					
	Average					
EXERCISE	1 minute					
	2 minutes					

Table 2. Breathing Rate (breaths/minute)

		Student 1	Student 2	Student 3	Student 4	Average
RESTING	Trial 1					
	Trial 2					
	Trial 3					
	Average					
EXERCISE	1 minute					
	2 minutes					

Table 3. Heart Rate (beats/minute)

		Student 1	Student 2	Student 3	Student 4	Average
RESTING	Trial 1					
	Trial 2					
	Trial 3					
	Average					
EXERCISE	1 minute					
	2 minutes					

Questions:

Analysis & Conclusions: Answer the questions below using your BACKGROUND information in the lab, as well as your lab data. ANSWER THE QUESTIONS IN COMPLETE SENTENCES.

1. How did exercise affect the time needed for the solution to change color? Explain why the color change occurred (How does BTB work?)

2. What can you conclude about the effect of exercise on the amount of carbon dioxide that is present in your exhaled breath? Why is this so?

3. What can you conclude about the effect of exercise on breathing rate? Why is this so?

4. What can you conclude about the effect of exercise on heart rate? Why is this so? What do your muscles need during exercise that the blood brings?

5. State whether your hypothesis was correct or incorrect and why. In doing so, discuss what you think is going on in the muscles of the body as muscle activity is increased. Address the need to get oxygen to the muscles and get rid of carbon dioxide, as well as how the muscles cells get the energy needed to continue contracting. What did you see? Anything you were not expecting? Something really awesome? Describe it here.

Reference: https://www.csub.edu/chemistry/_files/exerciseandcellularrespirationlabao.pdf

Chapter 9: Understanding of Cell Division: Mitosis and Meiosis (Animal and Plants)

Learning outcomes: After completing the current lab on mitosis, students should be able to:

- define the following terms: chromosome, spindle, centromere, centrosome, gametes, somatic, cytokinesis, cleavage furrow, homologues (homologous chromosomes),
- list the stages of the cell cycle in order
- describe the cellular events that occur during interphase (be sure to include the specific portions of interphase such as G1, S and G2)
- describe the overall process of mitosis (what is the purpose and the outcome of mitosis)
- describe the events of prophase of mitosis
- describe the events of metaphase of mitosis
- describe the events of anaphase of mitosis
- describe the events of telophase of mitosis
- explain what is meant by a diploid chromosome number versus a haploid chromosome number
- if given the chromosome number of the parent cell, determine what the chromosome number of the daughter cells would be after mitosis

Introduction

According to the cell theory of biology, all cells arise from pre-existing cells. In eukaryotes, this generally occurs by the process of mitotic cell division, which involves the separate but often coordinated processes of cytokinesis (division of the cytoplasm and organelles) and mitosis (division of the nucleus and associated DNA). Mitosis without the co-occurance of cytokinesis leads to the production of multinucleated cells, such as those found naturally in skeletal muscle and cardiac muscle. Mitotic cell division leads to the production of genetically identical daughter cells from existing parent cells. Mitotic cell division is used in asexual reproduction, growth, and

repair. Meiosis is another form of cell division in eukaryotes that leads to the production of gametes. While the process of mitosis is continuous process within the cell cycle (i.e., it doesn't occur in discrete steps), biologists are classifiers and tend to place things into discrete categories. Mitosis is commonly divided into four major phases: prophase, metaphase, anaphase, and telophase. You may find that some accounts of mitosis further subdivide the process to include prometaphase between prophase and metaphase. In this exercise, we will consider prometaphase a component of prophase.

In these laboratory activities, you will be examining real cells that have been frozen in time in the midst of undergoing the various phases of the cell cycle. To better examine the many cell cycle stages that occur with real cells, we use cells that are undergoing a high amount of cell division. One great source of rapidly dividing animal cells is in early embryonic development. A blastula is a ball of cells in an early embryonic phase and is continuing to undergo rapid cell divisions. After egg and sperm meet, the fertilized ovum rapidly begins to divide and will go through this blastula stage. Since we are interested in studying cell division in humans (a type animal), and it would be unethical to examine human blastula, fish blastula are commonly used.

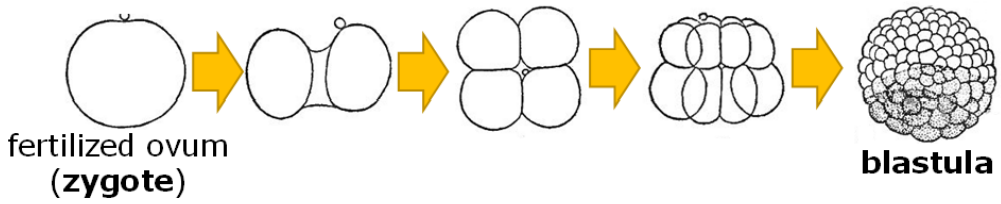


Figure 9.1: Development of fish blastula.

Real cells can look quite different than the illustrated versions of cells in their cell cycle stages. To assist with recognizing these stages, examine the table above showing images of real animal cells. A description of features that can be used to identify cells in each stage is also provided. Note that these microscope slides are prepared using stains that stain the DNA (chromosomes/chromatids) a color that makes it more obvious such as purple, blue, or pink. The

images above have the DNA stained dark purple.

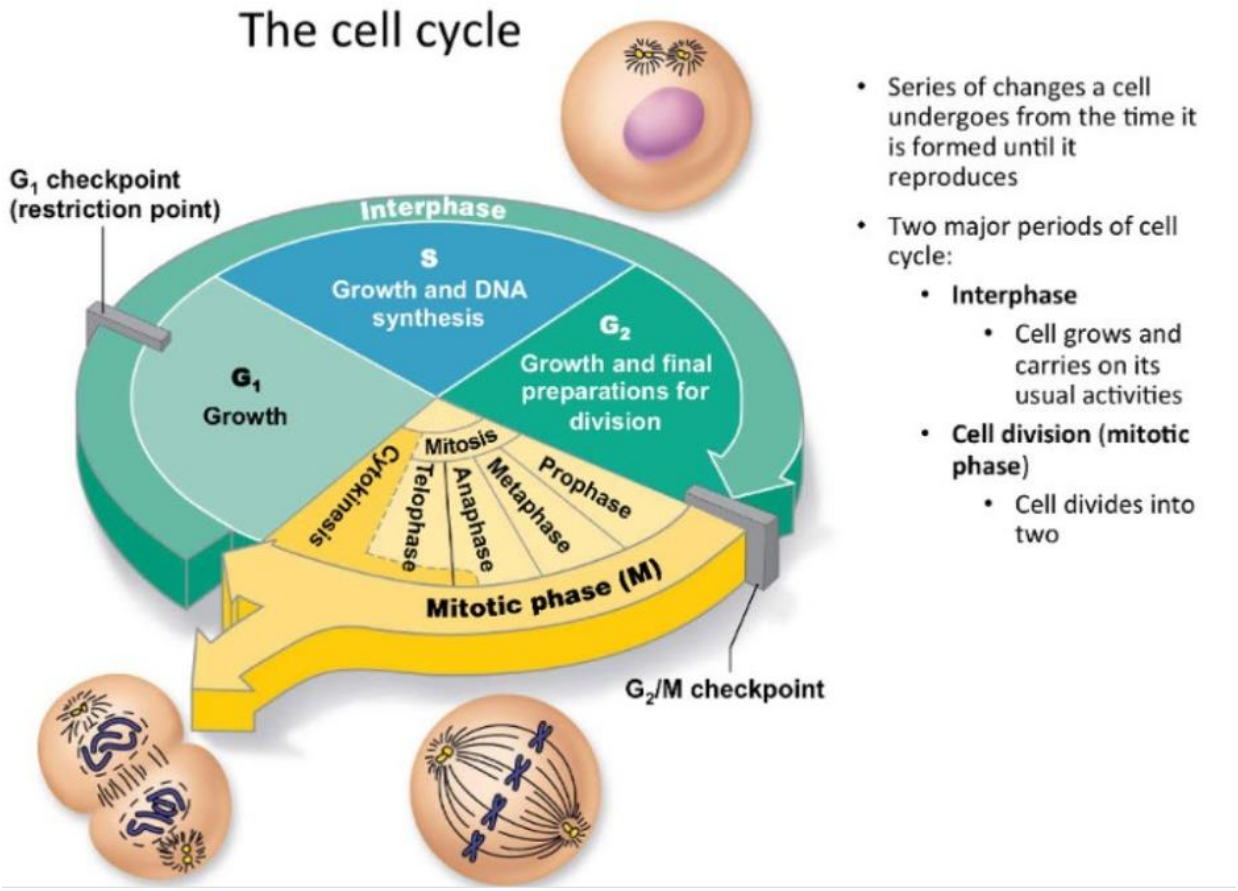


Figure 9.2: Cell Cycle

Write main characteristic features of following stages.

Interphase

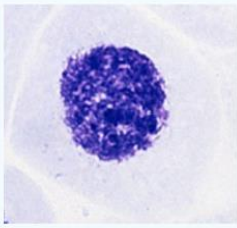
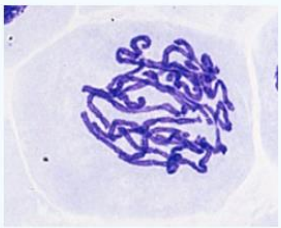
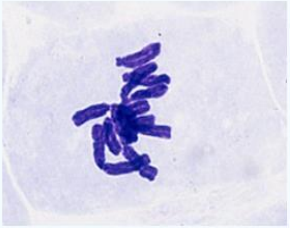
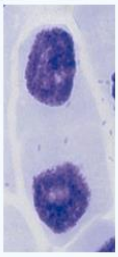
Prophase

Metaphase

Anaphase

Telophase

Real cells can look quite different than the illustrated versions of cells in their cell cycle stages. To assist with recognizing these stages, examine the table above showing images of real animal cells. A description of features that can be used to identify cells in each stage is also provided. Note that these microscope slides are prepared using stains that stain the DNA (chromosomes/chromatids) a color that makes it more obvious such as purple, blue, or pink. The images above have the DNA stained dark purple.

Interphase	Prophase	Metaphase	Anaphase	Telophase
				
• Nucleus is present • Chromosomes are not condensed (cannot see individual thick strands) • Only one nucleus present	• Chromosomes are less confined in a circle structure (nucleus breaking down) • Chromosomes are condensed (can see individual thick strands) • Chromosomes are NOT in a line or pulling apart to opposite poles of the cell	• Chromosomes are condensed (can see individual thick strands) • Chromosomes are roughly lined up in a line • Chromosomes are NOT pulling apart to opposite poles of the cell	• Chromosomes are condensed (can see individual thick strands) • Chromosomes are pulling apart to opposite poles of the cell	• Two round nuclei inside one cell • Chromosome s are not condensed (cannot see individual thick strands)

Use the letters to match with the descriptions as appropriate. ***Some choices may be used more than once and some may not be used at all.***

A. mitosis
B. interphase
C. prophase
D. homologous chromosomes
E. telophase
F. anaphase
G. cytokinesis
H. sister chromatids
I. metaphase
J. S phase

_____ A phase when chromosomes line up in the center of the cell.
_____ A phase when the nuclear envelope begins to dissolve.
_____ A phase between cell divisions.
_____ A phase when chromosomes separate from one another.
_____ Nuclear division.
_____ A phase when DNA replication occurs.
_____ A phase when the DNA decondenses.
_____ Type of chromosomes that separate during mitosis.
_____ Division of the cytoplasm to produce 2 separate cells.
_____ A phase where the cell grows and organelles replicate.
_____ A phase where DNA condenses.
_____ A process that produces sister chromatids.
_____ A phase when the nuclear envelope reforms.

