General Biology
BioSci 100
Laboratory Manual

Building Knowledge through Experiments

COLLEGE OF THE CANYONS

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Preface

The purpose of creating this custom lab manual for BioSci 100 was to provide our students with a high quality, low cost learning resource. This project would not have been possible without the dedication and commitment of the faculty and staff in the Department of Biological Sciences and the Office of Distance and Accelerated Learning at College of the Canyons.

I am especially grateful to the following individuals for their contributions to the development of the original lab activities and the lab manual chapters: Kelly Burke, Janet Cetrone, Jeannie Chari, Amy Foote, James Glapa-Grossklag, Miriam Golbert, Elizabeth Hernandez, Kim Jesu, Jenna King, Jenny Leadbetter, John Makevich, Patricia Medina, Carri Musser, Gregory Nishiyama, Patricia Palavecino, Christopher Shane Ramey, Kathy Sloan and Jim Wolf.

For this 4th ed. of the lab manual, I am particularly grateful to Dr. Patricia Palavecino and Kelly Burke, for their time, expertise and talent in preparing much of the new content and artwork for the manual. I am also grateful to the Office of Distance and Accelerated Learning for their financial support of this project.

Dilek Sanver-Wang

Note to Students about the Reading Assignments

Each lab activity contains background information that is important for completing the lab activity and questions. However, the background information in the lab manual is not exhaustive and most of the lab activities contain additional required reading assignments from the course textbook. You are expected to complete the textbook reading assignments before coming to class in order to fully understand the theory behind each of the lab activities. You are encouraged to bring your textbook to lab each week so that you can complete the lab assignment and answer all the questions.

Each lab activity also lists some links to websites for optional background information. You are encouraged to look through these additional resources because they provide helpful illustrations, animations and videos to clarify and reinforce the lab material. The URL's are listed for each link; however, to make it easier to access the websites, all these links are also listed on the Biology Department website under "Student Resources" (click on the Bio 100 link at the bottom.) You may want to bookmark the Bio 100 page so that you can access the page easily throughout the semester.

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LAB SAFETY GUIDELINES

Each laboratory is a restricted area. Enrolled students may work in a lab only when there are authorized personnel present. Friends of students in lab classes will not be allowed to "visit" inside the laboratory. Students are not permitted into the storage rooms or prep areas unless given specific permission by their instructor or lab personnel. Ensuring safety in the laboratory is the responsibility of everyone working in the lab. Please follow these guidelines carefully.

GENERAL GUIDELINES

1. USE COMMON SENSE WHEN WORKING IN THE LAB.
2. Be prepared for your work in the lab. Read all procedures thoroughly before entering the lab. Follow all written and verbal instructions carefully. If you do not understand a direction or part of a procedure, ask the instructor before proceeding.
3. Do not eat, drink, or smoke in the lab. Do not use laboratory glassware as containers for food or beverages.
4. Always wear close-toed shoes in the lab.
5. Wear safety goggles whenever working with chemicals or when there is an impact risk.
6. Long hair should be tied back when working with flames, chemicals or dissections.
7. Observe good housekeeping practices. Work areas should be kept clean and tidy at all times. Keep aisles clear. Push your chair under the desk when not in use.
8. No open flames are permitted in the laboratory unless specifically indicated by the instructor. When burners or hot plates are being used, caution should be exercised to avoid thermal burns. If you sustain a thermal burn immediately flush the area with cold water and notify the instructor.
9. If there is a blood spill, immediately notify the instructor.
10. ANY ACCIDENTS OR INJURIES THAT OCCUR IN THE LAB MUST BE REPORTED TO THE INSTRUCTOR AT ONCE.
11. Familiarize yourself with the location of the Fire Extinguisher. There is a telephone in each lab for EMERGENCY USE ONLY. In case of emergency dial 7 (OR 77 at the Canyon Country Campus).
12. Broken glass is to be disposed of in the broken glass (sharps) container and reported to the instructor.
13. Keep hands away from face, eyes, mouth and body while using chemicals or preserved specimens. Wash your hands with soap and water after performing all experiments. Clean, rinse and wipe dry all work surfaces and apparatus at the end of the lab activity. Return all equipment to the proper area.
14. Handle all living organisms used in a lab activity in a humane manner.
15. Never use mouth suction to fill a pipette. Use a rubber bulb or pipette pump.
16. When removing an electrical plug from its socket, grasp the plug not the electrical cord. Hands must be completely dry before touching an electrical switch, plug or outlet.

HANDLING CHEMICALS

1. Wear safety goggles whenever working with chemicals.
2. Chemicals and biological stains should be used with caution. Follow specific instructions regarding all chemicals used during lab. Check the label on chemical bottles twice before removing any of the contents. Take only as much chemical as you need. Do not carry a chemical stock bottle to your work station or remove it from the chemical station.
3. If any chemical comes into contact with your skin, immediately flush the area with water for several minutes and notify the instructor.
4. Dispose of all chemical waste properly. Do not pour chemicals down the sink unless told to do so by your instructor. Check the label of all waste containers twice before adding your chemical waste to the container.

DISSECTIONS – Special Precautions

1. Students should consult with the instructor regarding the pros and cons of wearing contact lenses during dissections.
2. Safety glasses or other protective eyewear is recommended for all students performing dissections.
3. Protective gloves should be worn during dissections. If your skin comes in contact with a chemical preservative, immediately run water over the area and notify the instructor.
4. Do not remove preserved specimens from the laboratory.
5. Preserved biological materials are to be treated with respect.
6. When using scalpels and other sharp instruments, always carry them with the tips and points pointing down and away. Notify your instructor of any cuts or other injuries.

Lab 1: Lab Safety and the Scientific Method

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify and apply the correct lab safety procedures to follow for a variety of scenarios.
2. List and explain the steps of the Scientific Method.
3. Identify and provide examples of questions that can be answered scientifically.
4. Explain what distinguishes a good scientific hypothesis.
5. Define, give examples of, and identify dependent, independent and standardized variables.
6. Explain the importance of control treatments and replication.
7. Explain the difference between quantitative and qualitative data.
8. Conduct a simple experiment using the Scientific Method.

II. Background Information:

A. The Scientific Method:

Science is a search for an understanding about the way that things work in the natural world. Scientific inquiry is based on falsifiable **hypotheses**. This means that there is room for any assumption about the natural world to be shown false. Science is fluid and dynamic and changes as new information is introduced, examined and the best explanations are accepted. If a hypothesis cannot be shown to be potentially false, then that hypothesis cannot be investigated using science. A scientific investigation depends on a set of procedures. These procedures or steps are known as the **scientific method**.

1. Observation: An initial observation is made about a phenomenon in the natural world.
2. Question: A question is asked about the phenomenon.
3. Hypothesis: A possible explanation of the phenomenon, or answer to the question is proposed. Oftentimes, the scientist will make a more specific **prediction** based on the more general hypothesis that has been proposed.

It is very important that the hypothesis that is proposed is scientifically testable and potentially falsifiable. For example, the hypothesis: “Picasso is the greatest painter of all time” is not scientifically testable because it is a subjective statement. However, the hypothesis could be changed to make it testable, for example: “Picasso’s paintings are the most valued based on auction prices.”

4. Experiment: An experiment is designed to test the hypothesis.
5. Results: Data are collected in an objective manner.
6. Conclusion: The results are analyzed and the alternate hypothesis is accepted or rejected.

Using the rules of the scientific method ensures that an investigation will be designed so that results can be reviewed in an objective manner and the experiment replicated by others. The ability to repeat an experiment is essential to the validity of its results. If a tested hypothesis can be shown true in repeated testing, it may be that the information will be added to the general body of knowledge that is science. By the way, negating a hypothesis is often just as valuable as is accepting one to be true.

A good test isolates a single factor or variable for examination. Sometimes this is very difficult to do. A crucial step in designing experiments is to identify the variables and treatment groups.

B. Elements of an Experiment:

1. Independent Variable:
This is the variable that is changed by the investigator. This variable is chosen because the investigator predicts that changing it will impact the dependent variables and a functional relationship can be established.

2. Dependent Variable:
This is the variable that is measured, counted or recorded by the investigator. It is the factor that varies in response to conditions manipulated with respect to the independent variable.

3. Standardized Variables:
These are the variables that are kept equal in all treatments so that any changes in the dependent variable can be attributed solely to changes in the independent variable.

4. Experimental Treatment:
The experimental treatment is the one where the independent variable is manipulated.

5. Control Treatment:
A control treatment is one where the independent variable is either eliminated or set at a standard value. The results of the control treatment are compared to the results of the experimental treatment to determine if manipulating the independent variable had a measurable effect on the dependent variable.

6. Replication:
It is important for an experiment to be repeatable; this increases our confidence that that the observed results are due to changes in the independent variable. All biological systems contain natural variability and will therefore respond in slightly different ways each time an experiment is performed. By repeating the experiment multiple times, or by increasing the number of organisms that are experimented on, the investigator can obtain an average value that more accurately reflects the true result.

In certain situations, it is not possible to directly manipulate the independent variable or control all the variables, such as with natural disasters (e.g. an oil spill), naturally occurring phenomena (e.g. the changing of the seasons, phases of the moon), etc. In those cases, the researcher will try to gather as much data as possible before and after the phenomenon and

try to determine which variables were standardized and which variables need to be accounted for in the analysis of the data.

The results of an experiment are the recorded change in the dependent variable. Data are reported in objective terms that allow for independent interpretation by anyone reading the report. The preferred method of reporting data is the presentation of results in tables and graphs that provide a quick and clear overview of any observed effects. There are two types of data: qualitative and quantitative.

D. Types of Data:

1. Qualitative Data:

These kinds of data include qualities such as color, smell and taste. These are subjectively perceived and can be difficult to express in an objective manner. While everyone conducting the experiment may agree that the solution changed color, there may be variation in what individuals identify as blue, light blue, etc.

2. Quantitative Data:

These kinds of data include qualities that can be measured objectively such as weight, volume, length and temperature. Quantitative data have a number associated with them and can be reported in universally accepted metric units. This makes it easy for others to interpret the results.

Both types of data are valid and important. In some instances an experiment may result in just one or the other type of information. Oftentimes, the investigator will collect and report both types of data.

III. Reading Assignments:

A. Required background reading

Campbell Essential Biology (7^{th} ed.): pp.4-10 (The Process of Science)

B. Recommended background reading (optional):

1. UC Berkeley - Understanding Science
http://undsci.berkeley.edu

2. Baruch's Biology Lab Safety Tutorial
http://www.baruch.cuny.edu/tutorials/weissman/biolab/

IV. Pre-Lab Questions:

1. You have been asked to conduct an experiment with different flavors of ice cream.
a. List one example of a scientifically testable hypothesis about ice cream.

b. List one example of a hypothesis about ice cream that cannot be tested scientifically and explain why it cannot be tested scientifically.

2. Consider the following experiment:
A researcher wants to find out if spraying apples with pesticide affects the vitamin levels in those apples. For this experiment, identify:

a) the independent variable: _____

b) the dependent variable: _____

c) **two** standardized variables: _____

d) the control treatment: _____

e) the experimental treatment(s): _____

3. In each of the following lab safety scenarios listed below, briefly identify how proper lab safety procedures were not followed and provide a safer alternative.

a) Cindy broke a test tube. Carefully she picked up the broken pieces with one hand and placed them in her other hand. Then she dumped the glass pieces into the wastebasket.

b) Frankie was unsure how to get a chemical from a large flask into a small test tube. He poured the liquid as best he could, but still spilled some on his hand and the table. He quickly washed his hands and wiped the table so the teacher didn't find out. Later, he noticed that his hand felt itchy and he worried that the chemical he spilled on himself might be dangerous.

c) Heather walked into lab late and missed the instructor's explanation about the day's activity. She had not read the lab beforehand, so she skimmed it quickly and began the experiment on her own. She did not understand parts of the lab but did not ask any questions to avoid getting into trouble with the instructor. Her experimental results looked very different from her classmates'.

d) James is cleaning up his workbench and finds a beaker containing an unlabeled clear liquid that his partner left behind. Not sure what it is, he carefully pours it down the sink and washes the beaker.

V. Lab Exercise:

Materials

Per Group:	Per Room:
• 1 sealed box containing four items • 1 container with an array of possible content items • 1 unsealed, empty box	• Triple beam balances • Magnets

Procedure

A. Black Box Experiment

The "black box" can be used as a model for scientific inquiry. Frequently, scientists can see what elements go into a process and they can likewise identify the results of the process, but they are unable to see the process itself; that is the "black box." Scientists must devise methods of figuring out what occurs in the "black box."

In the following activity, you will use the scientific method to answer the following question: "Which items are located in the sealed box?"

1. Each group of students should select a sealed box. (Do NOT open the box at any point during this investigation!)

2. Note the assortment of possible items located in the container provided. There are FOUR of the possible items in your sealed box.

3. As a group, you must devise a way to determine what is in the sealed box. Each time you make a guess as to what might be in the box and test it to determine if you are correct, you are conducting a "mini" experiment. Complete the following table and questions as you proceed (an example has been provided for clarification). You may need fewer or more rows to reach a conclusion about the items in your sealed box.

Hypothesis / Prediction	**Experimental Procedure**	**Results/Data**	**Data qualitative or quantitative?**	**Hypothesis supported or refuted?**
1.There is a marble, compass, paperclip and magnet in the sealed box	*Place all four items in the empty box, weigh box and test it with a magnet.*	*The two boxes don't weigh the same and the items are not attracted to the magnet.*	*Qualitative (magnetic) and Quantitative (weight)*	*Hypothesis is refuted. Need to try different combination of items.*
2.				
3.				
4.				
5.				
6.				

If your hypothesis and your result are the same, then your original hypothesis has been **supported**. If your result is different from your hypothesis, then your hypothesis has been **refuted**.

In any scientific inquiry, investigators use all the tools available at their disposal to collect the most accurate data they can and analyze their results to reach a conclusion. They then report their results, along with the statistical significance of their results, which indicates the likelihood that the observed results were in fact due to the experimental manipulations.

When you have determined the four items that are likely to be in your sealed box (**without opening the box**), answer the following questions. Your instructor may ask you to share your results with the rest of the class when all the groups have finished their experiment.

a. What other tools could you use (that were not available today) that would help you in answering your scientific question?

b. Based on your experiment, how confident are you that your results are accurate?

c. How could you increase your confidence and/or the accuracy in your results with the experimental tools that are provided?

B. Designing an Experiment

Background information:

Peanut is an annual herbaceous plant that can grow between 30cm to 50cm (1-1.6 ft) tall. The botanical definition of a "nut" is a fruit whose ovary wall becomes very hard at maturity. Using this criterion, the peanut is not a true nut, but rather a legume, and so it belongs to the same family as bean and peas. Like most other legumes, peanuts harbor symbiotic nitrogen-fixing bacteria in their root nodules. Peanut pods develop underground, an unusual feature known as ***geocarpy***, where the plant flowers grow above ground but the fruits develop below ground.

After fertilization, a short stalk at the base of the ovary (termed a pedicel) elongates to form a thread-like structure known as a "peg". This peg grows down into the soil, and the tip, which contains the ovary, develops into a mature peanut pod (Fig. 1.1).

Since peanuts are able to fix nitrogen, they do not require fertilizers with nitrogen, and they can also improve soil fertility. Therefore, they are valuable in crop rotations. They grow well in light, sandy, and loamy soil.

Peanuts need warm weather throughout the growing season to develop well. They can grow with as little as 350 mm (14 in) of water, but for best yields need at least 500 mm (20 in) of water.

Fig. 1.1 Diagram illustrating how a peanut plant grows.[1]

[1] Wikimedia Commons: http://commons.wikimedia.org

1. You want to investigate in what type of soil pH, peanuts will grow better. Therefore, you will design a hypothetical experiment to investigate the following question: Do peanuts grow better in acidic, neutral or basic soil? In the space below write a summary of your experiment starting with the statement of your **hypothesis**.

2. Identify the following elements of your peanut growth experiment:

a. independent variable: _____

b. dependent variable: _____

c. control treatment: _____

d. experimental treatment(s): _____

e. standardized variables: _____

3. Indicate the kind of data will you collect and if it will be quantitative or qualitative. (Hint: you need to decide how you would specifically measure peanut growth to answer this question.)

4. Determine what kind of graph you will use to present your data clearly, and what variables they will include. Draw a sample graph with data that **support your proposed hypothesis**.

VI. Post-Lab Questions

1. Consider the following question: *Does eating a sweet snack (e.g. candy) cause more weight gain than eating an oily snack (e.g. potato chips)?*

State a scientifically testable hypothesis to answer the question.

2. Identify the following elements of your snack experiment:

a. independent variable: _____

b. dependent variable: _____

c. control treatment(s): _____

d. experimental treatment(s): _____

e. standardized variables: _____

3. How would the results of your experiment be affected if these variables were not standardized?

4. Indicate the kind of data will you collect and if it will be quantitative or qualitative.

Lab 2: Scientific Measurements

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify the standard metric units of weight, length, volume and temperature.
2. Make measurements using the metric system.
3. Convert values between different metric units as well as between the imperial and metric units of measurement.
4. Identify the names and functions of laboratory equipment.
5. Make measurements using laboratory equipment.
6. Present data in appropriate and correctly labeled graphs.
7. Use graphical data to predict experimental results.

II. Background Information[1]:

Systems of measurement have a long and diverse history. In the United States, we typically use the United States Customary System, which evolved from a common ancestor to the imperial units traditionally used in Great Britain. This system merged components of the Anglo-Saxon and Roman systems of measurement. The Anglo-Saxon system had an agrarian foundation of barleycorns. Legally, an inch was defined as three barleycorns. Needless to say, the system is a bit confusing and the barleycorn standard led to some measurement differences. While the imperial system is now standardized, conversion between units can still be a bit awkward (e.g., 12in=1ft and 3ft=1yd).

Although the United States continues to hold on to the US Customary System, most nations (including Great Britain) have converted to the International System of Units (SI), which is based in the metric system. Some imperial measurements remain in limited use in Canada, India, Malaysia, Sri Lanka, South Africa and Hong Kong.

Scientific measurements are made using the metric system, which allows scientists to communicate their results in a standardized manner. Quantitative measurements are important in science because they allow for greater precision and improve our ability to communicate information. For example, the terms “tall” and “taller” mean little; however, “29.6 meters tall” and “5.6 centimeters taller” convey more precise information. The purpose of this lab exercise is to familiarize you with the system of quantitative measurement that you will be using throughout this and future science courses.

A. The Metric System

The metric system is based on units of 10. The standard units of measurement in the metric system are meter (m) for length, liter (L) for volume, gram (g) for mass and Kelvin (K) for temperature. The more commonly used standard unit for temperature, however, is degrees Celsius (ºC). A gram was originally defined as the mass of 1mL (or ml) of water at 4ºC.

[1] Washington State Open Course Library: http://opencourselibrary.org/

Furthermore, 1mL = 1 cm^3.
Whether measuring length, volume, or mass, the prefixes listed below are used to designate the relationship of a unit of measure to the base unit (i.e., m, L, or g).

Prefix	Prefix Abbreviation	Decimal equivalent	Exponential equivalent
Kilo	k	1000	10^3
Centi	c	0.01	10^{-2}
Milli	m	0.001	10^{-3}
Micro	μ	0.000001	10^{-6}

Converting between metric units of measure:
Many students try to jump into unit conversions by moving the decimal place to the left or right. If you are familiar with the metric system, this may work for you. However, common mistakes include moving the decimal in the wrong direction. A more reliable method for unit conversion is the use of conversion factors and unit cancellation. For example, if you are asked to convert 2km to cm, begin by considering the conversion factors that you know.

1. If starting with 2km, we need to multiply by a conversion factor to eliminate the *km*. We need to divide *km* by *km* to eliminate this unit (*km* divided by *km* equals 1). Therefore, the multiplier is the conversion factor between *km* and *m* in fraction form:

$$\frac{1000m}{1\ km}$$

with *km* in the denominator. So, we can multiply our original measure by the conversion factor:

$$2km \times \frac{1000m}{1km} = 2000m$$

which cancels out the *km* and leaves us with *m* in the numerator.

2. But we're not done! We wanted to convert to *cm*, not *m*; therefore, we need another conversion factor. We now have *m* and need *cm*. One meter is equal to 100cm. This conversion factor needs to be arranged to eliminate *m*. In fraction form, we need to divide by *m*, so our conversion factor becomes:

$$\frac{100cm}{1m}$$

Multiplying our new measure by this conversion factor leaves us with *cm*.

$$2000m \times \frac{100cm}{1m} = 200{,}000cm$$

3. Steps 1 and 2 could be combined as follows:

$$2km \times \frac{1000m}{1km} \times \frac{100cm}{1m} = 200{,}000cm$$

While perhaps tedious, using conversion factors for unit cancellation can help prevent mistakes when converting between units of measure.

Sample conversion factors for the metric system:

1kg = 1000g	1km = 1000m	1m = 1000000µm	1L = 1000ml
1g = 1000mg	1cm = 10mm	1km = 1,000,000mm	
1g = 1000000µg	1m = 100cm	1mm = 1000µm	

B. The Imperial System vs. the Metric System

The sample conversion factors listed below should illustrate that converting between imperial units, or imperial and metric units can be harder to do without memorizing a list of conversion factors and having a calculator handy. In this course, we will make all measurements in metric units and focus mainly on conversions between metric units.

Sample conversion factors for the imperial system:

1 foot = 12 inches
1 gallon = 128 fluid oz
1 mile = 5280 feet

Example conversion factors between the imperial and the metric system:

1 meter = 1.094 yards	1 inch = 2.54 centimeters
1 liter = 1.057 quarts	1 ounce = 28.35 grams
1 kilogram = 2.205 pounds	$^oF = (^oC \times 1.8) + 32$

C. Graphical Representation of Data

Scientists need to collect many types of data when conducting experiments and it is important to record those data as soon as the measurements are made, to avoid errors. A properly labeled data table is an essential tool in any investigation. However, it is often difficult to visualize any patterns in the data by simply looking at list of numbers, which is why it is important to graph the results. This will help you interpret your results more easily and help you determine whether or not your hypothesis was supported or refuted.

Line graphs are used to track changes over short and long periods of time (i.e. the rate at which a beaker of boiling water cools). Line graphs can also be used to compare changes over the same period of time for more than one treatment group (Fig. 2.1). Bar graphs, on the other hand, are used to compare outcomes between different categorical groups, for example the effectiveness of different toothpaste brands at preventing cavities (Fig. 2.2)

As you measure many things you might start to recognize that particular patterns of the **dispersion** of data exist. Often, when the data are put into categories, and graphed, the data points will form a normal or bell curve. Many types of data form this pattern, including the heights and weights of a group of people, as the sample size, or the number of people you measure, increases.

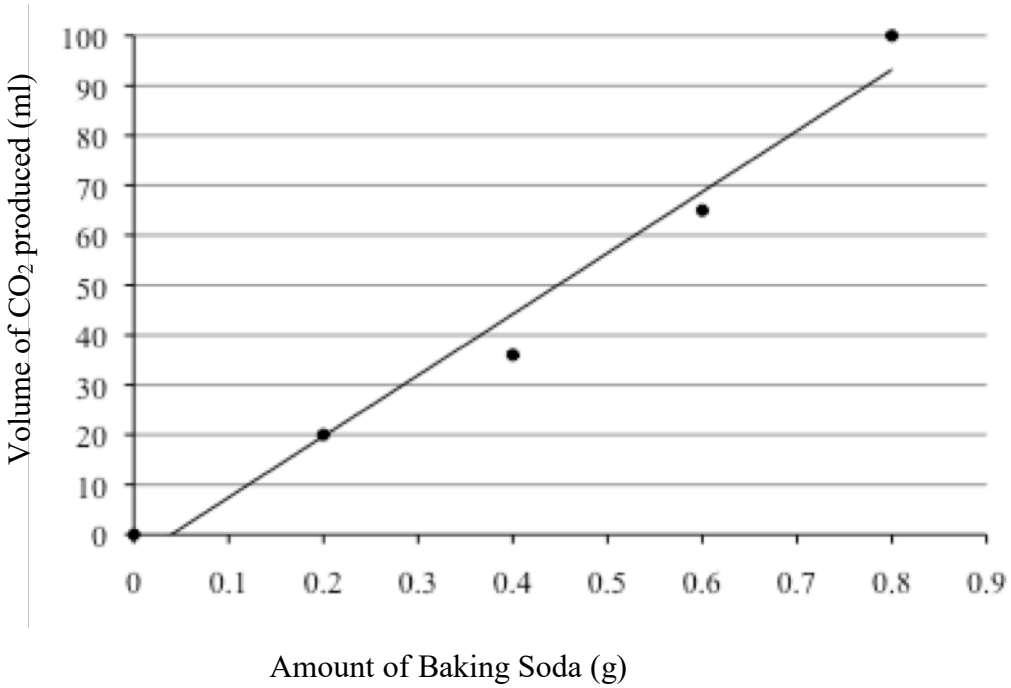


Fig. 2.1 Line graphs can be used to graph variables that are continuously changing (i.e. time or amount of baking soda).

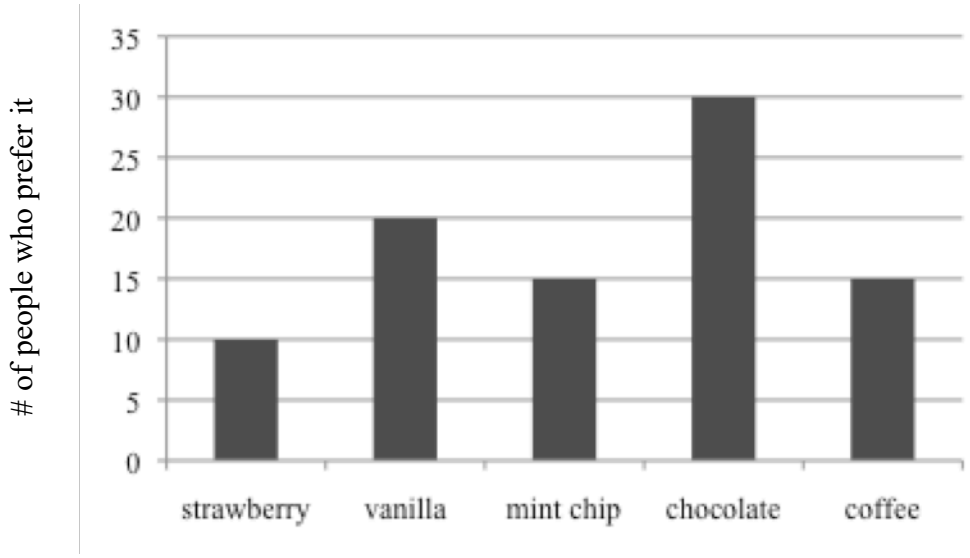


Fig. 2.2 Bar graphs can be used for comparing different groups of categorical data.

How to graph data and draw a best-fit line:

1. Collect data for the graph in a table format first. A graph has two sets of data: independent and dependent. The independent variable is what the investigator manipulates (or a variable that changes constantly, such as time). The dependent variable changes based on the independent variable.

2. Draw the area of your graph with the vertical and horizontal axes. The horizontal axis (x-axis) represents the independent variable. The vertical axis (y-axis) represents the dependent variable. Label each axis with appropriate units based on the data.

3. Determine the range of data for each axis by looking at the maximum and minimum values in your data set and dividing this range into equal sections (e.g. if you are measuring temperature and your values range from 5 to 55 ^{o}C, then your axis range can be 0-60 ^{o}C in increments of 5.)

4. Plot your data on the graph and using a ruler, draw a straight or curved line that appears to "fit" the data. One way to draw a best-fit line through your data points by hand (assuming that your data points do, in fact, show a linear pattern) is to adjust the ruler through your data points such that the distance between each of the data points and the line you are about to draw is minimized and that the number and distance of the points that lie above the line are equal to those below the line. Thus, depending on your data, you may end up with a best fit-line that passes through the majority of your actual data points.

III. Reading Assignments:

A. Required background reading
1. National Center for Education Statistics – Graphing Tutorial
https://nces.ed.gov/nceskids/help/user_guide/graph/whentouse.asp

B. Recommended background reading (optional):
1. The Metric System – Units, Definition and History
http://www.sciencemadesimple.com/metric_system.html

IV. Pre-Lab Questions:

1. Calculate the following using the conversion factors provided in the background reading.

a. 2 meters = _____ centimeters

b. 24.3grams = _____ micrograms

c. 24.3 milliliters = _____ liters

d. 6738 meters = _____ kilometers

2. Write the standard metric unit for:

a. temperature: _____

b. length: _____

c. volume: _____

d. weight: _____

3. Identify the numerical value of the following prefixes:

a. Kilo (k): _____

b. Centi (c): _____

c. Milli (m): _____

d. Micro (μ): _____

4. Which system (imperial or metric) is easier to use when doing conversions? Why?

5. You are interested in testing the following hypothesis: "Students who drink coffee before an exam will score higher on the exam than students who don't drink coffee."

a. For this experiment, identify the following:

Independent variable: _____

Dependent variable: _____

Two standardized variables: _____

Control treatment: _____

b. The independent variable should be plotted on the _____ axis and the dependent variable should be plotted on the _____ axis.

c. Would this experiment require the collection of quantitative or qualitative data? Justify your answer.

d. What kind of graph (line or bar graph) would be more appropriate in graphing the data? Why?

V. Lab Exercise:

Materials

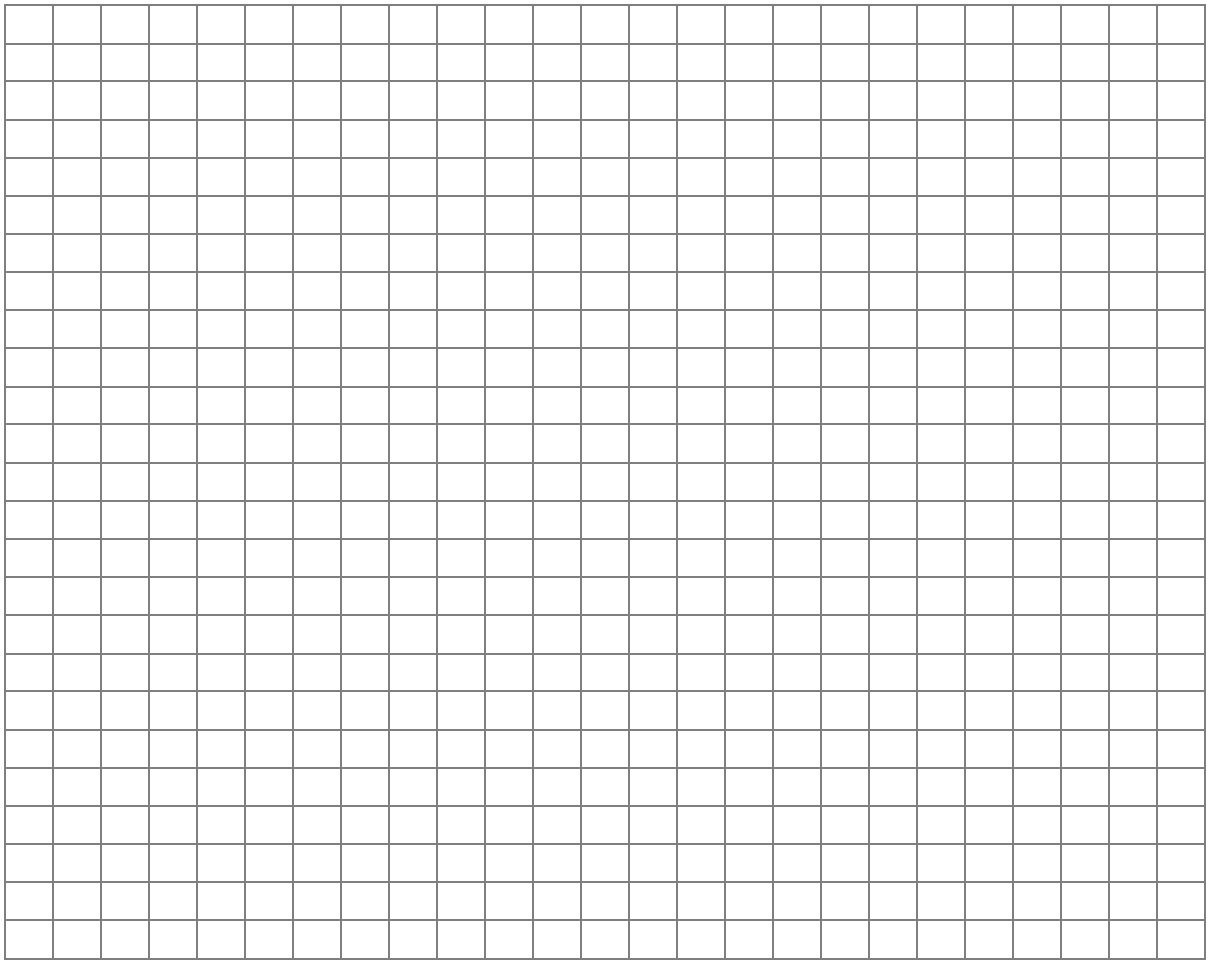
Per Group:	Per Room:
• Rulers • Graduated cylinders • 600mL beaker • Plastic pipettes • Container of water • 1 empty can of soda • Thermometer	• Triple beam balance • Digital scale • Salt • Vinegar • Baking soda • Spatula • Weigh boat

Procedure

A. Temperature Measurement

1. Fill a 600mL beaker with 500mL of tap water and heat it in the microwave for 2 min.
2. Use caution when removing the hot beaker from the microwave. Use the heat gloves provided. Your instructor may decide to complete steps 1-2 for each group.
3. Using the thermometer provided, immediately measure the temperature of the water in ^{o}C and record it under time 0 min. The thermometer should be suspended in the solution when taking the temperature rather than touching the bottom of the beaker.
4. Continue to record the temperature of the water in the beaker every 3 minutes for 33 min (or until the temperature no longer changes) in the data table below. Draw a graph of time (x-axis) vs. temperature (y-axis) and draw a best-fit line through your data points. Refer to the background information on how to draw a best-fit line.

Time (min)	Temperature (^{o}C)	Time (min)	Temperature (^{o}C)
0		18	
3		21	
6		24	
9		27	
12		30	
15		33	



B. Units of Length

1. Measure the length of this page in centimeters: _____ cm

2. Convert the length of the page into the following units:

   millimeters (mm): _____

   meters (m): _____

3. Measure the height of the 600mL beaker in millimeters: _____ mm

4. Convert the height of the beaker into the following units:

   centimeters (cm): _____

   meters (m): _____

   kilometers (km): _____

C. Units of Volume

1. Using the 1L graduated cylinder and an empty soda can, determine the volume of the soda can by filling the can with tap water and emptying it into the graduated cylinder.