Mitosis

Cells in your body reproduce at different rates. Skin cells reproduce frequently (about once per day); liver cells reproduce rarely (about once per year). Some specialized cells like nerve and muscle cells almost never reproduce and are in a special stage called G_0. The whole process of

mitosis, prophase to telophase, takes approximately 90 min. In plants, an area of rapid growth is the tips of roots. This exercise uses onion root tips to illustrate the amount of time spent in each phase of mitosis.

The Cell Cycle

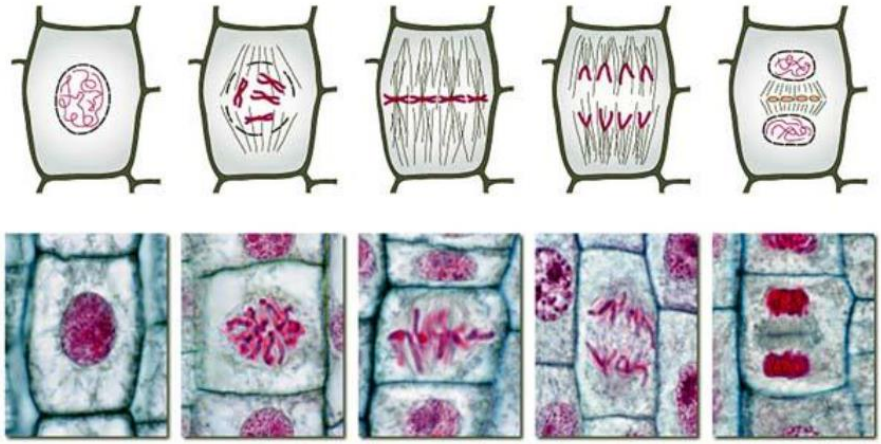
The cell spends a majority of its life in **interphase**, where it performs cell processes and copies its DNA. In interphase, the nucleus will appear as a dark structure near the center of the cell.

Mitosis begins with **prophase**, where the DNA in the nucleus condenses and chromosomes can be seen. The spindle forms, which moves chromosomes.

During **metaphase**, the chromosomes line up at the equator of the cell.

Chromatids (identical copies of chromosomes) separate during **anaphase**.

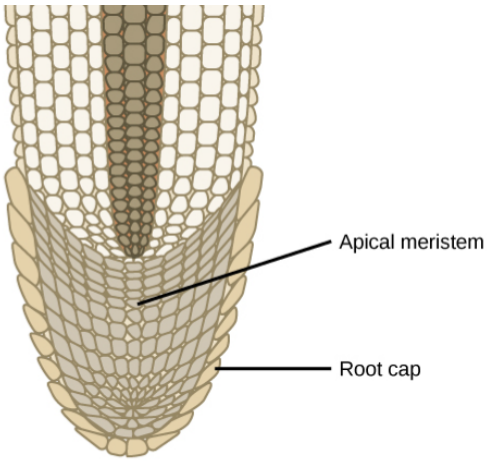
The last phase is **telophase**, where the nuclear membrane reforms around two new daughter cells and cytokinesis begins.



3. Label the phases of the cell cycle:

One of the basic tenets of biology is that all new cells come from living cells. New cells are formed by the process of cell division which includes both the division of the cell's nucleus, **mitosis**, followed by cytokinesis. Cell divisions are used by organisms to repair tissue, growth, and asexual reproduction. Cells created in this process are identical to the original.

Where does one find cells undergoing mitosis? It depends if you are studying plants or animals. Plants and animals differ in this respect. In most plants, the process of forming new cells is restricted to special growth regions called **meristems**. These regions usually occur at the tips of the stems and roots of the plant. Mitosis in animals can be viewed by looking at the growing embryo or at cells of tissue that rapidly divide.



1. Where do you look in a plant cell to find dividing cells?

2. Why do cells divide?

In plants, cell division cell division generally takes place in meristematic tissues where the plant is actively growing, at the apex of roots and shoots. An onion root may be divided roughly into four regions:

1. the root cap-protective tissue composed largely of dead cells
2. meristematic tissue-rapidly dividing cells located just behind the root cap
3. elongating cells-located immediately behind the meristematic tissue
4. mature and specialized cells-root hairs and other plant tissues

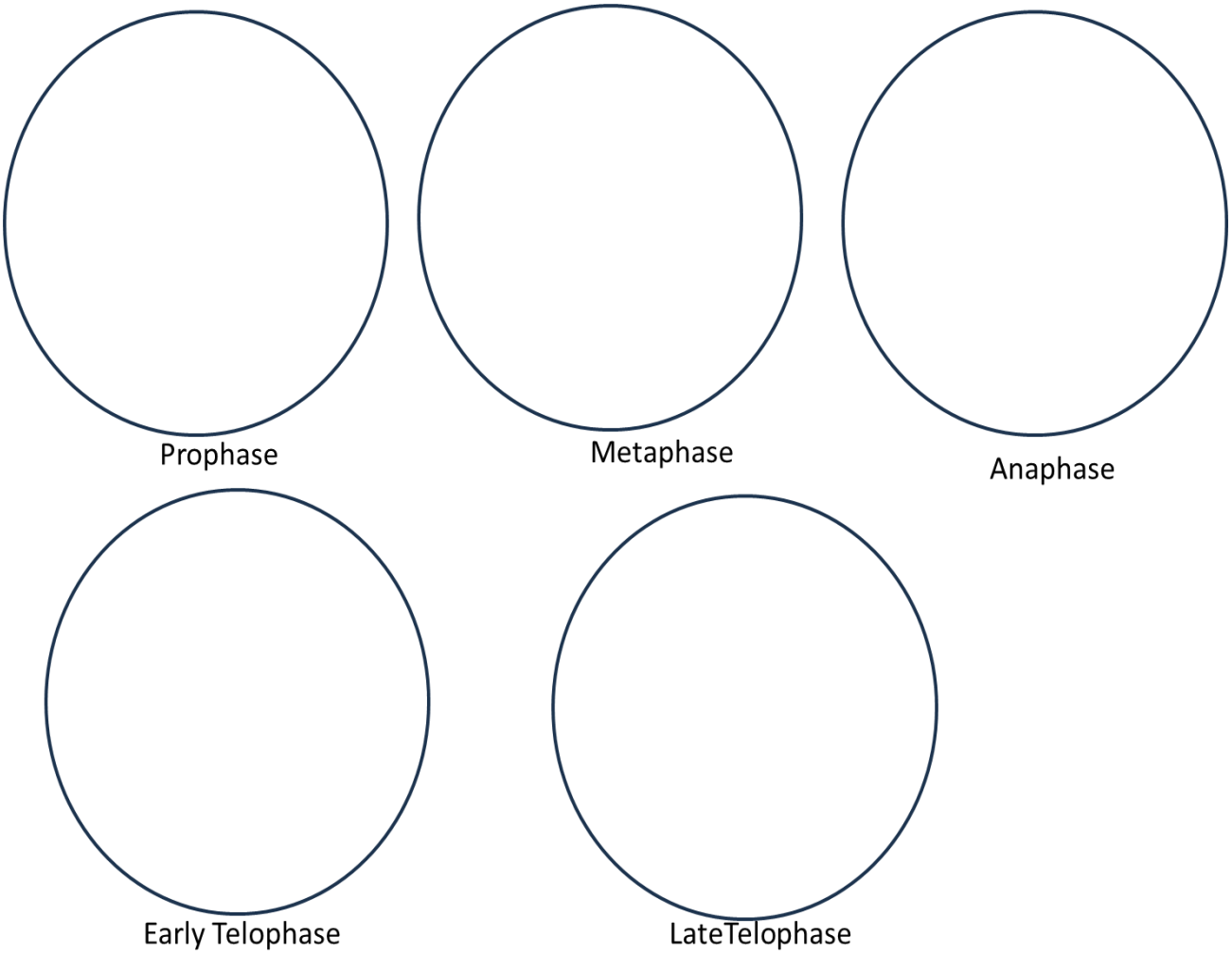
You will be provided a prepared slide of onion root tip to examine the different stages of the mitosis.

Materials: Compound microscope, Onion root tip slide

Procedure

1. The cells go in the appropriate temporal sequence through cell cycle
2. You are likely to use the same term multiple times.
3. While technically not a phase of mitosis, you may use “interphase” for some of the cells, but you should not use “cytokinesis.”
4. Hold the slide of the onion root up to the light and note that there are several longitudinal sections on the slide.
5. Examine all sections on the slide. Locate the meristematic tissue to observe phases of mitosis using high power. Find each phase of mitosis on the slide.

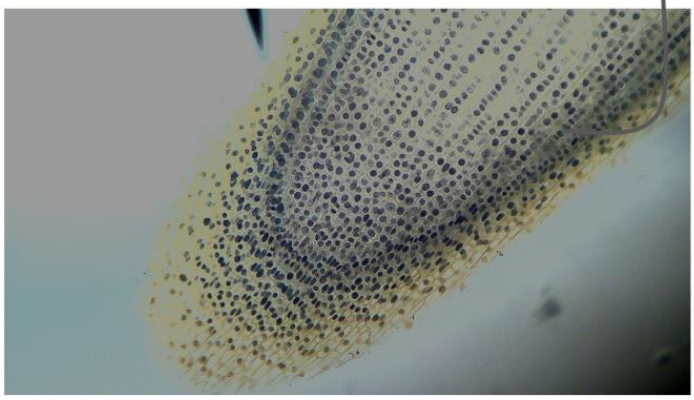
6. Work as a team to look at onion root tips under the microscope. Identify the phases of the cell cycle for 5 randomly chosen cells. Draw each stage inside circle.

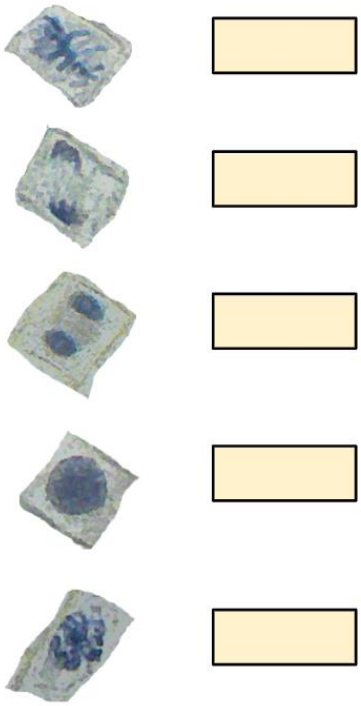


The images show (in no particular order) photos from a microscope slide of an onion root.

4. Label the phases.

5. Which phase do you think is the hardest to identify? Why?





Meiosis: Most cells in multicellular organisms like plants and animals are diploid. A diploid cell has two sets of chromosomes that form homologous pairs. A cell with only one set of chromosomes are considered haploid. Gametes, like the egg and sperm in humans, are examples haploid. Gametes fuse during sexual reproduction to form a zygote which is once again diploid with one set of chromosomes from each parent. Meiosis is a process that starts with one diploid cell and creates four haploid cells. Meiosis is similar to mitosis and must have the cell's DNA replicate before it can begin. This creates chromosomes that are made up of two sister chromatids connected by a centromere. Unlike mitosis, meiosis requires two rounds of division to get half the number of chromosomes into all of the daughter cells.

Meiosis begins with meiosis I when homologous pairs of chromosomes will be split. The stages of meiosis I are similarly named to the stages in mitosis and also have similar milestones:

- prophase I: homologous pairs come together to form tetrads, nuclear envelope disappears, spindle forms (crossing over may also happen during this phase)
- metaphase I: tetrads line up at the equator following the law of independent assortment
- anaphase I: homologous pairs are pulled apart

- telophase I: cytoplasm divides, nuclear envelope may or may not reform. The nuclei now only have 1 set of (duplicated) chromosomes) .