   Volume = _____ mL

2. Calculate the volume of the soda can using measurements of length. To calculate the volume of a cylinder, measure the height (h) and radius (r) of the soda can in centimeters (cm). You will need to decide whether to use the inner diameter or outer diameter of the soda can, which will affect the magnitude of your calculated volume.
3. Use the following formula to calculate the volume: $V = \pi r^2 h$. ($\pi = 3.14$)
   Because the measurements were taken in centimeters, the volume will be in cubic centimeters (cm^3).

   Measurements:

   r = _____ cm; h _____= cm

   Calculations:

   Volume = _____ cm^3

4. Compare the measured volume (mL) with the calculated volume (cm^3).
   (Remember that for water at 4°C, 1g = 1mL = $1cm^3$.)

   Is the measured volume (mL) nearly the same as your calculated volume (cm^3) (Hint: think about whether you used the inner or outer diameter, and how that might have affected your result)?

   If not, what explanations can you provide for the observed difference?

D. Units of Mass

1. Measure the mass of water that a soda can holds by completing these steps:
   Place the empty soda can on the triple-beam balance and record its mass. Then fill the can with water and record the mass of the full can. Calculate the mass of the water by subtracting the mass of the empty can from the mass of the full can.

   Mass of empty soda can = _____ g

   Mass of soda can + water = _____ g

   Mass of water = _____ g

2. Compare the mass of water you measured (g) with your measured volume (mL).

   Are these values similar or different? What might be the reason for any differences?

Note: The term **weight** was avoided in the above discussion because **mass** is a quantity of matter, while weight depends on the gravitational field in which the matter is located. Thus, if you were on the moon you would weigh less, but your mass would be the same as on earth. Although it is technically incorrect, mass and weight are often used interchangeably.

3. Using the container of salt provided and a weigh boat, weigh out 5 grams of salt using the *triple beam balance* (the 5 g will include the mass of the weigh boat.) Be as precise as possible in your measurement.
4. Take the weigh boat with the salt you just measured and place it on the digital scale. Be sure to zero the scale before you begin. Record the mass that registers on the digital scale.

Is the value registered on the digital scale exactly 5g?

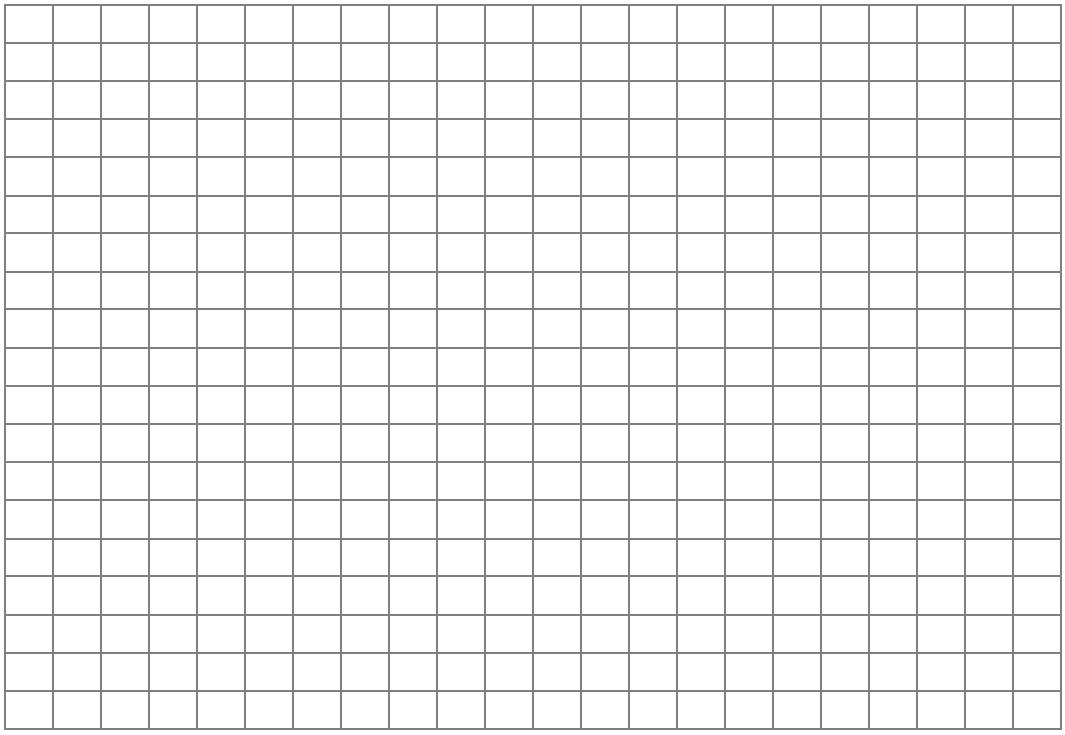
What are the benefits and limitations of using a triple-beam balance versus a digital scale to measure mass?

E. Graphing and Predicting the Rate of a Simple Reaction

1. Add 10mL of vinegar into a 100mL graduated cylinder. Weigh out 0.1g of baking soda using the weigh boat provided.
2. Add the baking soda to the vinegar and ***quickly*** observe and record the maximum volume of CO_2 bubbles that is produced from the reaction in the data table below.

Vinegar (mL)	Baking Soda (g)	Volume of CO_2 bubbles produced (mL)
10	0.1	
10	0.3	
10	0.5	
10	0.7	

3. Pour the completed reaction solution down the sink, and using a fresh batch of vinegar, repeat the experiment for the new amount of baking soda.
4. Do NOT put away your lab equipment yet. You need to conduct further experiments AFTER graphing your data.
5. Draw a graph of the amount of baking soda (x-axis) vs. volume of CO_2 bubbles (y-axis) and draw a best-fit line through your data points.



6. Using the best-fit line on your graph, predict the volume of bubbles that will be produced if you add 0.2g and 0.6g of baking soda to the reaction.

   Predicted volume of bubbles that will be produced for:

   0.2g of baking soda: _____ mL

   0.6g of baking soda: _____ mL

7. Repeat steps 1-3 of the experiment using 0.2g and 0.6g of baking soda and record your results below.

   Observed volume of bubbles that were produced for:

   0.2g of baking soda: _____ mL

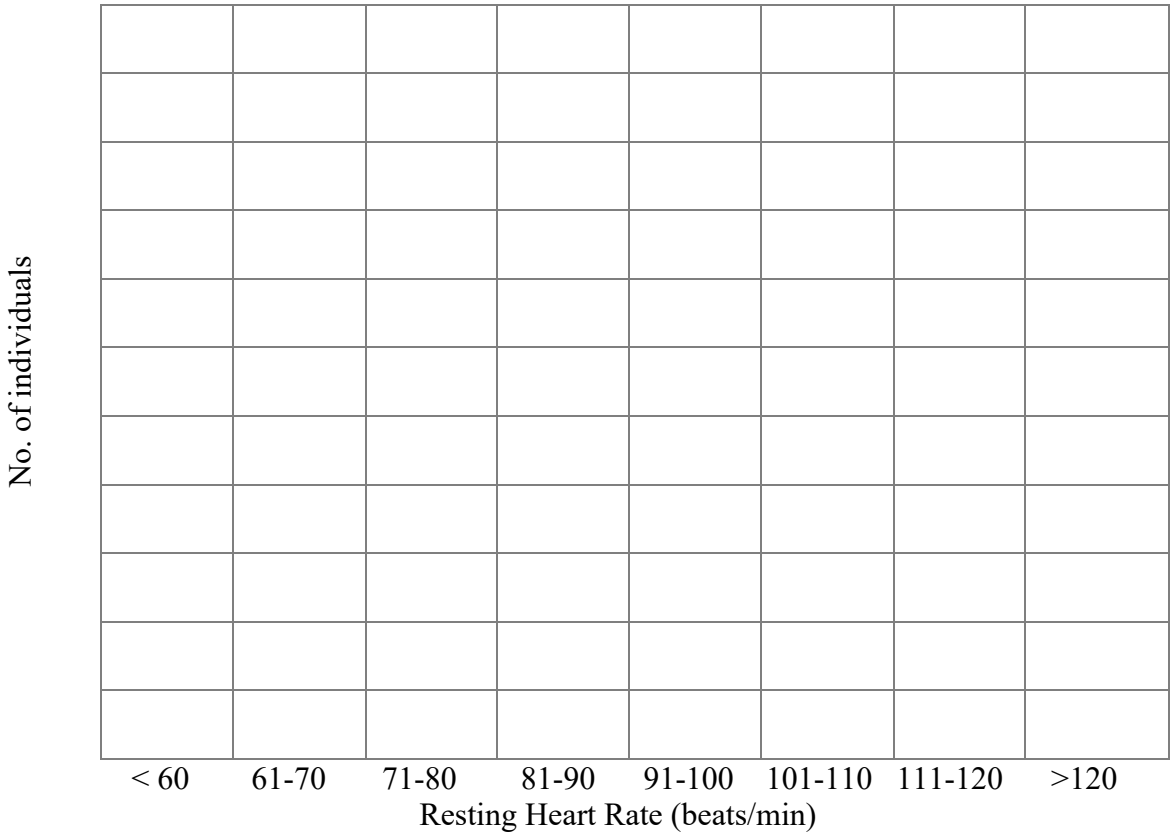
   0.6g of baking soda: _____ mL

Were your predictions correct? If not, how could you explain the discrepancy between your observed and expected results?

F. Dispersion of Data

1. For this activity, you will be determining the dispersion of data using the heart rate of your classmates.
2. Find a quiet spot to sit down and relax. Place two fingers on your carotid artery on your neck or on your vein in your wrist and count how many beats you feel in a minute. You can take a measurement for 15 seconds and multiply this number by 4.
3. Record your heart rate on the table or graph that your instructor has drawn on the board. Note how the dispersion of the data changes as more students record their results.
4. Draw a graph below showing the class data after everyone has recorded their heart rate on the board.

Graph of **Resting** Heart Rate for the class:



Does the graph show a normal distribution or is it skewed to one side?

How could the sample size affect the shape of the graph and the dispersion of data?

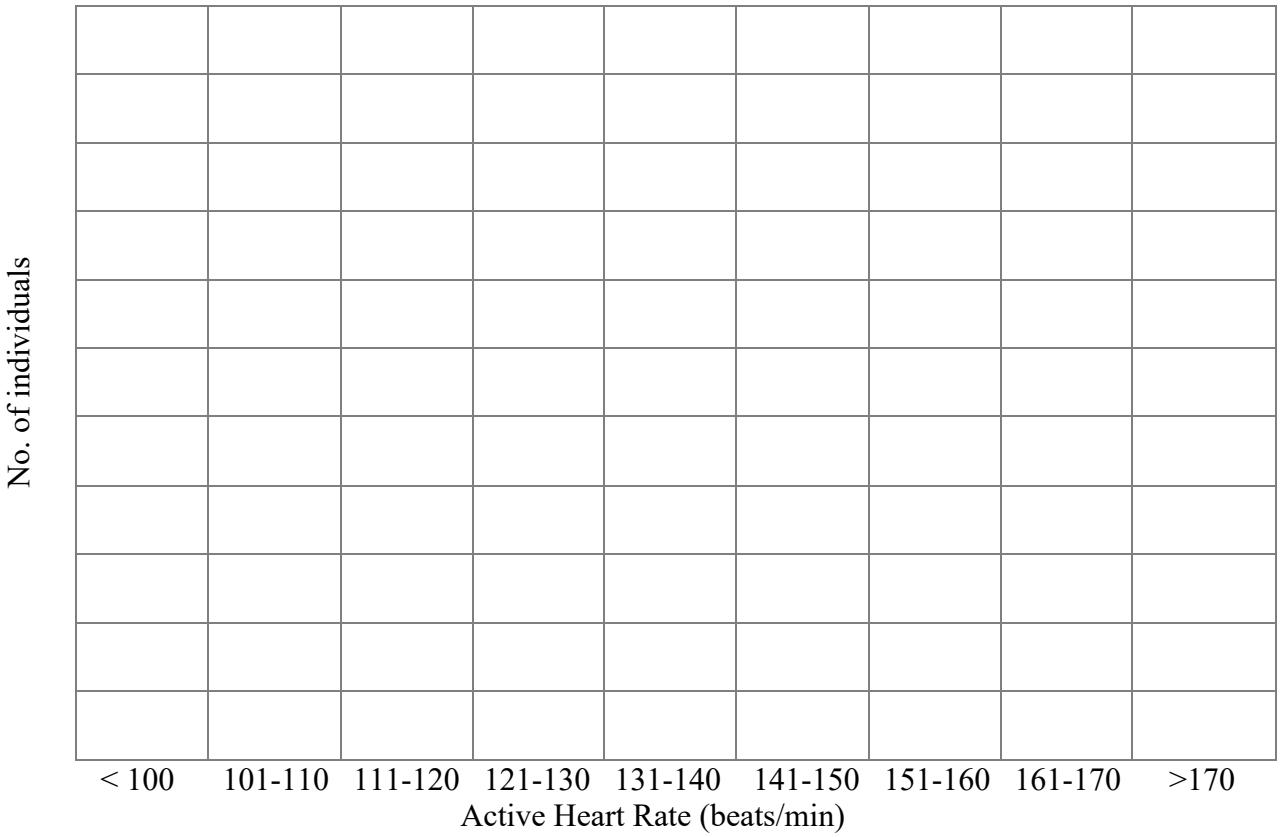
5. A healthy resting heart rate ranges between 60-100 beats per minute. Calculate the average heart rate for the class.

*Average heart rate for class:*_____

How does your heart rate compare with the class average?

6. Spend 1-2 min doing moderately rigorous activity outside (running in place, walking up and down the stairs quickly, jumping jacks, etc.) Your instructor may specify an activity for you to complete in order to standardize the procedure.
7. Measure your heart rate AFTER you have completed your activity and then repeat steps 3-4.

Graph of **Active** Heart Rate for the class:



Does the graph show a normal distribution or is it skewed to one side?

8. A healthy active heart rate ranges between 100-170 beats per minute depending on age. Calculate the average heart rate for the class.

*Average heart rate for class:*_____

How does your heart rate compare with the class average?

VI. Post-Lab Questions

1. Calculate the following using the conversion factors provided in the background reading.

a. 2 meters = _____ centimeters

b. 2 kilograms = _____ milligrams

c. 5000 micrometers = _____ meters

d. 1 millimeter = _____ micrometers

e. 30 liter = _____ milliliter

2. Calculate the following using the conversion factors provided in the background reading.

a. 3.5 yards = _____ meters

b. 4 pounds = _____ kg

c. 9 inches = _____ cm

d. 20 ^{o}C = _____ ^{o}F

3. Describe how you would draw a best-fit line through sample data points.

4. Explain what kinds of data are best presented in a line graph vs. a bar graph.

<u>Important Note:</u>
In preparation for Lab 3 (Macromolecules & Nutrition), you will need to record what you ate and drank for one of your meals. Refer to the pre-lab questions for Lab 3 for more information about the assignment.

Lab 3: Macromolecules and Nutrition

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Choose the appropriate tests to determine the presence of carbohydrates, protein and lipids in sample foods and analyze the results.
2. Calculate total and % Calories from carbohydrates, protein and fat in sample foods.
3. Analyze the nutritional value of various foods compared to nutritional guidelines.

II. Background Information[1]:

In an age where we have unlimited access to a wealth of information at our fingertips, the challenge we are faced with is making sense of that information and evaluating the accuracy of it. Nutritional information is a good example of this. Every day we are bombarded by news organizations, health magazines, self-help books and food company advertisements, all trying to tell us what to eat and what to avoid, what is the latest fad for losing weight, what is part of "a healthy breakfast", etc. In order to evaluate these claims objectively, it is essential to have an understanding of the basic chemistry of biological macromolecules and human nutritional requirements.

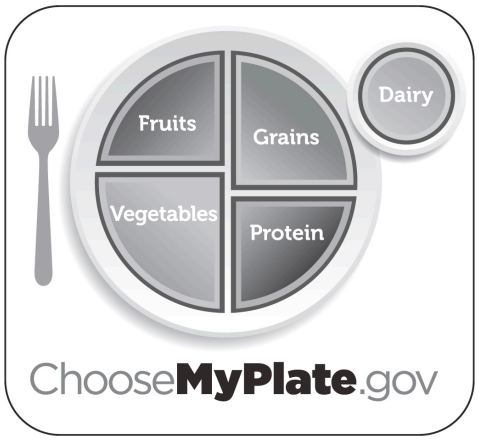


Fig. 3.1. In 2011, the USDA released the federal government's new food icon, MyPlate, to serve as a reminder to help consumers make healthier food choices.[2]

A. Macromolecules:

Macromolecules are very large molecules, formed of smaller subunits. In this lab, we will focus on the three macromolecules that are important energy sources for biological organisms: carbohydrates, proteins and fats.

[1] Nutrition.gov: http://www.nutrition.gov/smart-nutrition-101

[2] United States Dept. of Agriculture: www.choosemyplate.gov

Carbohydrates:

A carbohydrate is an organic compound that consists only of carbon, hydrogen, and oxygen. Your body uses carbohydrates (carbs) to make glucose, which is the fuel that gives you energy and helps keep everything going. Your body can use glucose immediately or store it in your liver and muscles for when it is needed.

In general, the smaller carbohydrate compounds are commonly referred to as sugars, which are found naturally in foods such as fruits, vegetables, milk, and milk products. Simple carbohydrates also include sugars added during food processing and refining.

Starch and dietary fiber are the two types of complex carbohydrates. Starch must be broken down through digestion before your body can use it as a glucose source. Quite a few foods contain starch and dietary fiber such as breads, cereals, and vegetables.

Proteins:

Proteins are part of every cell, tissue, and organ in our bodies. These body proteins are constantly being broken down and replaced. The protein in the foods we eat is digested into amino acids that are later used to replace these proteins in our bodies.

Protein is mainly found in the following foods: meats, poultry, and fish, legumes (dry beans and peas), tofu, eggs, nuts and seeds, milk and milk products, and grains.
Most adults in the United States get more than enough protein to meet their needs. It is rare for someone who is healthy and eating a varied diet to not get enough protein.

There are 20 different amino acids that join together to make all types of protein. Our bodies cannot make some of these amino acids so these are known as ***essential*** amino acids – it is essential that our diet provide these. A complete protein source is one that provides all of the essential amino acids, for example, meat, poultry, fish, milk, eggs, and cheese are considered complete protein sources. An incomplete protein source is one that is low in one or more of the essential amino acids. Complementary proteins are two or more incomplete protein sources that together provide adequate amounts of all the essential amino acids (e.g. rice and beans).

Fats:

Although the term ***lipid*** is sometimes used as a synonym for ***fats***, fats are actually a subgroup of lipids composed of triglycerides. Moreover, triglycerides that are solid at room temperature are often called ***fats*** while those that remain liquid at room temperature are called "***oils***." Triglycerides are also composed of carbon, hydrogen and oxygen atoms, but in different ratios than in carbohydrates. Triglycerides have long chains of carbon and hydrogen bonds, which creates the hydrophobic tail. This property prevents triglycerides from mixing readily with water and causes them to separate relatively easily in solution, a property that we will utilize in our experiment today.

Unsaturated Fats, Saturated Fats and Trans Fats:

Most of the fat that you eat should come from unsaturated sources: polyunsaturated fats and monounsaturated fats. In general, nuts, vegetable oils, and fish are sources of unsaturated fats. Polyunsaturated fats can also be broken down into two types:
Omega-6 polyunsaturated fats: these fats provide an essential fatty acid that our bodies need, but can't make (e.g. soybean oil, corn oil and safflower oil).
***Omega-3 polyunsaturated fats*:** these fats also provide an essential fatty acid that our bodies need (e.g. soybean oil, canola oil, walnuts, flaxseed and fish). In addition, omega-3 fatty acids, particularly from fish sources, may have potential health benefits.

Saturated fats are oftentimes the "solid" fats, but other saturated fats can be more difficult to see in the foods we consume. In general, saturated fat can be found in the following foods: high-fat cheeses, high-fat cuts of meat, whole-fat milk and cream, butter, ice cream and palm and coconut oils. Although animal fats are the primary source of saturated fat, palm and coconut oils, and cocoa butter are also important sources of saturate fat, and these are often added to commercially-prepared foods, such as cookies, cakes, doughnuts, and pies. Solid vegetable shortening often contains palm oils and some whipped dessert toppings contain coconut oil.

Trans fat increases the level of low-density lipoprotein (LDL or “bad”) cholesterol and decreases the level of high-density lipoprotein (HDL or “good”) cholesterol in the blood — which, in turn, can increase the risk of developing cardiovascular disease. Cardiovascular disease is the leading cause of death in both men and women in the U.S. The *Dietary Guidelines for Americans* recommends keeping the intake of trans fat as low as possible by limiting foods containing partially hydrogenated oils (a source of artificial trans fat). Eating foods with even small amounts of trans fat can add up to a significant intake over time.

The most common types of trans fats are found in foods that contain partially hydrogenated oil. Food manufacturers use artificial trans fat in food products because it is inexpensive and it increases the food’s shelf life, stability, and texture. Major contributors to artificial trans fat intake include fried items, savory snacks (like microwave popcorn), frozen pizzas, cake, cookies, pie, margarines and spreads, ready-to-use frosting, and coffee creamers.

Sodium:

Sodium occurs naturally in most foods. The most common form of sodium is sodium chloride, which is table salt. Milk, beets, and celery also naturally contain sodium. Sodium is also added to many food products. Some of these added forms are monosodium glutamate (MSG), sodium nitrite, sodium saccharin, baking soda (sodium bicarbonate), and sodium benzoate. These are in items such as Worcestershire sauce, soy sauce, onion salt, garlic salt, and bouillon cubes. Processed meats like bacon, sausage, and ham, and canned soups and vegetables also contain added sodium. Fast foods are generally very high in sodium.

Table salt is 40% sodium; 1 teaspoon of table salt contains 2,300 mg of sodium. Healthy adults should limit sodium intake to 2,300 mg per day. Adults with high blood pressure should have no more than 1,500 mg per day.

B. Food Calories

When speaking of the energy content of a macromolecule we often refer to calories or Calories. There is a big difference between discussing "calories" and "Calories." A calorie is defined as the amount of heat needed to raise the temperature of 1g of water by 1°C. Notice that the word "calorie" is spelled with a lower case letter "c." This is how a physicist would describe a calorie. Nutritionists measure the amount of energy in food in Calories (with a capital letter "C'), which is equivalent to 1 kcal or 1000 calories (lower case "c"). The amount of Calories per gram of each macromolecule is listed below.

1 gram of carbohydrate = 4 Calories
1 gram of protein = 4 Calories
1 gram of fat = 9 Calories

You can see from the values above that one gram of fat contains more than double the amount of energy as a gram of carbohydrate or protein. By analyzing nutrition labels, it is possible to calculate the amount of Calories that each macromolecule contributes by multiplying the number of grams by the Caloric value. You can also calculate the % Calories by dividing the Calories from each macromolecule by the total Calories.

> Example: A candy bar contains 5g of carbohydrate, 4g of Protein and 2g of Fat. The Calories contributed by each macromolecule:
>
> Carbohydrate: 5g x 4 Cal = 20 Calories
> Protein: 4g x 4 Cal = 16 Calories
> Fat: 2g x 9 Cal = 18 Calories
>
> Total: 20 + 16 + 18 = 54 Calories
>
> % Calories from Carbohydrate: (20/54) x 100 = 37%
> % Calories from Protein: (16/54) x 100 = 30%
> % Calories from Fat: (18/54) x 100 = 33%

The *Nutrition Facts* label is designed to give consumers important nutritional information about a product and allow comparisons with other food. The serving size indicates the amount of food that the nutrition information applies to. However, a serving size according to a package may bear little resemblance to the amount of the food that most people eat at a time.

When doing Calorie calculations using the Nutrition Facts label, it is important to also look at the serving size. For example, a bag of chips may be 2 servings, so to calculate the total and % calories for the entire bag (if that is what is being asked in the question), you will need to multiply the values by 2.

C. Chemical Analysis of Food

The presence of various biological macromolecules can be determined using the following tests:

Test Name	Macromolecule it tests for:	If macromolecule is present:	If macromolecule is absent:
Benedict's	Simple sugars (except sucrose)	Color turns yellow, red-orange or green	Color stays clear blue
Iodine	Starch	Color turns black	Color stays yellow/orange
Biuret	Protein	Color turns violet	Color stays light blue
Sudan	Fat (Lipid)	Food stains red-orange or dark pink	Food does not stain red-orange or dark pink
Paper	Fat (Lipid)	Paper turns translucent (semi-transparent)	Paper stays opaque (not transparent)

III. Reading Assignments:

A. Required background reading

Campbell Essential Biology (7th ed.): pp.40-47 (Macromolecules), and pg. 78 (Food Calories).

B. Recommended background reading (optional):

1. 2015–2020 Dietary Guidelines for Americans
http://health.gov/dietaryguidelines/2015/

2. FDA: How to Understand and Use the Nutrition Facts Label
http://www.fda.gov/food/ingredientspackaginglabeling/labelingnutrition/ucm274593.htm

IV. Pre-Lab Questions:

1. List the three types of macromolecules that provide energy for biological organisms and the number of Calories provided by 1g of each macromolecule.

2. A package of peanut butter crackers contains 7g of fat, 16g of carbohydrates and 4g of protein.

a) Calculate the Calories from each macromolecule.

b) Calculate the % Calories for each macromolecule.

3. An unknown food item is tested using Iodine, Biuret, Sudan, Benedict's and paper. The results are listed below. Fill in the table with the required information.

Test	Observed result	What macromolecule does it test for?	Is the macromolecule present or absent?
Biuret	Violet		
Paper	Translucent		
Iodine	Yellow		
Benedict's	Blue		
Sudan	Orange		

4. During the week prior to this lab, you will choose a meal and record what you ate and how much. If you choose a restaurant meal or packaged food, you should record the nutritional information that is posted. If you choose a home-cooked meal, you should record the approximate amount of each food and drink item you consumed. During the lab, you will be asked to calculate the total and % Calories of the meal you have recorded.
Note: If possible, bring in the empty, cleaned food containers (or photos of them) and/or restaurant nutritional information pamphlets with you to lab.

You may also use a free mobile app (i.e. MyFitnessPal) to help keep track of your meal.

Circle: Breakfast / Lunch / Dinner
Note: You may need fewer or more rows than those listed; use only as many rows as you need to complete your meal.

Food / Drink Item	Weight of carbohydrate, protein & fat contained in each item.

V. Lab Exercise:

Materials

Per Group:	Per Room:
• 150ml beaker • 2 plastic pipettes • glass stir rod • bottle of DI water • china marker • test tube clamp • test tube rack • 6 test tubes • 1 watch glass • 2 filter paper circles	• 100ºC water bath • Iodine • Biuret • Sudan • Benedict's solution • paper • goggles • rubber gloves • experimental foods • sample nutritional information sheets

Procedure

A. Chemical Analysis of Sample Foods

1. Fill approx. 1/3 of one of your test tubes with a sample food item that is available.
2. Label the test tube with your group name and place it in the 100ºC water bath for 10 min.
3. Prior to conducting your experiment, formulate a hypothesis about which macromolecules you would expect to find in your chosen food substance. Record your hypothesis in the table below.
4. Remove the test tube and observe the layers. There may be a distinct top, yellowish layer and a bottom layer, or there may be just a single layer. The bottom layer may contain solid precipitate (i.e. chip crumbs). One of the layers may be a liquid and the other one a solid. Write or draw your observation of the layers in the data section of the table below.
5. **Paper test:** Remove 1 drop from the top of your sample food solution using a pipette, place it onto a piece of paper and rub it in. **Wait for the paper to completely dry.** Observe and record whether the paper is translucent (+) or opaque (-).
6. **Iodine test:** Place 5 drops of your sample food solution into a clean test tube. Add 5 drops of iodine to it and mix well. Immediately observe and record whether the solution turns black (+) or remains yellow/orange (-) (the color will fade after a few minutes).
7. **Biuret test:** Place 5 drops of your sample food solution on to a watch glass. Add 7 drops of Biuret to it and mix well. Wait 5 min. and then observe and record whether the solution turns violet (+) or remains light blue (-). ***Note: the color change may be subtle; for best results, observe the solution against a white background.***
8. **Sudan test:** Add 2 drops of your sample food solution onto a small piece of filter paper. If your food sample has a solid precipitate at the bottom, be sure to include it onto the filter paper. Soak the paper for 3 min in the Sudan solution. Rinse the filter paper in the room temperature water bath for 1 min. Observe and record whether the sample food stained red-orange (+) or did not change color (-).

9. **Benedict's test:** Place 10 drops of your sample food solution into a clean test tube and add 10 drops of Benedict's solution to the test tube. Heat your test tube in the 100°C water bath for **exactly 2min**. Remove your test tube and immediately observe and record whether the solution turns red/orange (+) or remains blue (-).

Sample Food 1:

Food substance tested:	
Question:	*Which macromolecules (carbohydrate, protein, fat) does the food contain?*
Hypothesis (indicate which macromolecules you expect your sample to contain):	
Results: **Visual observation of layers (describe or draw):**	
Paper test result (transparency):	
Sudan test result (color):	
Biuret test result (color):	
Iodine test result (color):	
Benedict's test result (color):	
Conclusion: (Which macromolecules are present in this food item?)	

Were there any results that surprised you? If yes, what might be the reason(s) behind your observed result?

10. When you finish analyzing your sample food 1, wash all your materials and repeat steps 1-9 for your second sample food. You may be asked to share your results with the rest of the class.

Sample Food 2:

Food substance tested:	
Question:	*Which macromolecules (carbohydrate, protein, fat) does the food contain?*
Hypothesis (indicate which macromolecules you expect your sample to contain):	
Results: **Visual observation of layers (describe or draw):**	
Paper test result (transparency):	
Sudan test result (color):	
Biuret test result (color):	
Iodine test result (color):	
Benedict's test result (color):	
Conclusion: (Which macromolecules are present in this food item?)	

Were there any results that surprised you? If yes, what might be the reason(s) behind your observed result?

B. Analysis of a Diet

1. Using your food log from the pre-lab questions section, complete the following table for your meal. You may not need to use all the rows provided.

2. For each food/drink item, enter the amount of carbohydrate, protein and fat in grams in cells a-c, then calculate the calories for each macromolecule in cells d-f.

3. Add all the cells labeled (d) and enter the value into cell (g) for the total Calories from carbohydrates. Repeat for cells labeled (e) and enter the value into cell (h) for the total Calories from protein. Repeat for cells labeled (f) and enter the value into cell (i) for the total Calories from protein.

4. Add cells g-I and enter the value into cell j to get the total Calories for your meal. Finally, to calculate the % Calories from carbohydrates, calculate: (g/j)x100. Repeat the same procedure for protein (h/j)x100 and fat (i/j)x100.

Meal Calorie and % Calorie calculations:

Food / Drink Item		Carbohydrate	Protein	Fat
1.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
2.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
3.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
4.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
5.	Weight (g):	a)	b)	c)
	Calories:	d)	e)	f)
Total calories from each macromolecule:		g)	h)	i)
Grand total calories from all macromolecules combined:		j)		
Percentage calories from each macromolecule:		k)	l)	m)

CDC recommended nutritional guidelines:
Calories from carbohydrates: 45-65%
Calories from protein: 10-35%
Calories from fats: 20-35%

Compare the % Calories that you calculated for your sample meal with the CDC recommended values (listed above). In what ways are they similar and/or different?

Does the meal you recorded in your food log accurately represent the proportion of carbohydrate, protein and fat you consume in the rest of your meals? If not, how are they different?

C. Analysis of Sodium (Salt) and Sugar in Sample Foods

1. Observe the sample food containers on display and match the pre-measured amounts of sodium and sugar to each of the sample foods.
2. The nutrition labels have been purposefully covered. You will have to rely on your own judgment to complete the activity as accurately as possible.
3. Enter your guesses in the "predicted amount" columns in the data tables below and then check your answers with the key provided and make any necessary corrections in the "actual amount" columns below.

Sodium (Salt):

Food/drink item	Predicted sodium amount (mg)	Actual sodium amount (mg)

Sugar:

Food/drink item	Predicted sugar amount (g)	Actual sugar amount (g)

How accurate were you in your predictions? Did any of the answers surprise you?

VI. Post-Lab Questions

1. Use the nutrition label below to answer the following questions.

PLAIN YOGURT		
Nutrition Facts		
4 servings per container		
Serving Size		1 cup (227g)
Amount per Serving		% Daily Value
Total Fat	9g	14%
Saturated Fat	5g	25%
Trans Fat	0g	
Cholesterol	30mg	10%
Sodium	115mg	5%
Total Carbohydrates	12g	4%
Dietary Fiber	0g	0%
Total Sugars	11g	
Includes 0g Added Sugars		
Protein	8g	16%

a) Complete the following table for one serving of yogurt.

Nutrient	**Total Calories**	**% Calories**
Carbohydrate		
Protein		
Fat		

b) Compare the % Calories for carbohydrate, protein and fat in the plain yogurt with the CDC recommended values (listed on pg. 47). Do they fall within or outside the recommended range?

c) Based on these results, is this serving of yogurt a healthy snack choice? Why or why not?

d) Percent Daily Value (DV) on the Nutrition Facts label is a guide to the nutrients in one serving of food. For example, if the label lists 5% for sodium, it means that one serving provides 5% of the sodium you need each day.
Why do you think there is no % Daily values listed for **Trans Fat**?

Lab 4: Enzymes

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Define the following terms: substrate, product, active site and catalyst.
2. Explain how enzymes function as catalysts.
3. Explain how environmental factors such as temperature, pH and salt concentration can affect the functioning of enzymes.
4. Design an experiment to test the effect of environmental factors on enzyme function and analyze the results of your experiment.
5. Explain the purpose of using a spectrophotometer.

II. Background Information:

A. Enzymes[1]:

Enzymes speed the rate of chemical reactions. A **catalyst** is a substance involved in, but not consumed in, a chemical reaction. Enzymes are proteins that catalyze biochemical reactions by lowering the activation energy necessary to break the chemical bonds in reactants and form new chemical bonds in the products (Fig. 4.1). Catalysts bring reactants closer together in the appropriate orientation and weaken bonds, increasing the reaction rate. Without enzymes, chemical reactions would occur too slowly to sustain life.