  Meiosis II will see the sister chromatids split apart. This process is just like mitosis. The names of the stages are the same as mitosis, but they have the number 2 after them (prophase 2, metaphase 2, anaphase 2, telophase 2). The main difference is that the DNA does not go through replication before the start of meiosis 2.

  Materials and Procedure: String, 4 different colors of paper (preferably light blue, dark blue, light green, dark green), Ruler or Meter Stick ,Scissors, Marker, 4 paper clips ,Tape

- Using 1 m piece of string, make a circle on your desk to represent the cell membrane. Using a 40 cm piece of string, make another circle inside the cell for the nuclear membrane.

- Cut 1 strip of paper that is 6 cm long, and 4 cm wide from each color of paper (one light blue, one dark blue, one light green, and one dark green) Fold each of the four strips of paper in half, lengthwise. Then place the folded strips of each color inside the nucleus to represent a chromosome before replication. The light and dark strips of the same color represent homologous chromosomes. At one end of the dark blue strip write a large B (brown eyes) on the light blue make a lower case b (blue eyes). On the dark green at a tip write T (for tall) and on the light green write a lower case t (short)

- Modeling interphase: to represent DNA replication, unfold each paper strip and cut in half lengthwise. The two pieces that result from cutting each strip represent the chromatids. Attach the two identical chromatid strips at the center with a paperclip, so an X is formed. Each paper clip represents a centromere.4

- Modeling prophase 1: remove the nuclear envelope and put it aside. Place the light and dark blue chromosomes side by side and the light and dark green chromosomes side by side. Simulate crossing over by measuring and cutting a 2 cm tip for a light blue strip that includes the letters you drew on them earlier. Do the same with a dark blue strip. Tape the light blue tip to the dark blue strip and vice versa. Repeat this process for the light and dark green chromosomes.

- Modeling metaphase 1: Place four 10 cm strings inside the cell, so that two strings extend from one side into the center of the cell and two strings extend from the opposite side into the center of the cell. The string represents the spindle fibers. Tape a string to the centromere of each chromosome with tape. Move the chromosomes to the center of the cell. Make sure that the strings attached to the two blue chromosomes come from opposite sides of the cell (same for the two green chromosomes).

- Modeling anaphase 1: Grab onto the ends of the strings on both sides of the cell, and slowly pull the strings in opposite directions, so the chromosomes move to opposite ends of the cell.
- Modeling telophase 1: Remove the string from each centromere. Place a 40 cm piece of string around each group of chromatids, forming two nuclei. Place a 1 m piece of string around each cell, forming two membranes. You now have 2 different daughter cells.

MEIOSIS II

- Modeling prophase 2: Remove the strings that represent the nuclear membrane in both cells. Attach a 10 cm piece of string to each chromatid.
- Modeling metaphase 2: Move the chromosomes to the center of each cell, so they are lined up at the equator. Make sure the strings attached to the two strips in each chromosome come from opposite sides of the cell.
- Modeling anaphase 2: Grab onto the strings on both sides of each cell, and pull them slowly in opposite directions. The strips should separate. Only one of the chromatids should have the paper clip still attached to it.
- Modeling telophase 2: Remove the strings and paper clips. Each strip of paper now represents a chromosome. Place a 40 cm. piece of string around each group of chromosomes, forming four nuclei. Place a 1m string around each cell, forming four separate cells with only one chromosome in each.

1. What process did you model when you cut the strips in half in interphase?

2. What is the function of your paper clip? Why is it used to represent a centromere?

3. What is the purpose of placing the light and dark strips of the same color side by side?

4. How many chromosomes are in each cell at the end of meiosis I? Describe what each part of your model represents.
5. What is the diploid chromosome number of the original cell in your model? How many homologous pairs did you make?

6. If a cell with a diploid number of 8 chromosomes undergoes meiosis, draw what the cell looks like after Telophase 1.

7.

8. What would happen to an offspring if cells did not undergo meiosis before sexual reproduction?

9. How does crossing over change diversity of traits in a population?

10. Predict what would happen if homologous chromosomes did not pair in prophase I. Use your model to show this.

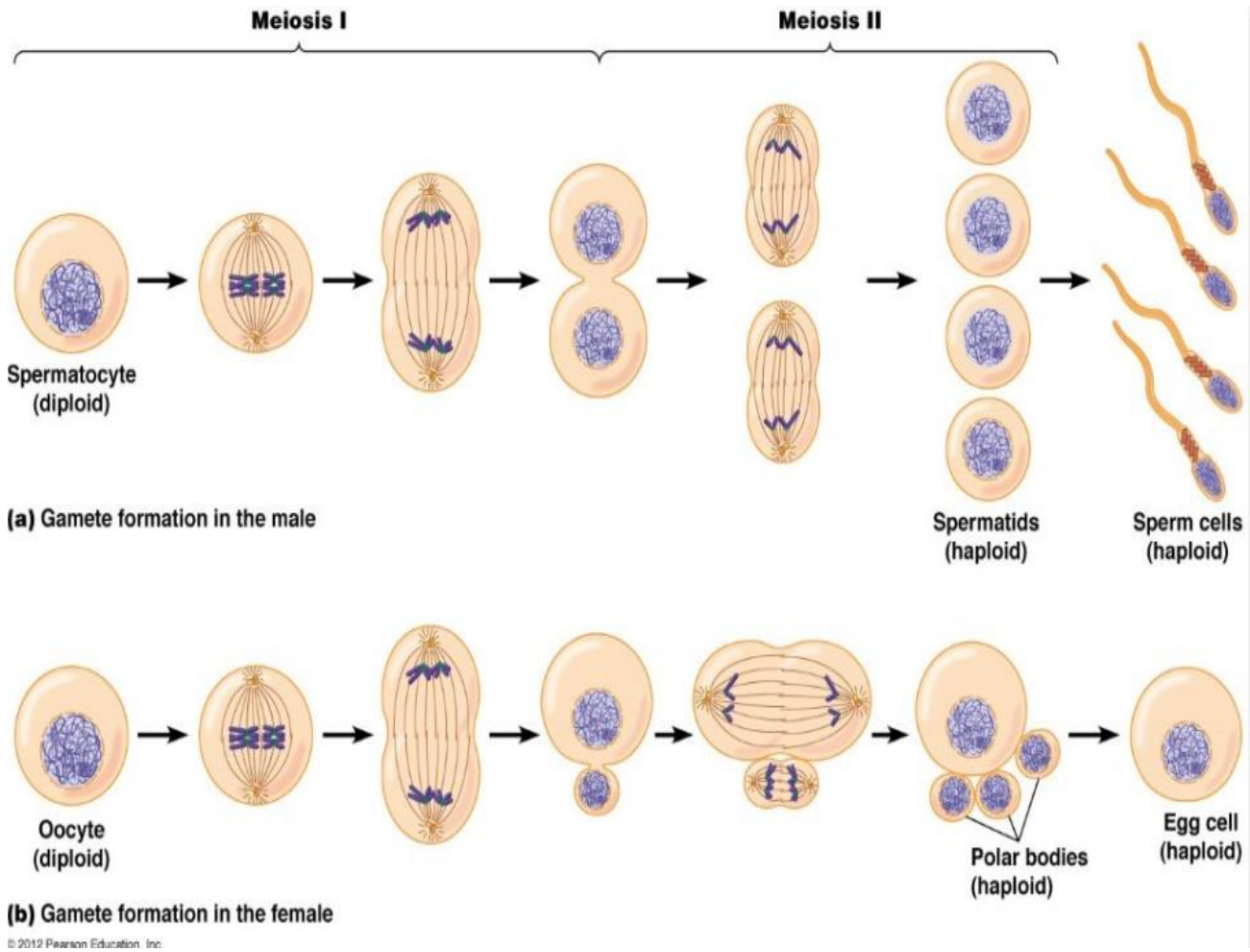


Figure 9.3: Different stages of Meiosis I and Meiosis II

Answer Following questions:

1. What is the purpose of Meiosis?

   _____

2. What are homologous chromosomes?

   _____

3. Which stage are there homologous chromosomes lined up down the middle of the cell?

   _____

4. Which stage are there sister chromatids lined up down the middle of the cell?

   _____

5. What does it mean to be diploid?

   _____

6. Give an example of a diploid cell _____

7. What does it mean to be haploid?

   _____

8. Give an example of a haploid cell _____

9. At the end of Meiosis I
   a. How many cells are there? _____
   b. Are they haploid or diploid? _____

10. At the end of Meiosis II
    a. How many cells are there? _____
    b. Are they haploid or diploid? _____

11. **Drawing Conclusions:** Look at your four gametes. How does meiosis lead to increased genetic variation? (consider crossing over)

    _____

    _____

    _____

12. **Understanding:** What is meant by the statement that Meiosis results in gene segregation?

13. **Predicting:** How would the gametes be affected if a pair of chromatids failed to separate in the second meiotic division?

_____

_____

_____

Name of Phase	Description
	Homologous chromosomes pair up and form tetrad
	Spindle fibers move homologous chromosomes to opposite sides
	Nuclear membrane reforms, cytoplasm divides, 4 daughter cells formed
	Chromosomes line up along equator, not in homologous pairs
	Crossing-over occurs
	Chromatids separate
	Homologs line up alone equator
	Cytoplasm divides, 2 daughter cells are formed

Chapter 10: Understanding of Genetics: Patterns of Inheritance, Mendelian Genetics, Genotype and Phenotype

Learning outcomes: After the completion of this section, students will be able to:

- Differentiate between genotype and phenotype
- Describe how alleles determine a person's traits
- Summarize Mendel's experiments and relate them to human genetics
- Explain the inheritance of autosomal dominant and recessive and sex-linked genetic disorders

Introduction:

We have discussed the events that lead to the development of a newborn. But what makes each newborn unique? The answer lies, of course, in the DNA in the sperm and oocyte that combined to produce that first diploid cell, the human zygote. Each human body cell has a full complement of DNA stored in 23 pairs of chromosomes. The image below shows the pairs in a systematic arrangement called a karyotype. Among these is one pair of chromosomes, called the sex chromosomes, that determines the sex of the individual (XX in females, XY in males). The remaining 22 chromosome pairs are called autosomal chromosomes. Each of these chromosomes carries hundreds or even thousands of genes, each of which codes for the assembly of a particular protein—that is, genes are "expressed" as proteins. An individual's complete genetic makeup is referred to as his or her genotype. The characteristics that the genes express, whether they are physical, behavioral, or biochemical, are a person's phenotype. You inherit one chromosome in each pair—a full complement of 23—from each parent. This occurs when the sperm and oocyte combine at the moment of your conception. Homologous chromosomes—those that make up a complementary pair—have genes for the same characteristics in the same location on the chromosome. Because one copy of a gene, an allele, is inherited from each parent, the alleles in these complementary pairs may vary. Take for example an allele that encodes for dimples. A child may inherit the allele encoding for dimples on the chromosome

from the father and the allele that encodes for smooth skin (no dimples) on the chromosome from the mother.