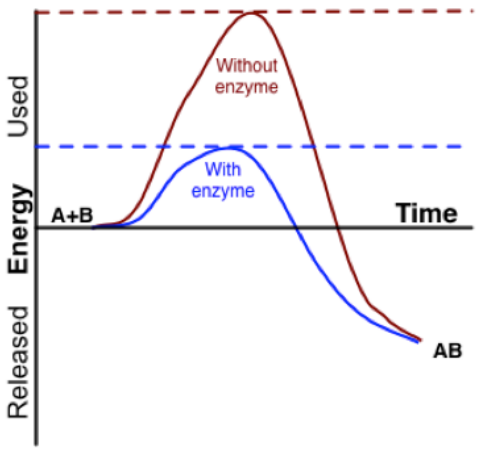


Fig. 4.1. Enzymes catalyze chemical reactions by lowering the activation energy necessary for a chemical reaction to proceed.[2]

The functionality of an enzyme is determined by the shape of the enzyme. The area in which bonds of the reactant(s) are broken is known as **the active site**. The reactants of enzyme catalyzed reactions are called **substrates**. The active site of an enzyme recognizes, confines,

[1] Washington State Open Course Library: http://opencourselibrary.org/

[2] Wikimedia Commons: http://commons.wikimedia.org

and orients the substrate in a particular direction (Fig. 4.2). Enzymes are substrate specific, meaning that they catalyze only specific reactions. For example, proteases (enzymes that break peptide bonds in proteins) will not work on starch (which is broken down by the enzyme amylase). Notice that both of these enzymes end in the suffix -ase. This suffix indicates that a molecule is an enzyme.

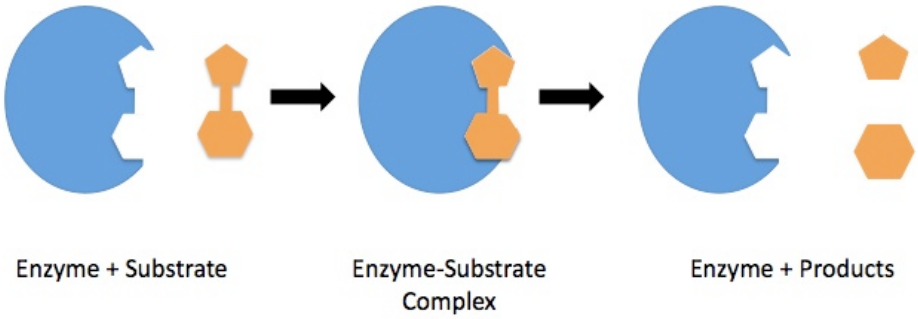


Fig. 4.2. A diagram illustrating a substrate binding to the active site of an enzyme. After the product is formed and released from the active site, the enzyme can accept a new substrate molecule.[3]

B. Environmental Effects on Enzyme Activity:

Environmental factors may affect the ability of enzymes to function. Enzymes function best when they are operating within optimal environmental conditions such as within a specific range of temperatures and pH (Fig. 4.3). It is important to note that enzymes in different organisms, or even in different parts of the same organism function optimally under different conditions. For example, the enzymes in your muscle cells may function better under close to neutral pH whereas the enzymes that break down the food in your stomach function optimally under acidic conditions (Fig. 4.4).

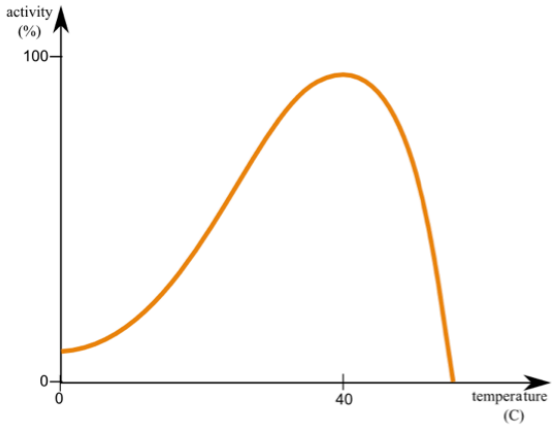


Fig. 4.3. The relationship between temperature and enzyme activity. Different enzymes will have peak activity at different temperatures.[4]

[3] Wikimedia Commons: http://commons.wikimedia.org

[4] Wikimedia Commons: http://commons.wikimedia.org

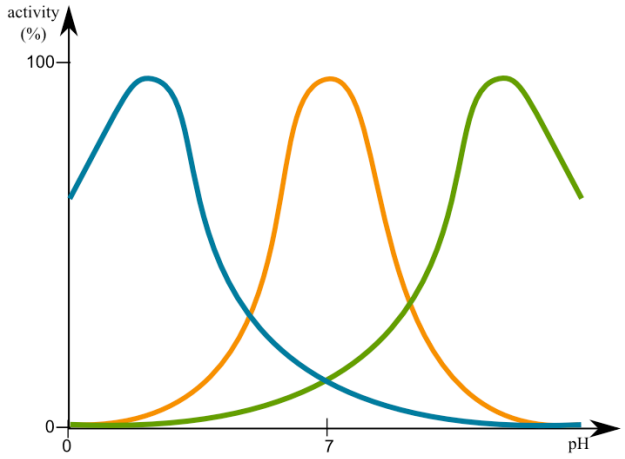


Fig. 4.4. The relationship between pH and enzyme activity for three different enzymes. Note that each enzyme demonstrates peak activity at a different pH.[5]

C. Catecholase and Catechol

Small amounts of the substrate **catechol** occur naturally in fruits and vegetables, along with the enzyme **catecholase**. Upon mixing the enzyme with the substrate and exposure to oxygen (e.g. when a potato or apple is cut and left out), the colorless catechol oxidizes to a reddish-brown product called **quinone**. Quinone has antimicrobial properties, which slows the spoilage of wounded fruits and other plant parts.

Reaction:	Catechol + (substrate)	Catecholase → (enzyme)	Quinone (product)
Color or source:	colorless	potato extract	brown

The enzyme can be inactivated by adding an acid, such as lemon juice, and slowed with cooling. Cooking denatures the enzyme because high heat causes the shape of the enzyme, particularly the active site, to change and no longer be functional. Covering cut fruit in an airtight seal also prevents the browning reaction because oxygen is necessary for the enzyme to break down the substrate.

D. Catalase and Hydrogen Peroxide

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of **hydrogen peroxide** to **water and oxygen**. It is a very important enzyme in protecting the cell from oxidative damage.

Catalase catalyzes the following reaction: $2H_2O_2$ (hydrogen peroxide) $\rightarrow$ 2 $H_2O + O_2$ Adding hydrogen peroxide and observing the reaction can demonstrate the presence of catalase in a microbial or tissue sample. The production of oxygen can be seen by the

[5] Wikimedia Commons: http://commons.wikimedia.org

formation of bubbles. This easy test, which can be seen with the naked eye, without the aid of instruments, is possible because catalase has a very high specific activity, which produces a detectable response, as well as the fact that one of the products is a gas.

E. Amylase and Starch

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch but little sugar, such as rice and potatoes, may acquire a slightly sweet taste as they are chewed because amylase degrades some of their starch into simple sugars. The pancreas and salivary gland make amylase hydrolyze dietary starch into disaccharides and trisaccharides, which are converted by other enzymes to glucose to supply the body with energy. One way to observe the production of simple sugars like maltose and glucose is by using a Benedict's test (refer to Lab 3).

Amylase catalyzes the following reaction:
starch (polysaccharide of glucose molecules) → maltose (disaccharide of glucose molecules)

F. Quantifying Enzyme Activity using the Spectrophotometer:

A **spectrophotometer** is an instrument that is used to measure the amount of light that is transmitted or absorbed as it passes through a solution (Fig. 4.5). According to Beer's law, the amount of light absorbed by the solution is proportional to the concentration of the absorbing material. Thus, spectrophotometry is one method of quantifying reaction rate when the substrate is colorless and the product has color; the darker the product color, the greater the absorbance will be, which would indicate that the reaction magnitude is greater.

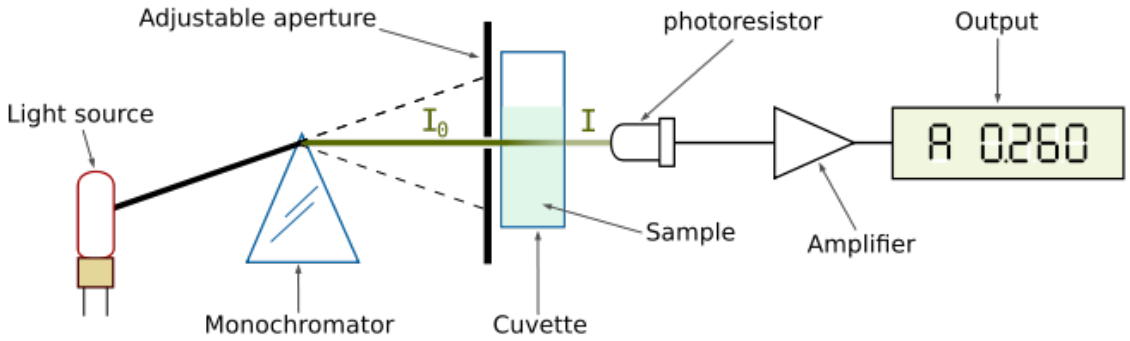


Fig. 4.5. A diagram of how a spectrophotometer works by passing a beam of light through a sample solution located inside a cuvette. The amount of light absorbance is a quantifiable measure of reaction rate in our experiment.[6]

[6] Wikimedia Commons: http://commons.wikimedia.org

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7th ed.): pp.80-82 (Enzymes)

B. Recommended background reading (optional):
1. McGraw Hill Online Learning Center: How Enzymes Work
http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

2. Properties of Enzymes and Use in Industries
https://www.bbc.com/education/guides/zdt4jxs/revision/1

3. Enzymes and Digestion
http://www.bbc.co.uk/schools/gcsebitesize/science/add_aqa_pre_2011/enzymes/enzymes_and_digestion1.shtml

IV. Pre-Lab Questions:

1. a) Enzymes are an example of which macromolecule? _____

b) What are the subunits that make up enzymes? _____

2. a) The location that a substrate binds to an enzyme is called: _____

b) List <u>two</u> environmental variables that may cause an enzyme to denature:

c) Explain how an enzyme functions as a catalyst of chemical reactions.

d) Provide one explanation as to why denatured enzymes cannot catalyze chemical reactions.

3. For the **catecholase** experiment that we will be conducting today, identify the following:

a) What is the substrate that catecholase breaks down? _____

b) What is the product that is formed? _____

c) How can we observe enzyme activity with this reaction?

4. For the **catalase** experiment that we will be conducting today, identify the following:

a) What is the substrate that catalase breaks down? _____

b) What is the product that is formed? _____

c) How can we detect enzyme activity with this reaction?

5. For the **amylase** experiment that we will be conducting today, identify the following:

a) What is the substrate that amylase breaks down? _____

b) What is the product that is formed? _____

c) How can we detect enzyme activity with this reaction?

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• 10 test tubes • 6 cuvettes • 10ml graduated cylinder • Three 1ml plastic pipettes • 150 ml beaker • glass stir rod • squirt bottle with DI water • china marker • test tube clamp	• Potato • Blender • Digital scale and weigh boat • Cutting board, knife and vegetable peeler • 500ml flask, cheesecloth and funnel • 250ml graduated cylinder • Catechol solution • Spectrophotometer and "blank" cuvette • 100° 37° and room temperature water baths • ice bath • pH 4, 8 and 12 solutions • 0%, 10% and 25% salt solutions • Benedict's, starch, amylase and catalse solutions • liver

B. Procedure

1. Isolation of the Catecholase Enzyme:

One group should conduct the following procedure for the whole class:

1. Peel a potato and dice it into small pieces (approx. $2cm^3$).
2. Weigh out 100g of the diced potato and place it in a blender along with 150ml of tap water. Blend for about 1min until the mixture is smooth.
3. Place the extra, diced potato in a beaker and cover it with water to prevent it from browning.
4. Filter the slurry in the blender through several layers of cheesecloth held within a funnel into a flask and keep the flask covered.
5. This is the stock solution of the **catecholase enzyme** that all the groups will use. If more enzyme solution is needed, the above steps should be repeated with the remaining diced potato.
6. Wash all the equipment used to prepare the enzyme solution immediately.

Important Note: The stock solution of potato extract should be used as quickly as possible since the solution will start to oxidize and turn brown when exposed to air.

2. Catecholase Control Experiments:

The purpose of the following control experiments is to observe the color of the solution in the i) absence and ii) presence of the enzyme. A **negative control** ensures that there is no effect when there should be no effect. A **positive control** ensures that there is an effect when there

should be an effect. If the positive control does not produce the expected result, there may be something wrong with the experimental procedure and the experiment should be repeated.

Negative Control Experiment:

1. Add 1ml of water to a clean test tube.
2. Add 10 drops of catechol to the tube.
3. Gently mix the solution by holding it upright and gently tapping the bottom.
4. Wait 1 min and observe the color of the solution.

Positive Control Experiment:

1. Add 1ml of potato extract (containing catecholase) to a clean test tube.
2. Add 10 drops of catechol to the tube.
3. Gently mix the solution by holding it upright and gently tapping the bottom.
4. Wait 1 min and observe the color of the solution.

Complete the data table and answer the questions below. Be sure to answer steps 1-3 before conducting each control experiment. Complete rows 4-6 in the data table after the experiment is completed.

	Negative Control	Positive Control
1. Is the substrate present?		
2. Is the enzyme present?		
3. Your hypothesis (predict whether the product will form and what the final color will be):		
4. Color of solution after **1 min.**		
5. Was the product formed?		
6. Was your hypothesis supported?		

If you observed the solution in the negative control turning reddish-brown, what would this suggest?

If you observed the solution in the positive control remaining colorless, what would this suggest?

3. Spectrophotometer Calibration Instructions:
(Check with your instructor to see if you need to calibrate the spectrophotometer before taking measurements.)

1. For the second part of today's lab, you will quantify the color change in the experiment using a spectrophotometer (review the background information to understand how a spectrophotometer works).
2. You will need to calibrate the spectrophotometer before you begin. You can then record all your data for one experiment without having to the repeat the calibration every time.
3. Make sure the wavelength control is set to 480nm.
4. Remove the aluminum foil from the "**blank**" cuvette (located next to the spectrophotometer), open the lid of the specimen holder, insert the "blank" cuvette into the machine and close the lid.
5. Push the 100% transmittance button.
6. Remove the "blank" cuvette, cover it with the foil and return it to its original location.
7. Place your sample solution in the specimen holder, close the lid and ***quickly*** record the value for ***absorbance*** value on your data sheet (the value may not stabilize since more product will continue to form and the solution will continue to get darker over time). Repeat this last step with all your experimental samples.

4. Testing the Effect of Environmental Factors on Catecholase Enzyme Activity

1. You may be asked to complete ALL or SOME of the following experiments (***check with your instructor before you begin***).
2. Be sure to check your experimental protocol with your instructor ***before*** you proceed and fill in the data table for each experiment that you conduct.
3. Remember to **record your hypothesis before you begin each experiment.**
4. You may need to complete one experiment, record your results, discuss your results with your instructor and wash your glassware ***before*** you begin the next experiment.

<u>Experiment 1: Temperature</u>

1. Add 5ml of water to each of three, clean test tubes.
2. Add 1ml of potato extract (which contains catecholase) to each test tube.
3. Label the tubes near the top with your group name and the temperature of the water bath using a china marker.
4. Place each tube in its corresponding water bath for 20min.
5. At the end of 20min, remove ONE of your test tubes from one of the water baths and place it at your table.
6. Add 10 ***drops*** of catechol to the test tube.
7. Gently mix the solution by holding it upright and tapping the side of the test tube.
8. Transfer the contents of the test tube into the small cuvette and record the absorbance value using the spectrophotometer (see instructions above). Do **NOT** mark the cuvette – it will affect the spectrophotometer readings.
9. Pour out the contents of the cuvette into the **catechol waste** container and wash it.
10. Repeat steps 5-9 for each of the other test tubes in the remaining water baths.

Experiment 1: Temperature

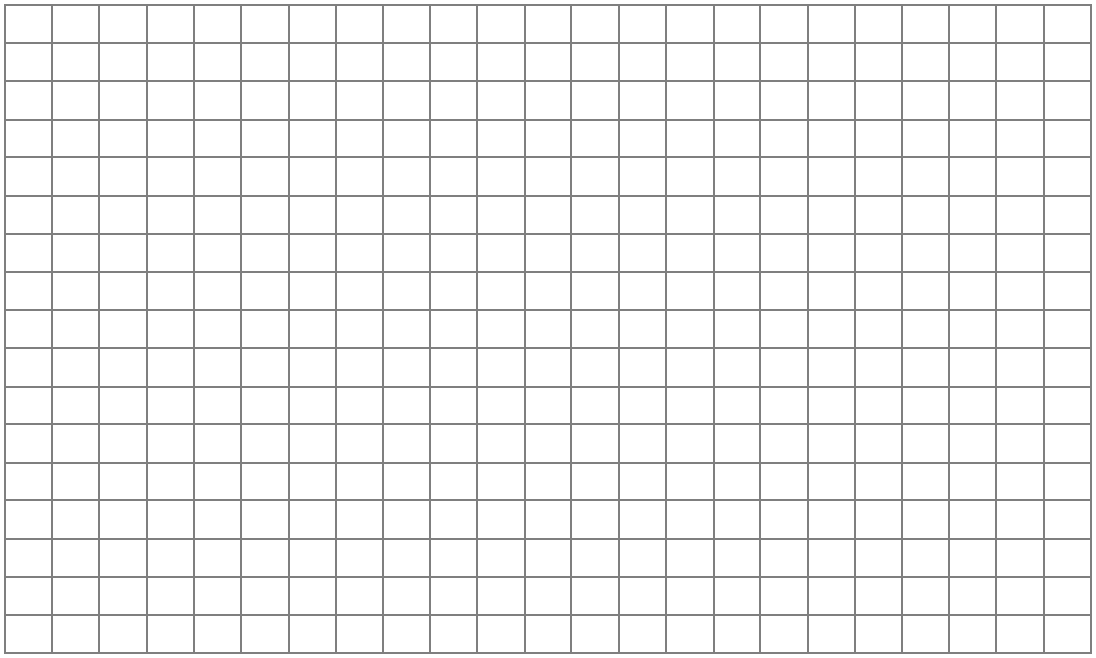
<table>
<tr><td>Hypothesis (Predict how temperature will affect enzyme activity):</td><td colspan="2"></td></tr>
<tr><td>Experimental design changes:
(Explain any changes to the procedure listed above, or any problems you encountered)</td><td colspan="2"></td></tr>
<tr><td rowspan="4">Results:
(Record the absorbance for each temperature tested)</td><td>Temperature (°C)</td><td>Absorbance</td></tr>
<tr><td>20</td><td></td></tr>
<tr><td>37</td><td></td></tr>
<tr><td>100</td><td></td></tr>
</table>

Graphing your results for Experiment 1:

Plot temperature on the x-axis and absorbance on the y-axis.