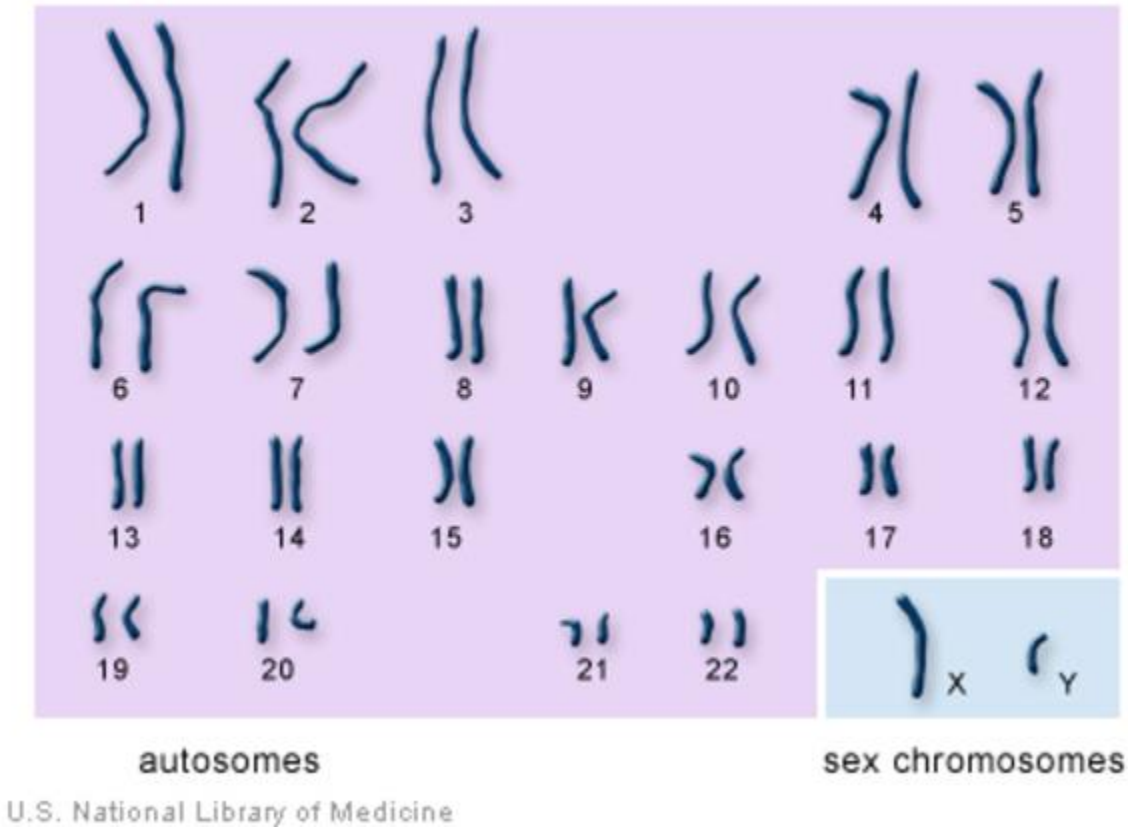


Figure 10.1 Karyon of a human male. Human chromosomes. They have been stained and arranged in order of decreasing size. The 22 autosomes are numbered by size. The other two chromosomes, X and Y, are the sex chromosomes. This picture of the human chromosomes lined up in pairs is called a karyotype. Figure 1. Each pair of chromosomes contains hundreds to thousands of genes. The banding patterns are nearly identical for the two chromosomes within each pair, indicating the same organization of genes. As is visible in this karyotype, the only exception to this is the XY sex chromosome pair in males. (credit: National Human Genome Research Institute)

Although a person can have two identical alleles for a single gene (a homozygous state), it is also possible for a person to have two different alleles (a heterozygous state). The two alleles can interact in several different ways. The expression of an allele can be dominant, for which the

activity of this gene will mask the expression of a nondominant, or recessive, allele. Sometimes dominance is complete; at other times, it is incomplete. In some cases, both alleles are expressed at the same time in a form of expression known as codominance.

In the simplest scenario, a single pair of genes will determine a single heritable characteristic. However, it is quite common for multiple genes to interact to confer a feature. For instance, eight or more genes—each with their own alleles—determine eye color in humans. Moreover, although any one person can only have two alleles corresponding to a given gene, more than two alleles commonly exist in a population. This phenomenon is called multiple alleles. For example, there are three different alleles that encode ABO blood type; these are designated I^A, I^B, and *i*.

Over 100 years of theoretical and experimental genetics studies, and the more recent sequencing and annotation of the human genome, have helped scientists to develop a better understanding of how an individual's genotype is expressed as their phenotype. This body of knowledge can help scientists and medical professionals to predict, or at least estimate, some of the features that an offspring will inherit by examining the genotypes or phenotypes of the parents. One important application of this knowledge is to identify an individual's risk for certain heritable genetic disorders. However, most diseases have a multigenic pattern of inheritance and can also be affected by the environment, so examining the genotypes or phenotypes of a person's parents will provide only limited information about the risk of inheriting a disease. Only for a handful of single-gene disorders can genetic testing allow clinicians to calculate the probability with which a child born to the two parents tested may inherit a specific disease.

Mendel's Theory of Inheritance

Our contemporary understanding of genetics rests on the work of a nineteenth-century monk. Working in the mid-1800s, long before anyone knew about genes or chromosomes, Gregor Mendel discovered that garden peas transmit their physical characteristics to subsequent generations in a discrete and predictable fashion. When he mated, or crossed, two pure-breeding pea plants that differed by a certain characteristic, the first-generation offspring all looked like one of the parents. For instance, when he crossed tall and dwarf pure-breeding pea plants, all of the offspring were tall. Mendel called tallness dominant because it was expressed in offspring

when it was present in a purebred parent. He called dwarfism recessive because it was masked in the offspring if one of the purebred parents possessed the dominant characteristic. Note that tallness and dwarfism are variations on the characteristic of height. Mendel called such a variation a trait. We now know that these traits are the expression of different alleles of the gene encoding height.

Mendel performed thousands of crosses in pea plants with differing traits for a variety of characteristics. And he repeatedly came up with the same results—among the traits he studied, one was always dominant, and the other was always recessive. (Remember, however, that this dominant–recessive relationship between alleles is not always the case; some alleles are codominant, and sometimes dominance is incomplete.)

Using his understanding of dominant and recessive traits, Mendel tested whether a recessive trait could be lost altogether in a pea lineage or whether it would resurface in a later generation. By crossing the second-generation offspring of purebred parents with each other, he showed that the latter was true: recessive traits reappeared in third-generation plants in a ratio of 3:1 (three offspring having the dominant trait and one having the recessive trait). Mendel then proposed that characteristics such as height were determined by heritable "factors" that were transmitted, one from each parent, and inherited in pairs by offspring.

In the language of genetics, Mendel's theory applied to humans says that if an individual receives two dominant alleles, one from each parent, the individual's phenotype will express the dominant trait. If an individual receives two recessive alleles, then the recessive trait will be expressed in the phenotype. Individuals who have two identical alleles for a given gene, whether dominant or recessive, are said to be homozygous for that gene (homo- = "same"). Conversely, an individual who has one dominant allele and one recessive allele is said to be heterozygous for that gene (hetero- = "different" or "other"). In this case, the dominant trait will be expressed, and the individual will be phenotypically identical to an individual who possesses two dominant alleles for the trait.

It is common practice in genetics to use capital and lowercase letters to represent dominant and recessive alleles. Using Mendel's pea plants as an example, if a tall pea plant is homozygous, it

will possess two tall alleles (*TT*). A dwarf pea plant must be homozygous because its dwarfism can only be expressed when two recessive alleles are present (*tt*). A heterozygous pea plant (*Tt*) would be tall and phenotypically indistinguishable from a tall homozygous pea plant because of the dominant tall allele. Mendel deduced that a 3:1 ratio of dominant to recessive would be produced by the random segregation of heritable factors (genes) when crossing two heterozygous pea plants. In other words, for any given gene, parents are equally likely to pass down either one of their alleles to their offspring in a haploid gamete, and the result will be expressed in a dominant–recessive pattern if both parents are heterozygous for the trait.

Because of the random segregation of gametes, the laws of chance and probability come into play when predicting the likelihood of a given phenotype. Consider a cross between an individual with two dominant alleles for a trait (*AA*) and an individual with two recessive alleles for the same trait (*aa*). All of the parental gametes from the dominant individual would be *A*, and all of the parental gametes from the recessive individual would be *a*. All of the offspring of that second generation, inheriting one allele from each parent, would have the genotype *Aa*, and the probability of expressing the phenotype of the dominant allele would be 4 out of 4, or 100 percent.

This seems simple enough, but the inheritance pattern gets interesting when the second-generation *Aa* individuals are crossed. In this generation, 50 percent of each parent's gametes are *A* and the other 50 percent are *a*. By Mendel's principle of random segregation, the possible combinations of gametes that the offspring can receive are *AA*, *Aa*, *aA* (which is the same as *Aa*), and *aa*. Because segregation and fertilization are random, each offspring has a 25 percent chance of receiving any of these combinations. Therefore, if an *Aa* × *Aa* cross were performed 1000 times, approximately 250 (25 percent) of the offspring would be *AA*; 500 (50 percent) would be *Aa* (that is, *Aa* plus *aA*); and 250 (25 percent) would be *aa*. The genotypic ratio for this inheritance pattern is 1:2:1. However, we have already established that *AA* and *Aa* (and *aA*) individuals all express the dominant trait (i.e., share the same phenotype), and can therefore be combined into one group. The result is Mendel's third-generation phenotype ratio of 3:1.

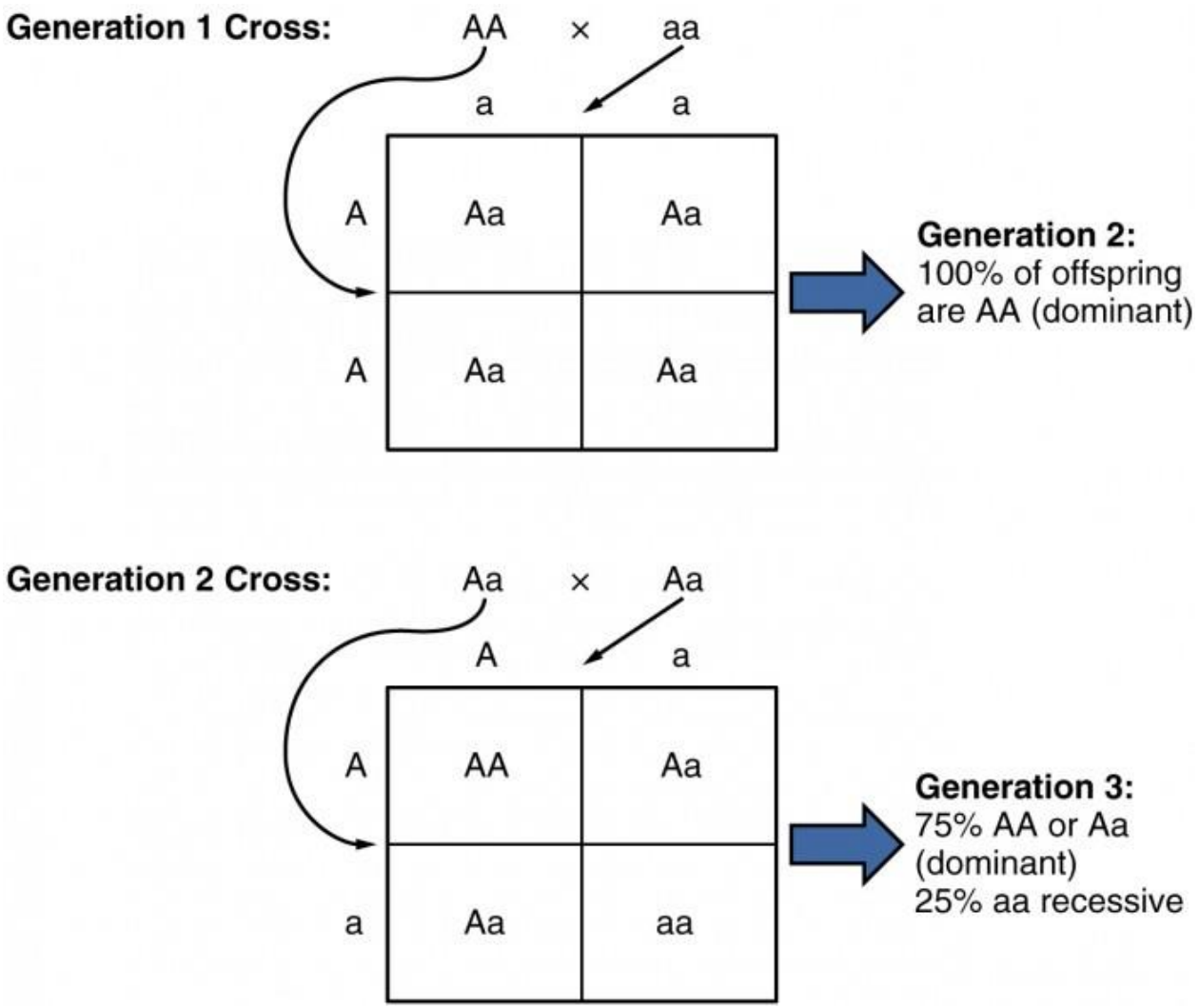


Figure 10.2: In the formation of gametes, it is equally likely that either one of a pair alleles from one parent will be passed on to the offspring. This figure follows the possible combinations of alleles through two generations following a first-generation cross of homozygous dominant and homozygous recessive parents. The recessive phenotype, which is masked in the second generation, has a 1 in 4, or 25 percent, chance of reappearing in the third generation.