What was your independent variable? _____

What was your dependent variable? _____



Conclusion: How did enzyme activity vary at the different temperatures?

Experiment 2: pH

1. Add 5ml of water to each of three, clean test tubes.
2. Add 1ml of potato extract (which contains catecholase) to each test tube.
3. Label the tubes with the pH using a china marker.
4. Add 30 ***drops*** of each pH solution to the corresponding test tube.
5. Check the final pH of the solution using pH paper (if available). Be careful not to touch or spill the solution.
6. Add 10 ***drops*** of catechol to each of the test tubes.
7. Gently mix the solution by holding it upright and tapping the side of the test tube.
8. Transfer the contents of the test tubes into small cuvettes and record the absorbance value using the spectrophotometer. Do **NOT** mark the cuvettes – it will affect the spectrophotometer readings.
9. Pour out the contents of the cuvettes into the **catechol waste** container and wash them.

Experiment 2: pH

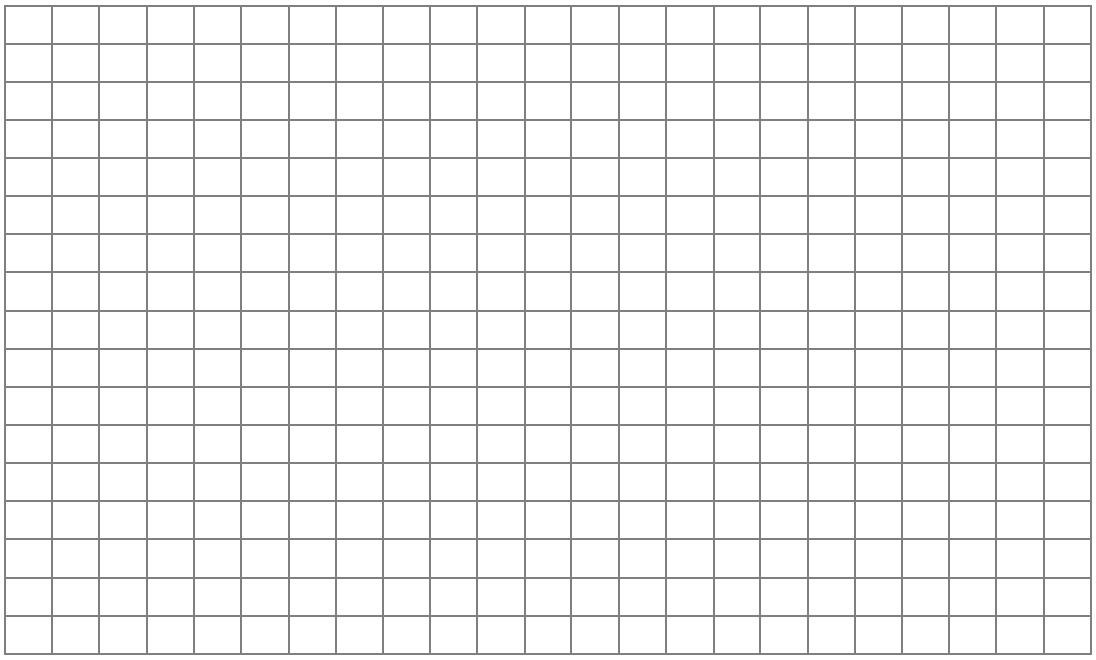
Hypothesis:		
Experimental design changes: (Explain any changes to the procedure listed above, or any problems you encountered)		
Results: (Record the final pH of each solution and the absorbance for each pH tested)	pH:	Absorbance:

Graphing your results for Experiment 2:

Plot pH on the x-axis and absorbance on the y-axis.

What was your independent variable? _____

What was your dependent variable? _____



Conclusion: How did enzyme activity vary with the different pH levels?

Experiment 3: Salt Concentration
(Time permitting - check with your instructor before proceeding with this experiment.)

1. Add 5ml of water to each of three, clean test tubes.
2. Add 1ml of potato extract (which contains catecholase) to each test tube.
3. Label the tubes with your group name and the salt concentration using a china marker.
4. Add 30 ***drops*** of each salt solution to the corresponding test tube. Let the test tubes sit at your counter for 10 min before proceeding with step 5.
5. Add 10 ***drops*** of catechol to each of the test tubes.
6. Gently mix the solution by holding it upright and tapping the side of the test tube.
7. Transfer the contents of the test tubes into small cuvettes and record the absorbance value using the spectrophotometer. Do **NOT** mark the cuvettes – it will affect the spectrophotometer readings.
8. Pour out the contents of the cuvettes into the **catechol waste** container and wash them.

Experiment 3: Salt Concentration

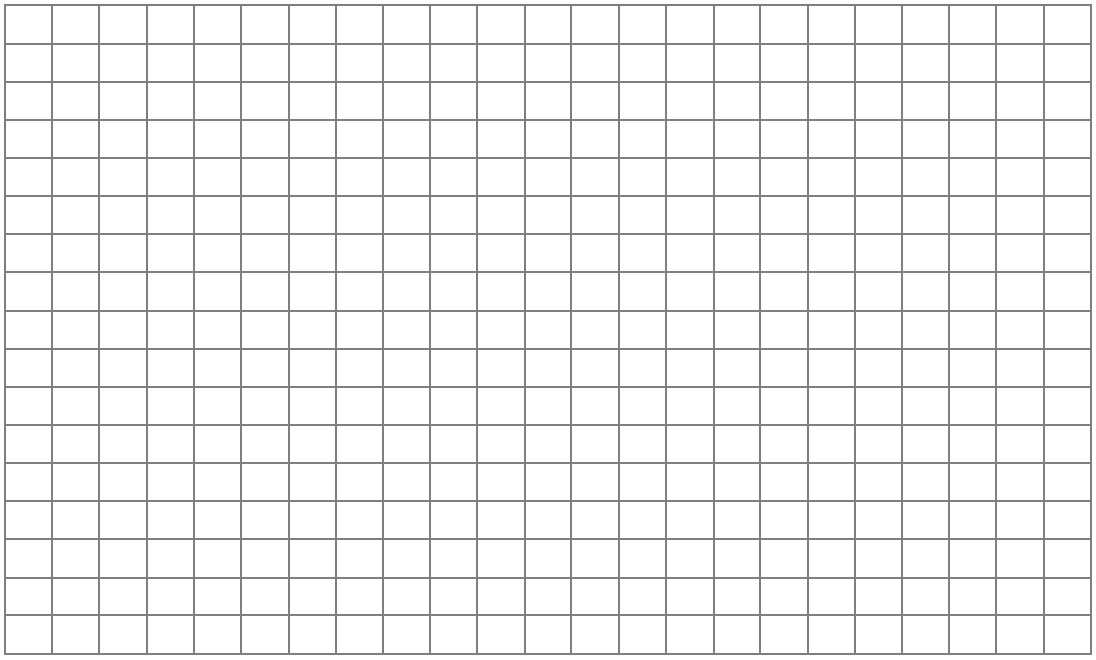
Hypothesis:		
Experimental design changes: (Explain any changes to the procedure listed above, or any problems you encountered)		
Results: (Record the absorbance for each salt concentration tested)	Salt Concentration:	Absorbance:
	0%	
	10%	
	25%	

Graphing your results for Experiment 3:

Plot salt concentration on the x-axis and absorbance on the y-axis.

What was your independent variable? _____

What was your dependent variable? _____



Conclusion: How did enzyme activity vary with the different salt concentrations?

5. Observing Amylase Enzyme Activity

1. Add 10 drops of starch solution (substrate) to each of two, clean test tubes.
2. Label one test tube "+ control" and the other one "- control." Mark both tubes with your group name or number.
3. Add 10 drops of amylase (enzyme) to the "+ control" test tube.
4. Add 10 drops water to the "- control" test tube.
5. Add 10 drops Benedict's solution to each tube.
6. Place both tubes in the 100°C water bath for 2min.
7. Remove your test tubes and immediately observe and record whether the solution turns red/orange (+) or remains blue (-).

	Negative Control	Positive Control
1. Is the substrate present?		
2. Is the enzyme present?		
3. Your hypothesis (predict whether the product will form and what the final color will be):		
4. Color of solution after boiling for **2 min.**		
5. Was the product formed?		
6. Was your hypothesis supported?		

6. Observing Catalase Enzyme Activity (Instructor Demo)

Your instructor will place a piece of liver and potato in separate containers and add hydrogen peroxide to each container. Liver and potato contain different amounts of the catalase enzyme. Refer to the background information and your experimental observations to help you answer the following questions.

	Liver	Potato
1. Is the substrate present?		
2. Is the enzyme present?		
3. Your hypothesis (predict whether the product will form in each sample):		
4. Observation of the reaction (Did bubbles form?)		
5. Was the product formed?		
6. Was your hypothesis supported?		

VI. Post-Lab Questions

1. Did the results of any of your enzyme experiments refute your hypotheses? If so, propose an explanation for each result that refuted your hypothesis.

2. You conduct an experiment to see how salt concentration affects catecholase enzyme activity. You conduct the experiment with 0%, 5%, 15% and 30% salt and measure the reaction rate at each concentration by recording the absorbance of light using a spectrophotometer. Refer to the data table below when answering the following questions.

Salt Concentration	Absorbance
0%	1.0
5%	0.8
15%	0.5
30%	0.1

a) What is the substrate that catecholase breaks down? _____ What is the product that is formed as a result of this reaction? _____

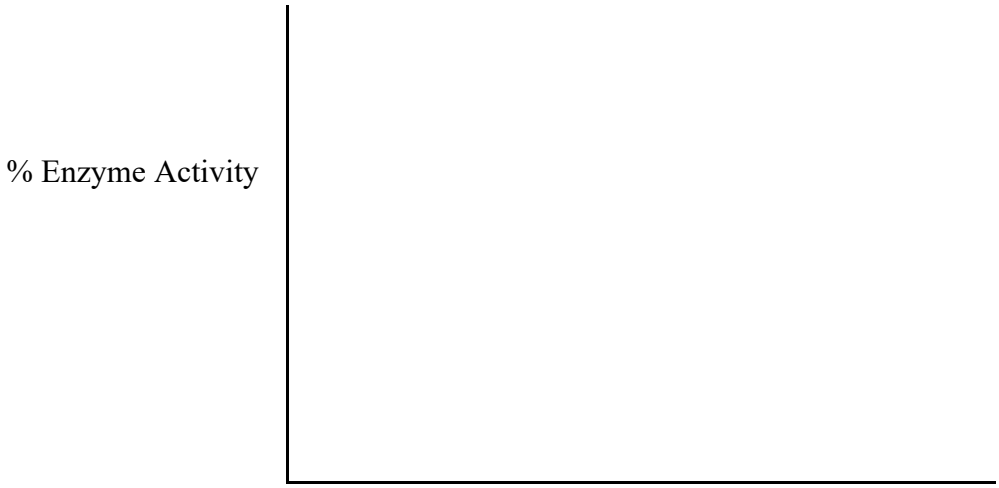
b) At which salt concentration is enzyme activity at an optimal level? _____

c) Provide one biological explanation of how salt concentration affects enzyme activity at 30%.

d) Graph the data in the space below, using appropriately labeled axes.

3. Some animals are **homeothermic**, meaning they maintain a constant body temperature (e.g. mammals) whereas other animals are **poikilothermic**, meaning their body temperature changes with the environmental temperature (e.g. many species of fish).

a) Predict and draw on the graph below what the % enzyme activity curve would look like for a homeothermic and a poikilothermic animal if their body temperature were to change. Be sure to label each curve as homeothermic or poikilothermic.



b) Provide a biological explanation for your prediction.

Lab 5: Photosynthesis and Cellular Respiration

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Write the equation for photosynthesis and cellular respiration.
2. Identify the necessary reactants and products of photosynthesis and cellular respiration.
3. Explain why fluorescence of chlorophyll occurs and describe how it fluoresces.
4. Explain how cresol red can be used as an indicator of photosynthesis and cellular respiration.
5. Explain how paper chromatography works and how to interpret the results.
6. Describe the pigments found in spinach.
7. Identify the elements of the photosynthesis experiments and analyze the results.

II. Background Information:

A. Photosynthesis:

Photosynthesis is a process used by plants algae, and many species of bacteria to convert the light energy captured from the sun into chemical energy that can be used to fuel the organism's activities. Photosynthetic organisms are called photoautotrophs, since they can create their own food. Photosynthesis uses carbon dioxide and water to produce glucose, and releasing oxygen as a waste product.

$$6\ CO_2 + 6\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2$$

The primary site of photosynthesis in plants is in the leaf, within the chloroplast. **Chlorophyll** is a green pigment found in cyanobacteria and the chloroplasts of algae and plants, and allows the absorption of light energy. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. However, it is a poor absorber of green and near-green portions of the spectrum, hence the green color of chlorophyll-containing tissues.

Photosynthesis consists of two reactions: the **light-dependent reaction** and the **light-independent reaction (or Calvin cycle)**. In the light-dependent reaction, solar energy is captured by chlorophyll and accessory pigments (such as carotenoids), and temporarily stored in ATP and NADPH. The energy in these molecules is then used to power the light-independent reaction. In the Calvin cycle, the enzyme Rubisco captures CO_2 from the atmosphere and forms glucose and other carbohydrate molecules.

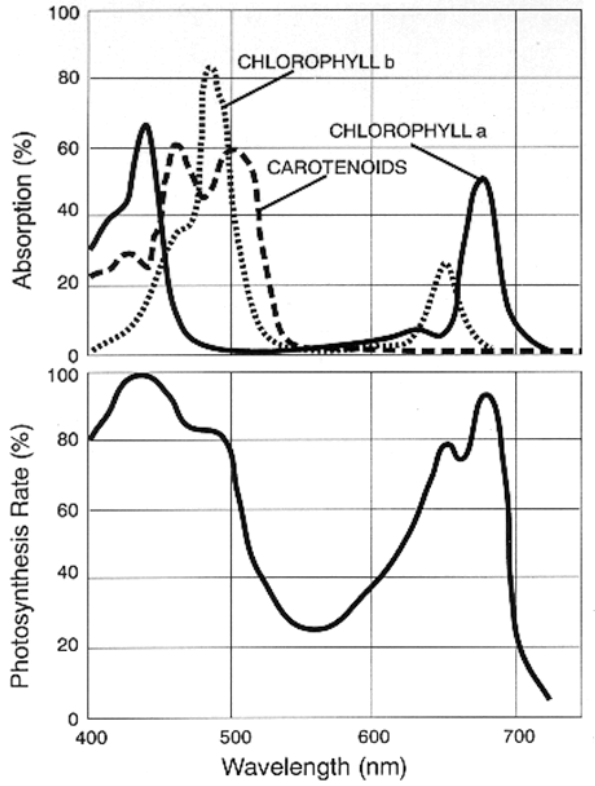


Fig. 5.1. (Top) The absorption spectrum of different light-absorbing pigments in plants. (Bottom) The action spectrum showing the photosynthetic activity of all the light absorbing pigments combined (400nm = violet, 500nm = blue, 550nm = green, 600nm = yellow and 650nm = red light)[1]

B. Cellular Respiration:

Cellular respiration is the set of the reactions that take place in the cells of organisms to convert biochemical energy from food into adenosine triphosphate (ATP), and then release waste products. The products of photosynthesis are the reactants of aerobic cellular respiration:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \sim 36\ ATP \text{ (estimates range from 30-36)}$$

Cellular respiration occurs in a series of steps: glycolysis is the first step, and occurs in the cytosol of cells in all living things. The product of glycolysis is pyruvate and is oxidized to acetyl-CoA in the presence of oxygen. If oxygen is present, acetyl-CoA enters the citric acid cycle (Kreb's cycle) in the mitochondria of eukaryotic cells. The final step in the process is oxidative phosphorylation, where the majority of ATP is produced and oxygen acts as the final electron acceptor, forming water as a waste product.

[1] Wikimedia Commons: http://commons.wikimedia.org

C. Measurement of Metabolic Activity

To determine whether a metabolic reaction is occurring, we can measure the reactants being consumed or products being formed (Think back to the enzyme lab where we measured quinone production by measuring the absorbance of light using the spectrophotometer.) Cresol red is a solution that contains a pH indicator. In a basic solution the color will be red and in an acidic solution the color will change to yellow. When carbon dioxide (CO_2) is mixed with water, it forms carbonic acid (H_2CO_3). By adding cresol red to water, then adding CO_2 to it, we can observe a change in color associated with a change in pH. If the addition of CO_2 is due to a metabolic process, such as cellular respiration, then the color change will indicate the occurrence of the metabolic process.

D. Chromatography:

Chromatography is a method commonly used to separate chemicals. With paper chromatography, which is the method we will use in this lab, a nonpolar solvent moves through a polar material (such as paper) that has a chemical sample placed at the starting end. The paper will attract polar molecules more strongly, causing them travel more slowly up the paper. On the other hand, nonpolar molecules will remain in the solvent mixture and travel further up the paper. How the pigment molecules are separated will depend on the chromatography material (paper is just one of many materials that can be used), the solvent, and the properties of the chemical pigments.

As mentioned earlier, plants contain a variety of photosynthetic pigments. We will use paper chromatography to separate the pigments from a spinach leaf and try to identify them using the pigment colors. The main pigments that are usually observed in spinach are chlorophyll (a and b), which are both green, and two types of accessory pigments called carotenoids. One of these accessory pigments is carotene and usually appears yellowish orange and the other is xanthophyll, which appears bright yellow.

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7th ed.): pp.92-100 (Cellular Respiration) and pp. 108-115 (Photosynthesis).

B. Recommended background reading (optional):
1. Photosynthesis Video by Paul Andersen:
http://www.youtube.com/watch?v=g78utcLQrJ4

2. Respiration Video by Paul Andersen:
http://www.youtube.com/watch?v=Gh2P5CmCC0M

3. Biology website by Paul Andersen:
http://www.bozemanscience.com/biology-main-page/

IV. Pre-Lab Questions:

1. Write the equation for photosynthesis:

2. a) Which wavelength (color) of light does chlorophyll absorb the ***least***? _____

b) What wavelengths (colors) of light does chlorophyll absorb the ***most***?

_____ and _____

3. Write the equation for cellular respiration.

4. a) What color would cresol become in a solution that contains water and carbon dioxide?

b) What is the reason for this?

5. What are the colors of the following pigments found in spinach?

a) chlorophyll a: _____

b) chlorophyll b: _____

c) carotene: _____

d) xanthophyll: _____

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• 2 plastic syringes • 1 hole puncher • 2 plastic cups • power strip • light bulb • aluminum foil • ruler • chromatography tube with solvent • test tube rack	• fresh spinach leaves • cutting board • chromatography paper • scissors • bicarbonate buffer solution • plain buffer solution • colored cups or plastic wrappers

B. Procedure

1. Fluorescence of Chlorophyll extract:

When chlorophyll absorbs light energy, electrons become excited and move to a higher energy state. If the photosystem within the plant does not harness that energy, the electrons will return to their original energy state, and in the process, release the light energy that they had absorbed. This experiment demonstrates the fluorescence of chlorophyll.

Use a solution of chlorophyll extract from spinach and shine a bright light on to it. You may need to turn off the ambient room light to see the results more clearly.

*What color does the chlorophyll extract fluoresce under the bright light?*_____

Provide a reason for this observation.

2. Observing Photosynthesis in *Elodea*:

Observe the two beakers containing the aquatic plant *Elodea* – one has been placed in light while the other has been left in the dark.

	Elodea in the dark	*Elodea* in the light
Indicate which is the + control and which is the - control		
Do you observe gas bubbles?		
What is the gas that is contained in the bubbles you observed?		

3. Measuring Photosynthetic Rate in *Elodea*:

Observe the two test tubes, one containing the aquatic plant *Elodea* and sodium bicarbonate buffer, and the other one containing only sodium bicarbonate buffer (no *Elodea*): Measure the amount of gas produced in each test tube and record your results below.

	Test tube with *Elodea*	Test tube without *Elodea*
Indicate which is the + control and which is the - control		
How much gas is produced?		

4. Use of Cresol Red as an Indicator of Photosynthesis and Cellular Respiration:

Observe and record how cresol red changes color when carbon dioxide is added to water.

Color of cresol red in the absence of CO_2: _____

Color of cresol red in the presence of CO_2: _____

Your instructor will place a sample *Elodea* plant in the cresol red solution that contains CO_2 and water. A similar setup will be prepared with the cresol red solution but without the *Elodea*. Observe and record what happens to the colors of the solutions over time in the data table below.

Is the beaker without the Elodea a positive or negative control? Why?

What color change would you expect to see in the beaker containing the Elodea after 90min? Why?

Time (min)	Color in beaker containing *Elodea*, cresol red and CO_2.	Color in beaker containing only cresol red and CO_2.
0		
45		
90		

Do your observations match your hypothesis? If not, provide an explanation for your observations.

5. Use of Chromatography to Separate Plant Pigments

You will be using paper chromatography today to separate plant pigments.

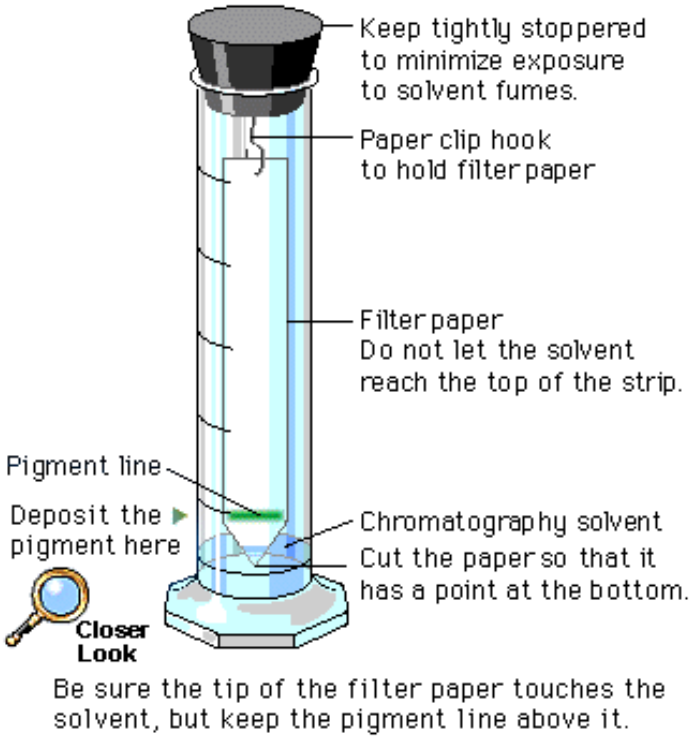


Fig. 5.2. Diagram of the paper chromatography setup.[2]

1. Cut a strip of chromatography paper long enough to hang in the test tube (with cork and hook) such that the bottom of the paper just touches the solvent.
2. Cut a point at the end of the paper. (Refer to the sample drawing or setup provided)
3. Place a spinach leaf near the end of the paper with the point and rub the leaf to transfer some of the pigment onto the paper.
4. Hang the paper with the pointed end towards the bottom in the test tube with solvent. Make sure you hang the paper such that the leaf rubbing comes close to, but does not touch the solvent.

[2] The Biology Place: http://www.phschool.com/science/biology_place/labbench/lab4/design1.html

5. Let the solvent move up the paper until it comes within a few centimeters from the top. Remove the paper and draw a line where the solvent stopped. Lay the chromatography paper on a paper towel and let it air dry for a couple of minutes.
6. Do NOT dump out your solvent, but leave it for the next class. Make sure the cork is on the test tube.
7. Observe your chromatography paper and locate the yellowish orange **carotene** at the top, the blue-green **chlorophyll a**, the yellow-green **chlorophyll b**. The remaining yellow bands are **xanthophylls**.
8. Draw your chromatography paper results below and label the plant pigments.

Chromatography experiment results:

6. Testing the Effect of Environmental Factors on Photosynthetic Rate:
Complete the following experiments. Be sure to check your experimental protocol with your instructor before you proceed and fill in the data sheet for each experiment that you conduct. Remember to record your hypothesis BEFORE you begin each experiment. You will need to complete one experiment, record your results, discuss your results with your instructor and wash your setup ***before*** you begin the next experiment. At the end of all of your experiments, wash and reset your workstation.
Note: If the weather permits, you should conduct your photosynthesis experiments outside (after preparing the syringes inside) and use natural sunlight rather than the lamps in the lab.

a. Presence of Light:

1. Using a hole-puncher, punch out 20 discs from a spinach leaf.
2. Pull the plungers out of two 35 ml syringes and place 10 discs in each syringe.
3. Replace the plungers making sure not to crush the discs.
4. Fill the two syringes with 20 ml of bicarbonate buffer solution.
5. To remove any trapped air from the leaf discs, firmly cover the open tip of the syringe with your thumb and pull on the plunger to produce a slight vacuum. Shake the syringe gently while pulling on the plunger to shake out any gas trapped within the discs. Because gas expands at a negative pressure, gases within the discs should escape.
6. Let go of the plunger while still keeping your finger firmly on the syringe tip. Push on the plunger to fill the vacated air spaces with the bicarbonate buffer solution. The discs should start to sink.
7. Remove your finger from the syringe tip and observe how many of the discs sink to the bottom. Repeat the procedure until most of the discs sink to the bottom.

8. Invert the syringes (syringe tip pointing upwards) and cover one syringe completely with foil and place both the covered and uncovered syringes standing on their plungers the same distance from the light source (approx. 3 cm).
9. Record the # of sunken discs at the beginning of the experiment in the data table below (you need to exclude any discs that don't sink from your calculations).
10. After ~20 min, count how many discs are floating in each syringe (you will need to subtract any discs that were floating before the experiment began).
11. Calculate the percentage of discs that are floating in each syringe:
(# new floating disks / total # sunken in the beginning) x 100.

b. Presence of CO_2:

1. Repeat the procedure listed in (1) but use the bicarbonate buffer (containing CO_2**)** in one syringe and the plain buffer (not containing CO_2**)** in the other syringe.
2. Place both syringes, uncovered, the same distance from the light source.
3. After ~20 min, count how many discs are floating in each syringe (you will need to subtract any disks that were floating before the experiment began).
4. Calculate the percentage of discs that are floating in each syringe.

c. Distance from Light Source (if conducting the experiments outside, skip this activity)

1. Repeat the procedure listed in (1) but place the two syringes at different distances from the light source (3cm and 20cm).
2. After ~20 min, count how many discs are floating in each syringe (you will need to subtract any disks that were floating before the experiment began).
3. Calculate the percentage of discs that are floating in each syringe.

Additional Experiments (time permitting):
d. Color of Light:

1. Repeat the procedure listed in (1) but use a different colored cup or plastic wrap to cover each syringe and change the color of light that reaches the leaf disks inside the syringe.
2. Place both syringes the same distance from the light source.
3. Record your experimental results in the data table below.

e. Type of Leaf

1. Repeat the procedure listed in (1) but use a different type of leaf for each syringe (old vs. young leaves, leaves from two different plants, etc.)
2. Record your experimental results in the data table below.

7. Experimental Results and Analyses:

Experiment a: Presence of Light

Question:	*Under which condition (light or dark) will the photosynthetic rate be higher?*	
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Light**	**Dark**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment b: Presence of CO_2

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Bicarbonate buffer (with CO_2)**	**Plain buffer (without CO_2)**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment c: Distance from Light Source

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Near (3cm)**	**Far (20cm)**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment d: Color of Light

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Light Color:**	**Light Color:**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

Experiment e: Type of Leaf

Question:		
Hypothesis:		
Independent Variable:		
Dependent Variable:		
Results:	**Leaf Type:**	**Leaf Type:**
# of sunken discs @ time = 0 min		
# of *new* floating discs @ time = 20 min (subtract # floating before exp. began)		
% floating disks (# new floating disks / # sunken @ beginning) x 100.		
Conclusion: (Compare photosynthetic rate for the control and experimental treatments and propose an explanation for your results)		

VI. Post-Lab Questions

1. Explain the purpose of each of the following steps in your spinach photosynthesis experiment:

a) Hole punching the leaves: _____

b) Adding bicarbonate solution to the syringe: _____

c) Exposing the disks to a vacuum in the syringe: _____

2. Why was the percentage of floating leaf disks a reasonable measure of photosynthetic activity in this experiment?

3. You conduct an experiment to see how color of light affects photosynthesis in spinach. Refer to the data table below when answering the following questions.

Color of light	% Floating disks
White	100%
Red	70%
Blue	80%
Green	10%

a) Which light is best for promoting photosynthesis in spinach? _____

b) Provide one biological explanation for why % floating disks is low under green light.

c) Graph the data in the space below, using appropriately labeled axes.

4. According to your chromatography experiment, how many different pigments does a spinach leaf contain? What are their names and colors?

5. If you placed an *Elodea* plant in a solution of cresol that was yellow due to the presence of carbon dioxide in water, and you placed this setup in the dark, would you expect the solution to turn red over time? Why or why not?

Lab 6: Microscopes and Cells

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Compare and contrast the characteristics of the compound light microscope and the dissecting microscope.
2. Identify the parts of the compound and dissecting microscopes.
3. Calculate the total magnification obtained by the compound and dissecting microscopes.
4. Operate and care for the microscopes and prepare wet mount slides of samples.
5. Explain how the field of view changes with different objective lenses.
6. Describe the main characteristics of Domain Bacteria, Archaea and Eukarya.
7. Visually differentiate, and explain the similarities and differences between: *Anabaena*, *Euglena, Paramecium, Amoeba*, animal cell, and plant cell.

II. Background Information:

In this lab you will be studying the different characteristics of prokaryotic and eukaryotic cells, however, before you begin looking at individual slides, you must learn how to use the dissecting and compound light microscopes to ensure the microscopes are not damaged during the lab activities.

1. Microscopy is the technical field of using a **microscope** to observe samples and objects that are too small to see with the human eye. A microscope magnifies the image of a specimen through the use of lenses and light. There are many different types of microscopes, and the properties of the images produced and the types of specimens that can be studied with each kind of microscope are different. In this lab we will focus on the dissecting microscope and the compound light microscope.

2. Dissecting Microscope
The dissecting microscope provides a three-dimensional view of a sample. As the name implies, this type of microscope can be used to conduct close work such as dissections, microsurgery, etc. and to look at objects that are too big to fit flat under a cover slip on a glass slide. Dual light sources allow for visualization of the specimen using both reflected and transmitted light, and can be adjusted depending on the specimen and on the features you want to observe. The path of light goes through two lenses: the **objective lens** (close to the object) and the **ocular lens** (close to the eye) located on the eyepiece (Fig. 6.1).

3. Compound Light Microscope
The compound light microscope contains a compound set of lenses that provides a much larger range of total magnification than the dissecting microscope (4X-1000X). The ocular lens typically has a magnification of 10X, and there are three or four objective lenses located on a revolving nosepiece depending on the microscope model. The smallest objective lens 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** (total area observed). As you

increase the magnification, the field of view decreases. Increasing the magnification requires more light or **illumination** in order to better observe the specimen. The compound light microscope depends on transmitted light to illuminate a thin section of a specimen. Consequently, only small organisms can be observed whole; larger specimens need to be sectioned into thin slices to allow enough light to pass through it.

The main parts of a microscope include the **base**, which rests on the table and the **arm**, which rises from the base and supports the **stage,** where the slide is placed and secured in place with the **stage clips**. The slide can then be moved using the **stage control knobs** located on the side of the stage. Most microscopes have a **condenser** located below the stage. It concentrates the light on the object and may be raised or lowered using the **condenser control knob**. Usually, the condenser should be raised to its highest position. There are two focusing knobs. The **coarse focus knob** is the larger knob and is used to bring objects into rough focus when using the smallest objective lens. The **fine-focus knob** is the smaller knob and is used to fine-tune the focus after the specimen has been located. **The fine-focus knob is the ONLY focus that should be used with the higher objective lenses.**

Fig. 6.1. A compound light microscope (left) and a dissecting microscope (right).[1]

[1] Wikimedia Commons: http://commons.wikimedia.org

4. Calculating total magnification:

Dissecting Microscope: The total magnification is simply the number that is aligned with the eyepiece. There is no additional calculation that needs to be made.

Compound (Light) Microscope: When calculating the total magnification of the specimen being observed under a compound microscope, use the following formula:

Total magnification = (ocular lens magnification) x (objective lens magnification)

The ocular lens magnification on the compound microscope is 10x.

Example: What is the total magnification when using the objective lens (4x)?

Total magnification = (10x) x (4x) = 40x

5. Cells[2]

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 general types of cells: **prokaryotic** and **eukaryotic**. These two words have their root in the Greek word karyon (nut), which refers to a cell's nucleus. The prefix pro- means "before" or "prior to", thus prokaryotic means "before having a nucleus." Prokaryotic cells do not have a membrane-bounded nucleus and their genetic material (DNA) is only loosely confined to a nuclear area within the cell. The prefix “eu”- means "true." The cells of eukaryotes have true, membrane-bounded nuclei containing their genetic material. Prokaryotic cells are generally smaller than eukaryotic cells (about ten times smaller) and lack membrane-bound organelles, whereas eukaryotic cells are compartmentalized by membrane-bound organelles with specialized functions.

Organisms belonging to the Domains **Bacteria** and **Archaea** are prokaryotic, whereas organisms belonging to the Domain **Eukarya** are eukaryotic. Organisms such as protists, fungi, plants and animals belong to Domain Eukarya.

a) Bacteria:

Present-day bacteria are found everywhere: in soil, in water, in ice, in boiling hot pools of water, even kilometers underground! Many bacteria are heterotrophic (consume organic compounds) while others are autotrophic (make their own food through photosynthetic). Morphologically, bacteria are either spherical (**cocci**), rod-shaped (**bacilli**), or spiral-shaped (**spirillum**). Furthermore, bacteria are often found in clusters or in chains. Some have one or more flagella. They are extremely small (approximately 1 to 2 μm in diameter). To view them with the light microscope, one must usually use an oil-immersion lens (100X). Even then, not much more than their basic shapes will be visible. Some of the photosynthetic bacteria, called **cyanobacteria**, are large enough to be seen at a total magnification of 400X.