1. Two parents have the genotype dd and DD for dimples. The presence of Dimples is dominant. What are the chances that their child will have dimples?

	D	D
d		
d		

2. Two parents have the genotype Cc and cc for cleft chins. The presence of a cleft chin is a recessive trait. What are the chances that their child will have a cleft chin?

	C	c
c		
c		

3. Two parents have eyebrows that are connected (unibrow). This describes their phenotype. The unibrow is a recessive trait. What is their genotype?

4. The gene resulting in the phenotype of flat fingernails is recessive. Rounded fingernails are dominant. Two parents have the genotype Ff and Ff. What is the phenotype of each parent?

What are the chances their child will have flat fingernails?

5. The one parent has hair on his or her toes; the other does not. Hair on the toes is a recessive trait. What are the possible genotypes of the parents?

6. The trait of a curved pinkie toe is a recessive trait. Both parents have curved pinkie toes. What are the chances that their child will have a straight pinkie toe?

Mendel's observation of pea plants also included many crosses that involved multiple traits, which prompted him to formulate the principle of independent assortment. The law states that the members of one pair of genes (alleles) from a parent will sort independently from other pairs of genes during the formation of gametes. Applied to pea plants, that means that the alleles associated with the different traits of the plant, such as color, height, or seed type, will sort

independently of one another. This holds true except when two alleles happen to be located close to one other on the same chromosome. Independent assortment provides for a great degree of diversity in offspring.

Mendelian genetics represent the fundamentals of inheritance, but there are two important qualifiers to consider when applying Mendel's findings to inheritance studies in humans. First, as we've already noted, not all genes are inherited in a dominant–recessive pattern. Although all diploid individuals have two alleles for every gene, allele pairs may interact to create several types of inheritance patterns, including incomplete dominance and codominance.

Secondly, Mendel performed his studies using thousands of pea plants. He was able to identify a 3:1 phenotypic ratio in second-generation offspring because his large sample size overcame the influence of variability resulting from chance. In contrast, no human couple has ever had thousands of children. If we know that a man and woman are both heterozygous for a recessive genetic disorder, we would predict that one in every four of their children would be affected by the disease. In real life, however, the influence of chance could change that ratio significantly. For example, if a man and a woman are both heterozygous for cystic fibrosis, a recessive genetic disorder that is expressed only when the individual has two defective alleles, we would expect one in four of their children to have cystic fibrosis. However, it is entirely possible for them to have seven children, none of whom is affected, or for them to have two children, both of whom are affected. For each individual child, the presence or absence of a single gene disorder depends on which alleles that child inherits from his or her parents.

Autosomal Dominant Inheritance

In the case of cystic fibrosis, the disorder is recessive to the normal phenotype. However, a genetic abnormality may be dominant to the normal phenotype. When the dominant allele is located on one of the 22 pairs of autosomes (non-sex chromosomes), we refer to its inheritance pattern as autosomal dominant. An example of an autosomal dominant disorder is neurofibromatosis type I, a disease that induces tumor formation within the nervous system that leads to skin and skeletal deformities. Consider a couple in which one parent is heterozygous for this disorder (and who therefore has neurofibromatosis), *Nn*, and one parent is homozygous for

the normal gene, *nn*. The heterozygous parent would have a 50 percent chance of passing the dominant allele for this disorder to his or her offspring, and the homozygous parent would always pass the normal allele. Therefore, four possible offspring genotypes are equally likely to occur: *Nn*, *Nn*, *nn*, and *nn*. That is, every child of this couple would have a 50 percent chance of inheriting neurofibromatosis. This inheritance pattern is shown in the table below, in a form called a Punnett square, named after its creator, the British geneticist Reginald Punnett.

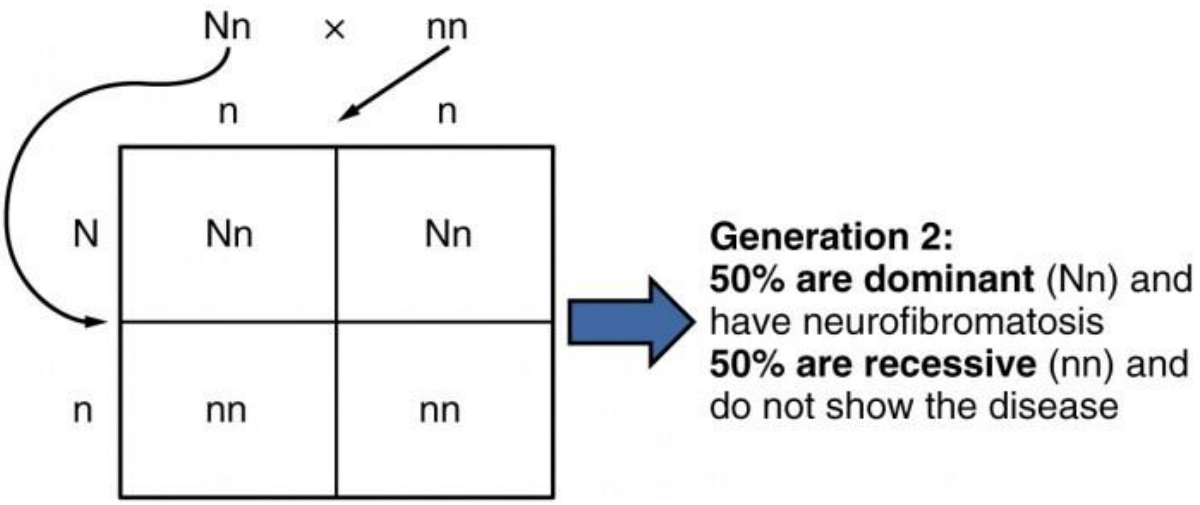


Figure 10. 3: Inheritance pattern of an autosomal dominant disorder, such as neurofibromatosis, is shown in a Punnett square.

Other genetic diseases that are inherited in this pattern are achondroplastic dwarfism, Marfan syndrome, and Huntington's disease. Because autosomal dominant disorders are expressed by the presence of just one gene, an individual with the disorder will know that he or she has at least one faulty gene. The expression of the disease may manifest later in life, after the childbearing years, which is the case in Huntington's disease (discussed in more detail later in this section).

Autosomal Recessive Inheritance

When a genetic disorder is inherited in an autosomal recessive pattern, the disorder corresponds to the recessive phenotype. Heterozygous individuals will not display symptoms of this disorder, because their unaffected gene will compensate. Such an individual is called a carrier. Carriers for

an autosomal recessive disorder may never know their genotype unless they have a child with the disorder.

An example of an autosomal recessive disorder is cystic fibrosis (CF), which we introduced earlier. CF is characterized by the chronic accumulation of a thick, tenacious mucus in the lungs and digestive tract. Decades ago, children with CF rarely lived to adulthood. With advances in medical technology, the average lifespan in developed countries has increased into middle adulthood. CF is a relatively common disorder that occurs in approximately 1 in 2000 Caucasians. A child born to two CF carriers would have a 25 percent chance of inheriting the disease. This is the same 3:1 dominant:recessive ratio that Mendel observed in his pea plants would apply here. The pattern is shown in the image below, using a diagram that tracks the likely incidence of an autosomal recessive disorder on the basis of parental genotypes.

On the other hand, a child born to a CF carrier and someone with two unaffected alleles would have a 0 percent probability of inheriting CF, but would have a 50 percent chance of being a carrier. Other examples of autosome recessive genetic illnesses include the blood disorder sickle-cell anemia, the fatal neurological disorder Tay–Sachs disease, and the metabolic **disorder phenylketonuria.**

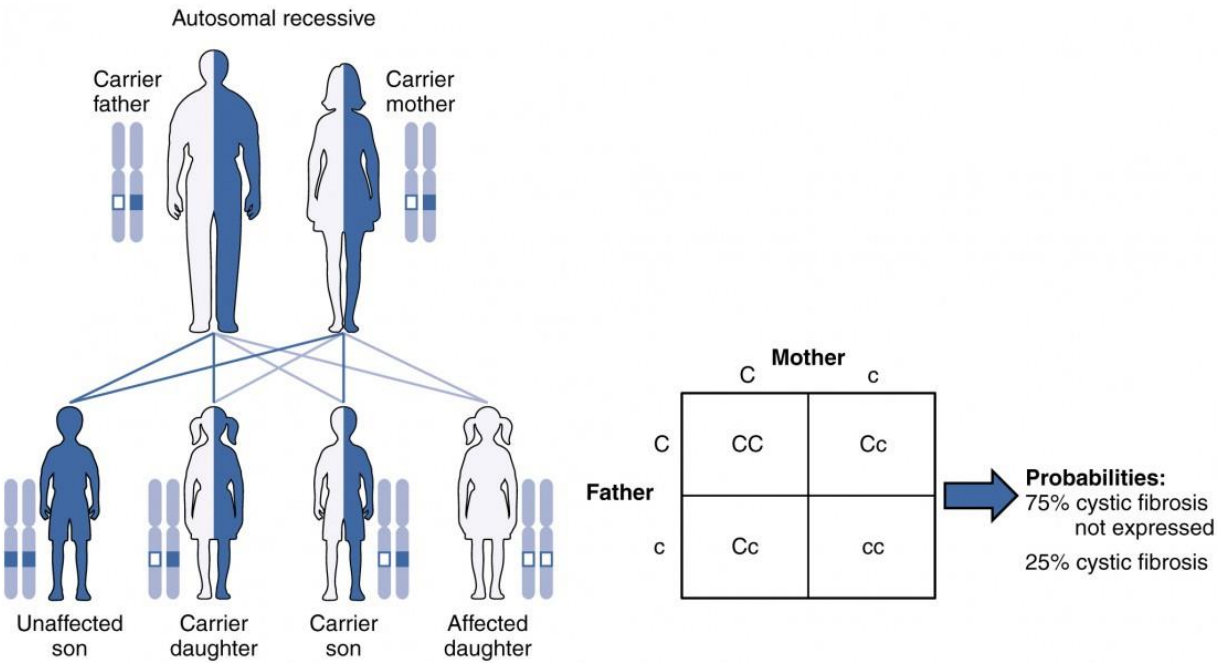


Figure 10. 4: The inheritance pattern of an autosomal recessive disorder with two carrier parents reflects a 3:1 probability of expression among offspring. (credit: U.S. National Library of Medicine)

X-linked Dominant or Recessive Inheritance

An X-linked transmission pattern involves genes located on the X chromosome of the 23rd pair. Recall that a male has one X and one Y chromosome. When a father transmits a Y chromosome, the child is male, and when he transmits an X chromosome, the child is female. A mother can transmit only an X chromosome, as both her sex chromosomes are X chromosomes.

X-linked Dominant Inheritance

When an abnormal allele for a gene that occurs on the X chromosome is dominant over the normal allele, the pattern is described as X-linked dominant. This is the case with vitamin D–resistant rickets: an affected father would pass the disease gene to all of his daughters, but none of his sons, because he donates only the Y chromosome to his sons. If it is the mother who is affected, all of her children—male or female—would have a 50 percent chance of inheriting the disorder because she can only pass an X chromosome on to her children. For an affected female,

the inheritance pattern would be identical to that of an autosomal dominant inheritance pattern in which one parent is heterozygous and the other is homozygous for the normal gene.

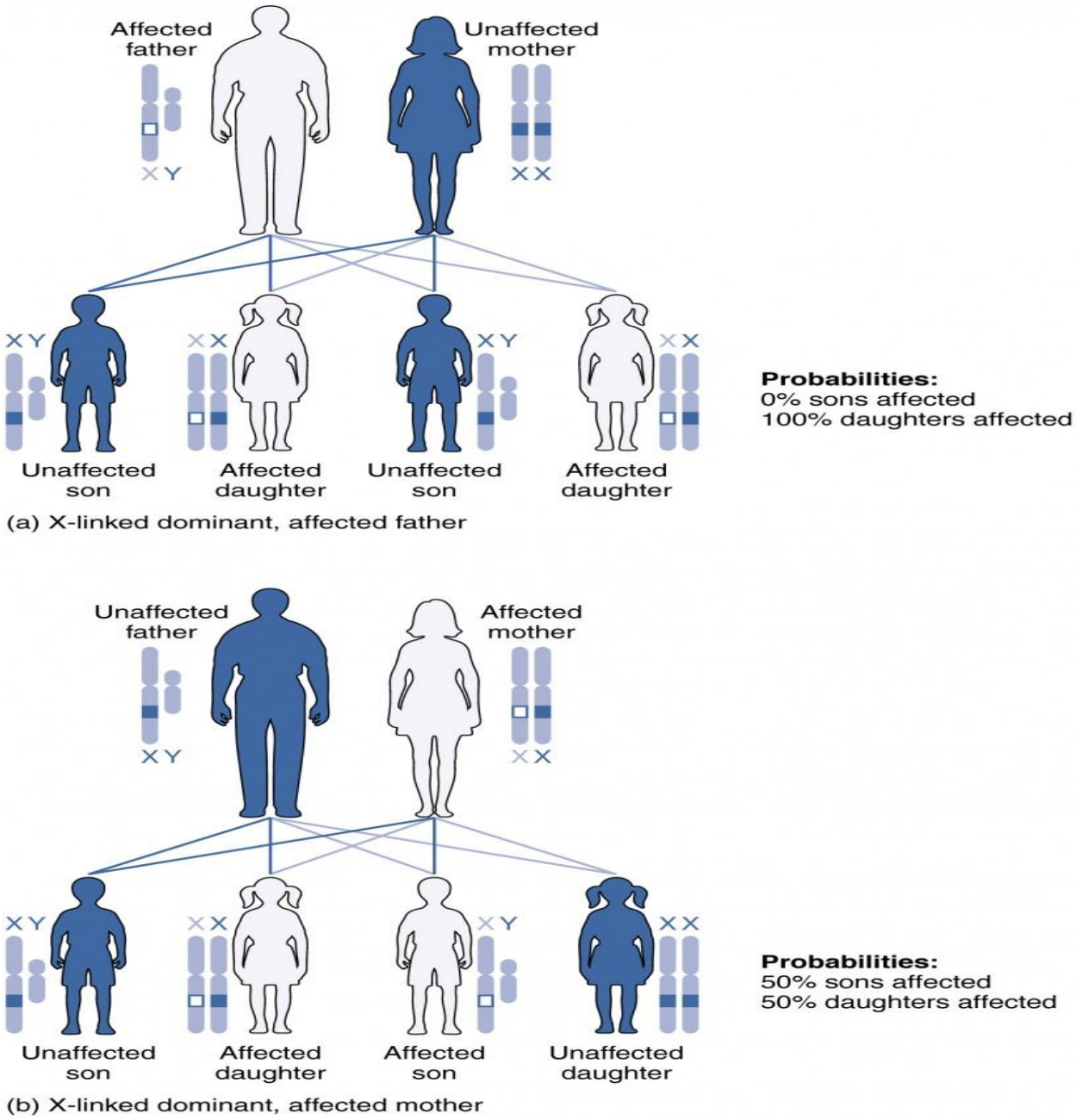


Figure 10.5:. Click for a larger image. A chart of X-linked dominant inheritance patterns differs depending on whether (a) the father or (b) the mother is affected with the disease. (credit: U.S. National Library of Medicine)

X-linked Recessive Inheritance

X-linked recessive inheritance is much more common because females can be carriers of the disease yet still have a normal phenotype. Diseases transmitted by X-linked recessive inheritance include color blindness, the blood-clotting disorder hemophilia, and some forms of muscular dystrophy. For an example of X-linked recessive inheritance, consider parents in which the mother is an unaffected carrier and the father is normal. None of the daughters would have the disease because they receive a normal gene from their father. However, they have a 50 percent chance of receiving the disease gene from their mother and becoming a carrier. In contrast, 50 percent of the sons would be affected. With X-linked recessive diseases, males either have the disease or are genotypically normal—they cannot be carriers. Females, however, can be genotypically normal, a carrier who is phenotypically normal, or affected with the disease. A daughter can inherit the gene for an X-linked recessive illness when her mother is a carrier or affected, or her father is affected. The daughter will be affected by the disease only if she inherits an X-linked recessive gene from both parents. As you can imagine, X-linked recessive disorders affect many more males than females. For example, color blindness affects at least 1 in 20 males, but only about 1 in 400 females.

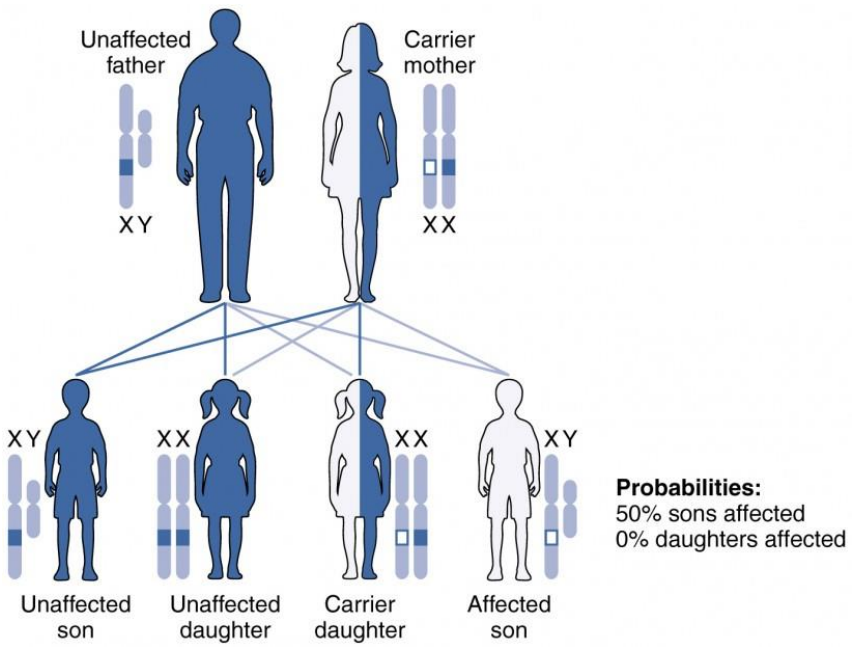


Figure 10.6: Given two parents in which the father is normal and the mother is a carrier of an X-linked recessive disorder, a son would have a 50 percent probability of being affected with the disorder, whereas daughters would either be carriers or entirely unaffected. (credit: U.S. National Library of Medicine)

Chapter 11: Understanding of DNA and Genetic Code

Learning outcomes: After completion of the current lab, students will be able to learn:

- Purify DNA from plant tissue.
- Model all stages of gene expression.
- Analyze DNA subjected to gel electrophoresis
- Understand the principle the polymerase chain reaction (PCR) and its application

Introduction

Deoxyribonucleic acid, or DNA for short, is a nucleic acid commonly referred to as "genetic material". As you learned in last week's laboratory, genes are actually specific segments of chromosomes which are simply really long pieces of DNA. So given the obvious importance of DNA we want to make sure you understand DNA structure, how DNA is copied in cells, and how specific genes are expressed into "gene products", which for most genes is a specific protein. While it would be good to learn about all these aspects of DNA before learning how to purify and analyze it in the laboratory, for practical reasons it will be necessary to do things the other way around. So let's start with gel electrophoresis.

Biotechnology is the use of artificial methods to modify the genetic material of living organisms or cells to produce novel compounds or to perform new functions. Biotechnology has been used for improving livestock and crops since the beginning of agriculture through selective breeding. Since the discovery of the structure of DNA in 1953, and particularly since the development of tools and methods to manipulate DNA in the 1970s, biotechnology has become synonymous with the manipulation of organisms' DNA at the molecular level. The primary applications of this technology are in medicine (for the production of vaccines and antibiotics) and in agriculture (for the genetic modification of crops). Biotechnology also has many industrial applications, such as fermentation, the treatment of oil spills, and the production of biofuels, as well as many household applications such as the use of enzymes in laundry detergent.

To understand the basic techniques used to work with nucleic acids, remember that nucleic acids are macromolecules made of nucleotides (a sugar, a phosphate, and a nitrogenous base). The phosphate groups on these molecules each have a net negative charge. An entire set of DNA molecules in the nucleus of eukaryotic organisms is called the genome. DNA has two complementary strands linked by hydrogen bonds between the paired bases. Unlike DNA in eukaryotic cells, RNA molecules leave the nucleus. Messenger RNA (mRNA) is analyzed most frequently because it represents the protein-coding genes that are being expressed in the cell.

Isolation of Nucleic Acids

To study or manipulate nucleic acids, the DNA must first be extracted from cells. Various techniques are used to extract different types of DNA (**Figure 11.1**). Most nucleic acid extraction techniques involve steps to break open the cell, and then the use of enzymatic reactions to destroy all undesired macromolecules. Cells are broken open using a detergent solution containing buffering compounds. To prevent degradation and contamination, macromolecules such as proteins and RNA are inactivated using enzymes. The DNA is then brought out of solution using alcohol. The resulting DNA, because it is made up of long polymers, forms a gelatinous mass. This method extracts all the nucleic acid within a cell. This includes **genomic DNA** (all the DNA in the genome), as well as RNA. If this DNA was to be used for further study, the RNA would often be digested with an enzyme to remove it.

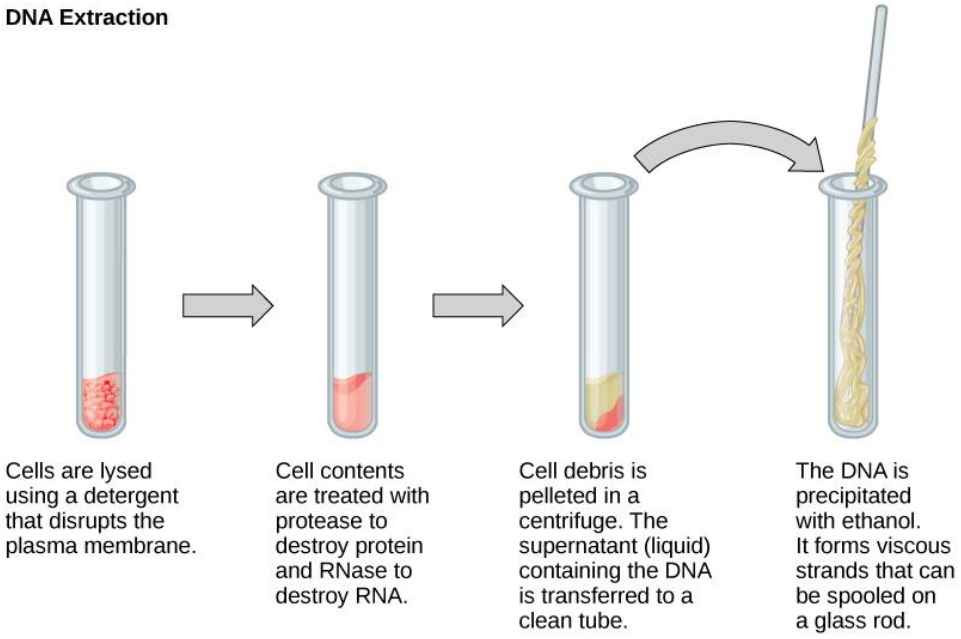
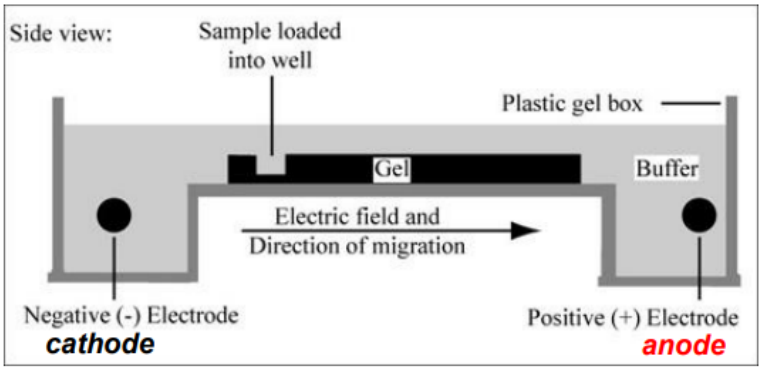


Figure 11.1 This diagram shows the basic method used for the extraction of DNA.

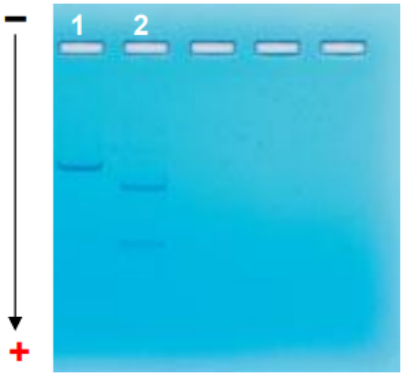
Relatively, RNA is studied to understand gene expression patterns in cells. RNA is naturally very unstable because enzymes that break down RNA are commonly present in nature. Some are even secreted by our own skin and are very difficult to inactivate. Similar to DNA extraction, RNA extraction involves the use of various buffers and enzymes to inactivate other macromolecules and preserve only the RNA.

Gel Electrophoresis

Below is a diagram of an **electrophoresis chamber** or "gel box" containing an **agarose gel** submerged in **electrophoresis buffer** (liquid that will conduct electrical current) as viewed from the side:



DNA molecules have a net negative charge for reasons you will learn later on. When a DNA sample is placed in the **well** of an agarose gel submerged in electrophoresis buffer and subjected to electrical current, the DNA will be drawn toward the positive electrode, the **anode** (colored ***red***), and away from the negative electrode, the **cathode** (colored ***black***). The rate at which DNA fragments move through the porous agarose gel matrix is inversely proportional to the length of the DNA fragment. In other words, the shorter the DNA fragment, the faster it will move through the gel. Likewise, the longer the DNA fragment, the slower it will move through the gel. The picture below shows an agarose gel (looking down onto the gel) after it has been run and stained to reveal the DNA fragments which are dark blue:



This gel reveals that the DNA sample loaded into the first well (lane 1) contains 9 bands and thus 9 DNA fragments of different sizes. The DNA samples in lanes 2 and 3 contain one and two different fragment sizes, respectively. The fragments that moved the furthest down the gel are the shortest and the fragments that moved the least distance are the longest. Keep in mind that each band you see on a stained gel is not a single piece of DNA but literally *billions* of copies of the same DNA fragment.

Because nucleic acids are negatively charged ions at neutral or alkaline pH in an aqueous environment, they can be moved by an electric field. **Gel electrophoresis** is a technique used to separate charged molecules on the basis of size and charge. The nucleic acids can be separated as whole chromosomes or as fragments. The nucleic acids are loaded into a slot at one end of a gel matrix, an electric current is applied, and negatively charged molecules are pulled toward the opposite end of the gel (the end with the positive electrode). Smaller molecules move through the pores in the gel faster than larger molecules; this difference in the rate of migration separates the fragments on the basis of size. The nucleic acids in a gel matrix are invisible until they are stained with a compound that allows them to be seen, such as a dye. Distinct fragments of nucleic acids appear as bands at specific distances from the top of the gel (the negative electrode end) that are based on their size (**Figure 11.2**). A mixture of many fragments of varying sizes appear as a long smear, whereas uncut genomic DNA is usually too large to run through the gel and forms a single large band at the top of the gel.

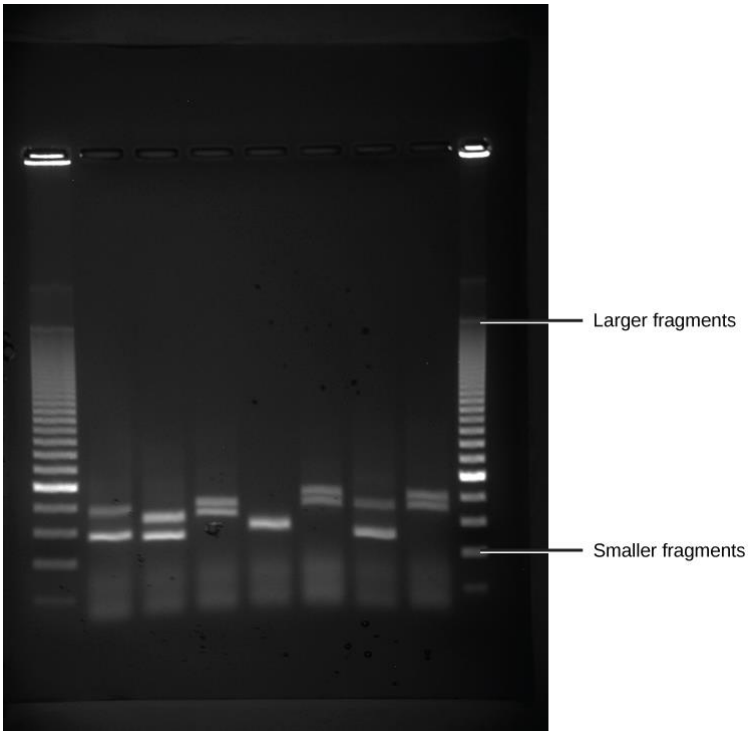


Figure: 11.2 Shown are DNA fragments from six samples run on a gel, stained with a fluorescent dye and viewed under UV light.

Polymerase Chain Reaction

DNA analysis often requires focusing on one or more specific regions of the genome. It also frequently involves situations in which only one or a few copies of a DNA molecule are available for further analysis. These amounts are insufficient for most procedures, such as gel electrophoresis. **Polymerase chain reaction (PCR)** is a technique used to rapidly increase the number of copies of one specific region of DNA for further analyses (**Figure11.3**). Typically the DNA that is used as the starting sample in a PCR reaction is genomic DNA, which would contain all the genes in the organism. PCR uses a special form of heat tolerant DNA polymerase, the enzyme that replicates DNA, and other short nucleotide sequences called primers that base pair to a specific portion of the DNA being copied. A PCR reaction does not copy the entire genome, rather it makes millions of copies of one specific region of interest.

- *Taq* DNA polymerase – this polymerase was isolated from *Thermus aquaticus*, which is a bacteria that lives at high temperatures in hot springs and deep sea vents. *Taq* polymerase has an optimum temperature of 70-80ºC and can survive nearly an hour at 95ºC. Like other DNA polymerases, *Taq* polymerase assembles nucleotides only in the 5′ to 3′ direction.
- Primers – Just like during DNA replication, *Taq* polymerase needs a free 3′ end to begin synthesis of the new DNA. Primers in a PCR reaction are man-made synthetic segments of DNA that match the ends of the sequence that the scientist is interested in amplifying. In order to synthesize both strands of the DNA double helix, a forward and a reverse primer must be added. These two primers have free 3′ ends that point towards the sequence of interest.

PCR is used for many purposes in laboratories. These include: 1) the identification of the owner of a DNA sample left at a crime scene; 2) paternity analysis; 3) the comparison of small amounts of ancient DNA with modern organisms; and 4) determining the sequence of nucleotides in a specific region. In all of these cases, the starting sample is genomic DNA. In some cases, the complete genome may not be present due to the DNA being old or broken down.

PCR involves the following basic steps:

1. Denaturation (94ºC): The sample is heated in a small plastic tube inside a thermocycler (which is able to rapidly change temperatures very accurately). Heating the sample to 94ºC causes the hydrogen bonds holding the DNA double helix to denature so the strands are separated (**Figure11.3** top). *Taq* polymerase is heat tolerant and does not denature at this temperature.

2. Annealing (54-60ºC): The sample is cooled so that the primers can anneal (base pair) with their complementary sequence (**Figure11.3;** middle). Because of the base pairing rules, primers can only anneal in the specific spot that contains their complementary base sequence. This allows scientists to choose which region of DNA will be amplified.
3. Extension (72ºC): The sample is warmed up to the optimum temperature for *Taq* polymerase. Taq extends off of the free 3′ end of both primers, producing a double helix (**Figure11.3**; bottom).

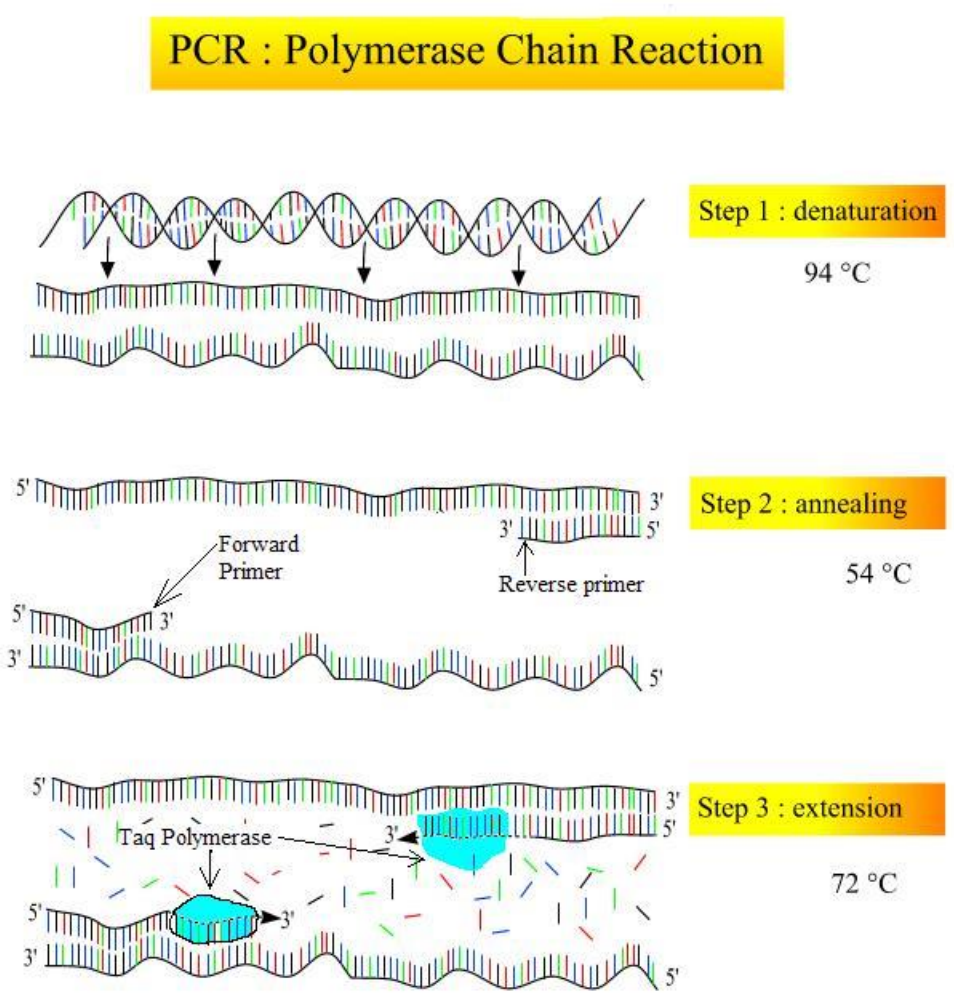


Figure: 11.3 The steps of a PCR reaction. The three basic steps of a PCR reaction are repeated multiple times ("cycles"). Each cycle causes a roughly 2x increase in the number of amplified copies of the specific section of interest. Remember that only one section of DNA is being amplified due to the specificity of the primers.

DNA Isolation

DNA is surprisingly easy to purify from plant and animal tissues. You may recall from an earlier lab that chromosomes, which are made of DNA, are stored in the nuclei of eukaryotic cells. So to purify chromosomal DNA you need to somehow release it from the cell nuclei and then separate it from all other cellular materials.

To do this will require three general steps:

1. Breaking open cells in the tissue to release the DNA from cell nuclei • this is done by mashing the tissue in a solution with detergent and salt to break up cell membranes
2. Removing the insoluble material (the DNA remains dissolved in liquid since it is soluble) • this is done by filtering the tissue "mash" and retaining the liquid portion
3. Precipitating the DNA in the liquid collected (making the DNA insoluble) • this is done by adding the right amounts of salt and alcohol to make the DNA insoluble

Now that you have a basic understanding of the principles involved, let's purify some DNA from strawberry tissue…

Carefully follow the steps below, and in about 5 minutes you will have isolated strawberry DNA!

1. Add 4 ml of detergent/salt solution to the strawberry slice in a Ziploc bag which should already be at your table.

2. Remove most of the air from the Ziploc bag, seal it, and thoroughly mash the strawberry slice in the detergent/salt solution (squeeze with hands or press on table top).

3. Set up a funnel over a glass beaker and line the funnel with 2 or more layers of cheese cloth (fold the cheese cloth into 2 or more layers).

4. Transfer as much of the strawberry mash as you can from the Ziploc bag to the cheese cloth in the funnel.

5. Allow the liquid from the strawberry mash to pass through the cheesecloth and collect in the beaker (you can squeeze the cheesecloth to help as much liquid as possible pass through).

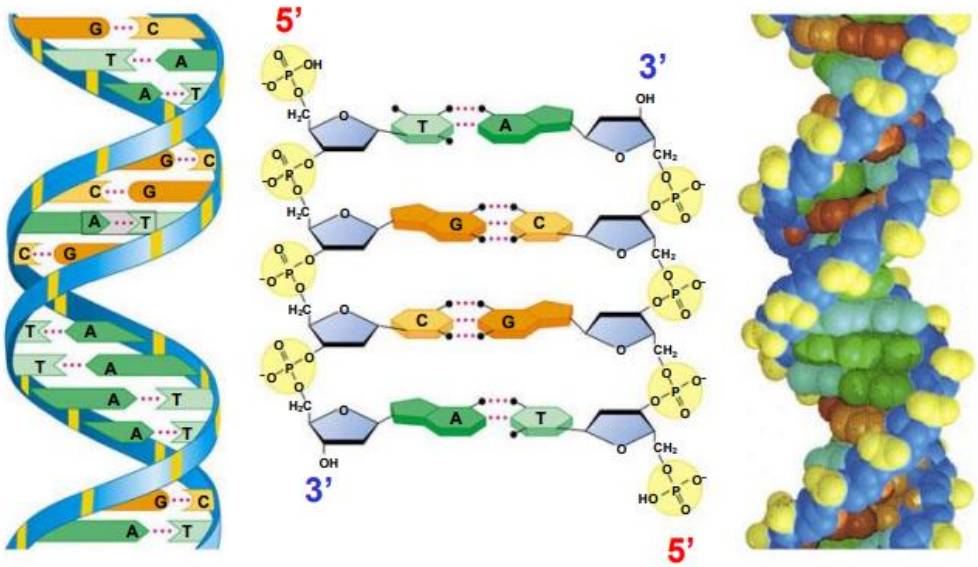
6. Transfer the liquid to a test tube and add one squeeze of ice cold ethanol from a transfer pipet. Do not mix.

7. Use a hooked glass rod to gently collect precipitated DNA at the interface of the ethanol and the strawberry liquid below.

8. After precipitating the strawberry DNA, use the hooked glass rod to remove it to the garbage, pour the remaining liquid down the sink; rinse out your test tube, beaker and funnel; and wash the cheesecloth with soap and water so it can be reused.

Now you have strawberry DNA like this:

Below are three different illustrations, each representing a double-stranded DNA molecule. Notice the base pairing between the two strands and that they are **anti-parallel** (the 5' and 3' ends are oriented in the opposite direction). The two strands also form a coil or **helix**, hence the reference to DNA as a double-stranded helix or simply a "**double helix**".



The Genetic Code: It is better idea that before we look at translation, you need to understand the genetic code. While this may seem like yet another thing you need to learn in biology class, having knowledge of the genetic code is truly a remarkable privilege. Scientists and thinkers from the past would have given anything to know what you are about to learn, arguably the most fundamental biological process there is: how genes store information, information in the form of a DNA sequence that can be expressed into proteins and passed on to the next generation. In other words, you are about to learn what genetic information actually means, not just for human beings, but for all life on earth. Once it was known that genes are made of DNA which somehow codes for proteins, a number of scientists set out to uncover the underlying genetic code. Since proteins are polymers of amino acids, they reasoned correctly that the sequence of nucleotides in DNA (or more specifically the sequence of bases) must somehow encode specific amino acids and their order in a protein. There are 20 amino acids used to make proteins. This fact was well known in the early 1960s when these investigations began, thus the genetic code was assumed to consist of at least 20 different short nucleotide sequences, presumably of the same length. Given that there are only 4 different one nucleotide sequences (A, C, G, T), 16 different two nucleotide sequences (AA, AC, AG, AT...), and 64 different three nucleotide sequences (AAA, AAC, AAG, AAT…), a code consisting of three nucleotide sequences was clearly the best candidate. Through some very clever experimentation the genetic code was soon discovered to be just that, a 3-nucleotide code which is read in a 5’ to 3’ direction. We now refer to each 3-nucleotide combination in the genetic code as a codon. The meanings of all 64 codons have been determined and are represented in the following chart:

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phe UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr UAA: Stop UAG: Stop	UGU, UGC: Cys UGA: Stop UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

This chart uses uracil (U) instead of thymine (T) since RNA, not DNA, is used to make proteins during the process of translation (though both nucleotides have the same meaning in the genetic code). The chart is organized to make it easy to find any particular codon. Codons in each row begin with the same base, codons in each column have the same second base, and codons in each box have a different third base.

If you look carefully you will notice that all 20 amino acids are represented in this code, with some being represented by only one codon (e.g., UGG for tryptophan), and others being represented by as many as six codons (e.g., leucine). You will also notice that there is a single start codon (AUG, which also codes for methionine), as well as three stop codons (UAA, UAG, UGA). The importance of these codons in the production of a polypeptide will be addressed as we move on to the final step of gene expression, translation, but not before you complete the next exercise.

Examine a chart of the genetic code and write all codons that specify the amino acid serine.

Determine the amino acid sequence encoded by the following mRNA sequence.

5’ – GCGUAUGACCGUCCGUCUUUAACGAAU – 3’

Appendix I

Conversion of Metric Units to English Units

Units of Length

The meter (m) is the basic unit of length.

$1\ \text{m} = 39.4$ inches (in)
$= 1.1$ yards (yd)
$= 3.28$ feet (ft)

$1\ \text{km} = 1000\ \text{m} = 10^3\ \text{m}$
$= 0.62$ mile (mi)

$1\ \text{cm} = 0.01\ \text{m} = 10^{-2}\text{m}$
$= 0.39\ \text{in} = 10\ \text{mm}$

$1\ \text{nm} = 10^{-9}\ \text{m} = 10^{-6}\ \text{mm}$
$= 10$ angstroms (Å)

$1\ \text{in} = 2.54\ \text{cm}$

$1\ \text{ft} = 30.5\ \text{cm}$
$= 0.305\ \text{m}$

$1\ \text{yd} = 0.91\ \text{m}$

$1\ \text{mi} = 1.61\ \text{km}$

Units of area are squared (two-dimensional) units of length.

$1\ \text{m}^2 = 1.20\ \text{yd}^2 = 1550\ \text{in}^2 = 1.550 \times 10^3\ \text{in}^2$

1 hectare = 10,000 square meters $(\text{m}^2) = 2.47$ acres

Measurements of area and volume can use the same units.

$1\ \text{m}^3 = 35.314\ \text{ft}^3 = 1.31\ \text{yd}^3$

$1\ \text{cm}^3$ (cc) $= 0.000001\ \text{m}^3 = 0.061\ \text{in}^3$

Units of Mass

The kilogram (kg) is the basic unit of mass. The kilogram is the only base-unit of the International System of Units (SI) with a prefix ("kilo," symbol "k").*

$1\ \text{kg} = 1000\ \text{g} = 10^3\ \text{g} = 2.2\ \text{lb}$

$1\ \text{g} =$ mass of $1\ \text{cm}^3$ of water at $4^\circ\text{C} = 0.035$ oz

Units of Volume

The liter (L) is the basic unit of volume. Units of volume are cubed (three-dimensional) units of length.

1 liter $= 1000\ \text{cm}^3$

1 liter $= 2.1$ pints $= 1.06$ qt

1 liter $= 0.26$ gal $= 1\ \text{dm}^3$

$1\ \text{mL} = 0.034$ fl oz

1 cup $= 240\ \text{mL}$

Units of Temperature

$5 \times$ degrees Fahrenheit $= (9 \times$ degrees Celsius$) + 160$

For example:

$40^\circ\text{C} = 104^\circ\text{F}$ (a hot summer day)
$75^\circ\text{C} = 167^\circ\text{F}$ (hot coffee)
$-5^\circ\text{C} = 23^\circ\text{F}$ (coldest area of freezer)
$37^\circ\text{C} = 98.6^\circ\text{F}$ (human body temperature)
$-40^\circ\text{C} = -40^\circ\text{F}$ (extremely cold day)

Appendix II

Some of the Major Differences between Prokaryotic and Eukaryotic Cells and between Plant and Animal Cells			
Prokaryote		**Eukaryote**	
		Animal	**Plant**
Exterior Structures			
Cell wall	Present (protein-polysaccharide)	Absent	Present (cellulose)
Cell membrane	Present	Present	Present
Flagella	May be present (single strand)	May be present	Absent except in sperm of a few species
Interior Structures			
ER	Absent	Usually present	Usually present
Ribosomes	Present	Present	Present
Microtubules	Absent	Present	Present
Centrioles	Absent	Present	Absent
Golgi complex	Absent	Present	Present
Other Organelles			
Nucleus	Absent	Present	Present
Mitochondria	Absent	Present	Present
Chloroplasts	Absent	Absent	Present
Chromosomes	A single circle of naked DNA	Multiple; DNA-protein complex	Multiple; DNA-protein complex
Vacuoles	Absent	Absent or small	Usually a large single vacuole

The End

HOPE YOU ENJOYED!!!!!