[2] Washington State Open Course Library: http://opencourselibrary.org/

b) Plant and Animal cells:
The cells of plants are eukaryotic and contain a large **central vacuole** used for storing water, pigments, and wastes. Within the cytoplasm are various types of plastids. These include the green-colored chloroplast, which is responsible for photosynthesis and others for storing starch or pigments. A **cell wall** composed of cellulose surrounds the plant cell and provides structural support. Animal cells are also eukayotic and contain many of the same organelles as plant cells, although they lack a large, central vacuole and plastids. Both plant and animal cells contain **mitochondria**, the organelles responsible for converting organic compounds into energy in the form of ATP to power cellular processes.

c) Protists
Protists are a large and diverse group of eukaryotic organisms that include unicellular, colonial and multicellular members that are not closely related through evolution. Protists have diverse life cycles, trophic levels, modes of locomotion and cellular structures. Protists live in almost any environment that contains liquid water. Many protists, such as algae, are photosynthetic and are vital primary producers in aquatic ecosystems. Other protists include pathogenic (disease-causing) organisms such as species of *Plasmodium*, which cause malaria. Members of the genus *Amoeba* move using extended cytoplasmic projections called **pseudopodia**. These structures can also be used to engulf food in a process called **phagocytosis**. *Euglena* is a genus characterized by having a **flagellum** (*pl.* **flagella**); a long, thin, whip-like projection that is used for locomotion. Some members of this genus are both autotrophic and heterotrophic; the cells contain chloroplasts for photosynthesis and they may also engulf food by phagocytosis when light is not available. *Paramecia* contain hair-like structures for movement called **cilia** and they are heterotrophic.

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7th ed.): pp.56-57 (Microscopes), pp. 58-70 (Cells), pp.300 (Bacterial shapes) and pp.307-310 (Protists).

B. Recommended background reading (optional):
1. Bacteria:
    Bozeman Science:
    https://www.youtube.com/watch?v=h-z9-9OOWC4

    Frank Gregorio – Introduction to the Bacteria
    https://www.youtube.com/watch?v=qCn92mbWxd4

2. Protists:
    Bozeman Science:
    https://www.youtube.com/watch?v=8deF3Rw4ti4

    Frank Gregorio – Introduction to the Protists:
    https://www.youtube.com/watch?v=0-6dzU4gOJo

IV. Pre-Lab Questions:

1. Calculate the total magnification for the following:

Ocular lens magnification	Objective lens magnification	Total magnification
	10x	
	40x	
	100x	

2. Circle the objective lens that would show the ***smallest field of view:*** ***4x*** / ***10x*** / ***40x***

3. List <u>two</u> similarities and <u>three</u> differences between prokaryotic and eukaryotic cells.

Similarities:

Differences:

4. Which two Domains contain organisms composed of prokaryotic cells?

_____ and _____

5. a) Which Domain contains organisms composed of eukaryotic cells? _____

b) What organisms belong to the Domain you listed in part 5a?

6. Number the following parts of the compound light microscope using the diagram below.

	Number		Number
Ocular lens		Stage	
Objective lens		Coarse focus knob	
Revolving nosepiece		Fine focus knob	
Light		Condenser	
Stage clips			

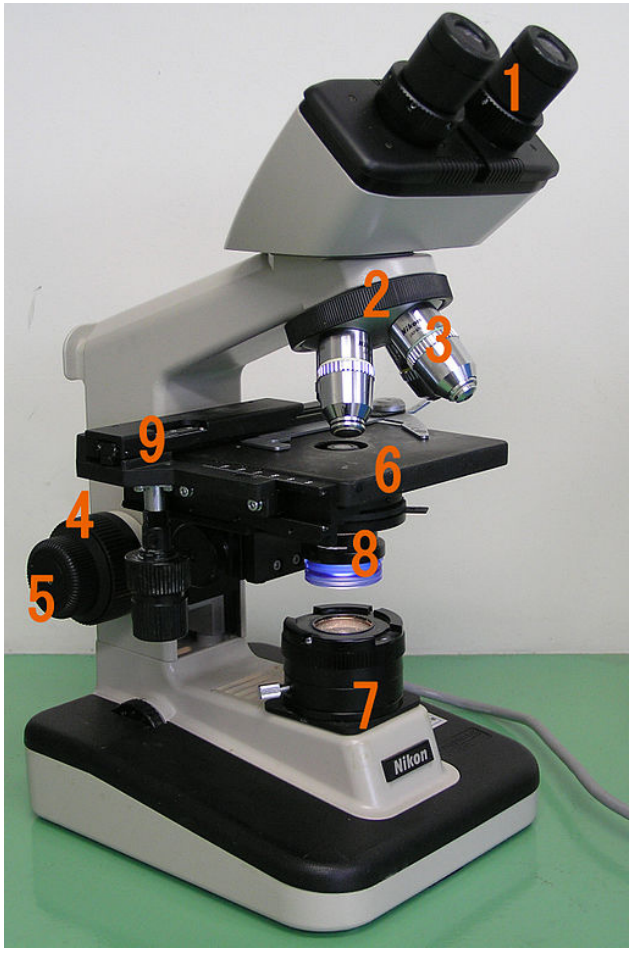


Fig. 6.2. The compound microscope.[3]

[3] Wikimedia Commons: http://commons.wikimedia.org

V. Lab Exercise:

A. Materials

Per Student:	Per Room:
• Compound microscope • Dissecting microscope • One glass slide • One cover slip	• Prepared slides: letter "e", *Euglena, Paramecium, Amoeba*, *Anabaena*, human cheek cell and bacterial shapes. • Live *Paramecium, Euglena, Anabaena* and mixed protist cultures • Photos of bacterial shapes • Yeast solution • Elodea plant • Scissors • Plastic pipettes • "Unknown" slides for students to ID

B. Procedure

1. Caring for the Microscope

1. Obtain a compound light microscope from the cabinet and carry it using both hands: one on the arm and the other one under the base.
2. Carefully, place the microscope on the table with the arm away from you. Do NOT drag the microscope on the table because the lenses may come loose and fall off.
3. If the lens is dirty, **Use ONLY lens cleaning solution and lens paper** (located on your lab bench) to clean it.
4. **NEVER** use a paper towel or other paper for cleaning the microscope lenses because it may scratch them.
5. **Do NOT touch the lenses;** body oils may also damage them.
6. For the safety of your eyesight, **keep both eyes open** when working with the microscopes (both monocular and binocular microscopes.) This will take some practice, but you will soon master the technique.
7. When you are finished with using your microscope refer to the following instructions before putting away your microscope:
   a) Remove the slide from the stage and return it to its proper place.
   b) Make sure the stage is clean and dry.
   c) Reposition the slide mount arm so that it does not extend out past the stage.
   d) Turn the revolving nosepiece to the lowest objective.
   e) Bring the stage all the way down (but don't force the coarse focus knob past its lowest point.)
   f) Turn off the light switch, unplug the electrical cord and wrap and secure it **ON THE SIDE** of the microscope. **NEVER** tuck the cord in between the base and the stage as this damages the control knobs.
   g) Cover the microscope and return it to the appropriate cabinet and numbered space with the microscope arm facing you.

2. Wet Mount Slide Preparation

Most specimens must be killed, fixed, sectioned, and stained for microscopy. These prepared slides contain a label on the side indicating the name and orientation of the specimen (i.e. "w.m." is whole mount, "c.s." is cross-section and "l.s." is longitudinal section). In addition to viewing prepared slides in this lab, you will also be viewing live organisms. If both prepared slides and live specimens are available for an organism, you may want to look at the prepared slide first so that you can more easily locate and observe the specimen before making a wet mount slide of the live organism. Oftentimes the prepared slide contains artificial staining to make the organism easier to find but masks the actual coloration of the organism.

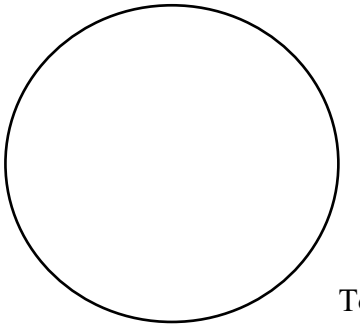
Follow the steps below to prepare a wet mount slide when asked to do so in the lab procedure.

1. Obtain a clean, blank glass slide.
2. 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.
3. Place a plastic 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.
4. When you are finished with your wet-mount, wash your slide and coverslip and reuse it for the next activity. At the end of the lab, wash your slide and coverslip and place it on the counter to dry.

3. Dissecting Microscope

a) Orientation: Letter "e" slide

1. Plug in the microscope and turn the light switch on. There are two light sources: the small knobs on either side of the arm control the two different lights.
2. Place the slide with the letter "e" on the stage and center it over the light source.
3. Use the focus knob on the side of the arm and focus on the object.
4. Practice using both lights to determine which light source provides the clearest view.
5. Turn the magnification knob (located at the top of the eyepiece) to the lowest magnification. The number that is pointing towards you indicates the total magnification.
6. Draw the orientation of letter "e" below as you observe it under the microscope.

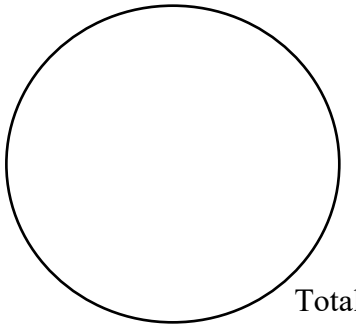


Total magnification: _____

4. Compound Light Microscope

a) Orientation: Letter "e" slide

1. Plug in the microscope and turn the light switch on.
2. Place the slide with the letter "e" on the stage and secure the slide by using the stage clips.
3. Position the letter "e" over the circular opening in the stage by using the stage control knobs.
4. By rotating the revolving nosepiece, allow the smallest objective to click into position for viewing.
5. Using the condenser height adjustment knob, make sure the condenser is all the way up under the stage.
6. While looking at the stage from the side, carefully turn the coarse adjustment knob to bring the stage up until it stops.
7. Look through the ocular lens and slowly turn the coarse focus knob away from you to lower the stage until you see the letter "e" clearly.
8. Fine focus your image by until the image is clear to your eyes.
9. Draw the orientation of the letter "e" as you observe it under the microscope.



Total magnification: _____

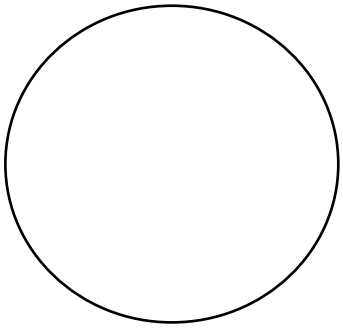
b) Cells

1. When looking at each of the specimens listed below, always start your examination using the smallest objective lens (scanning lens).
2. After you have located the specimen, switch to the medium objective to draw a detailed diagram of 1-2 representative organisms in the space provided and label as many of the cell structures as you can (plasma membrane, nucleus, etc.)
3. If prepared slides are provided for any of the listed organisms, observe and draw them first.
4. Prepare a wet mount slide of all the live specimens provided and make additional sketches and notes to compare the preserved specimens with the live ones.
5. If you cannot find any live specimens on the wet mount slide that you have prepared, clean your slide, get another sample and try again.
6. Many of the live specimens will be swimming around rapidly and may move out of view quickly so be prepared to go on a treasure hunt!
7. If you are having trouble finding the organisms, be sure to ask your instructor. You are responsible for being able to identify the main characteristics of each of the organisms that you observe in the lab.

Bacteria: *Anabaena*

Anabaena is the genus name of a group of cyanobacteria (photosynthetic bacteria). It is a colonial organism that grows in a filamentous strand composed of many cells that look like a string of pearls. Since *Anabaena* are photosynthetic, they contain chlorophyll, although the preserved specimens are stained various colors to make them more visible. There are occasional cells in the filament that are larger than the rest. These cells are called **heterocysts** and allow the bacteria to fix nitrogen from the environment, which is necessary for photosynthesis.

1. Observe the prepared slide of *Anabaena*. Draw several colonies below and **label the cell membrane and the heterocysts**.
2. Make a wet mount slide of the live specimens by taking a drop of sample from the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
3. Place a clean plastic coverslip over your sample.
4. Observe the live organisms and compare them with the preserved specimens.



Anabaena (total magnification: _____)

Would you expect to see nuclei in the cells of Anabaena? Explain why or why not.

_____

In what ways are the live and preserved specimens different?

_____

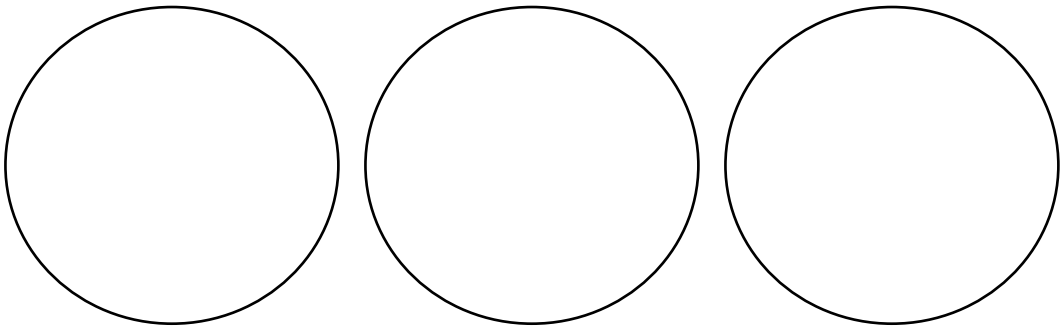
What is the color of the live colonies of Anabaena? _____

What does this suggest about whether these bacteria are autotrophic or heterotrophic? Why?

_____

Bacteria: Cell Morphology

1. Observe the three types of bacterial cell morphology (slides may be set up on display microscopes or you may need to obtain a slide with all three bacterial shapes and set it up yourself.) If you want, place a piece of hair on the slide to give you a sense of size of the bacteria. Most bacteria are less than 10um long, which is generally 10 times smaller than the width of a human hair!
2. Refer to the micrographs (photographs) of the different bacterial shapes. These were taken using an electron microscope, which has a much higher magnification than a light microscope.
3. Draw below the three bacterial shapes you observe on the compound scopes and record the total magnification for each drawing.



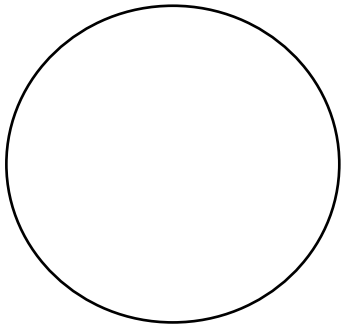
Spherical (Cocci) Rod-shaped (Bacilli) Spiral

Total magnification: _____ _____ _____

Protist: *Euglena*

Euglena is the genus name of single-celled protists that use **flagella** for locomotion. Some species in this genus are photosynthetic; others are heterotrophic and some species are both.

1. Observe the prepared slide of *Euglena*. Draw several individuals below and **label the cell membrane, nucleus and flagella (if visible)**.
2. Make a wet mount slide of the live specimens by taking a drop of sample from the **bottom** of the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
3. Place a clean plastic coverslip over your sample.
4. Observe the live organisms and compare them with the preserved specimens.



Total magnification: _____

What is the color of the live Euglena? _____

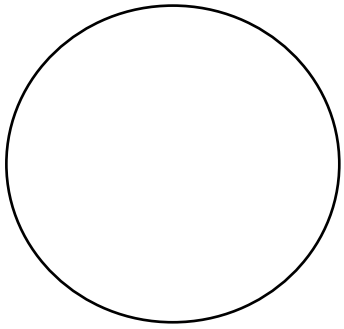
What does this suggest about whether these protists are autotrophic or heterotrophic? Why?

_____

Protist: *Amoeba*

Amoeba is the genus name of single-celled protists that use **pseudopodia** for locomotion. All members of this genus are heterotrophic.

1. Observe the prepared slide of *Amoeba*. Draw a sample individual below and **label the cell membrane, nucleus and pseudopods**.

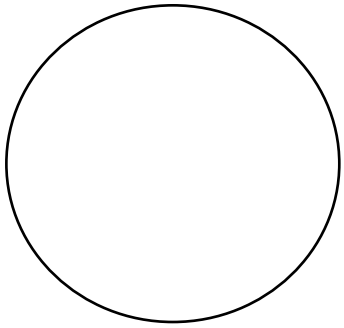


Total magnification: _____

Protist: *Paramecium*

Paramecium is the genus name of single-celled protists that use **cilia** for locomotion. In some species these cilia are grouped near one end of the organism and are used for feeding or "walking". All members of this genus are heterotrophic.

1. Observe the prepared slide of *Paramecium*. Draw a sample individual below and **label the cell membrane, nucleus and cilia (if visible)**.
2. Make a wet mount slide of the live specimens by taking a drop of sample from the **bottom** of the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
3. Place a clean plastic coverslip over your sample.
4. Observe the live organisms and compare them with the preserved specimens.
5. If you cannot find any Paramecia on your slide, clean your slide and obtain another drop of sample. Be sure to scan your entire sample.
6. If the Paramecia are swimming too fast for you to observe them, you can add a drop of Protoslo or Detain solution (located next to the live specimen) to slow their movement.



Total magnification: _____

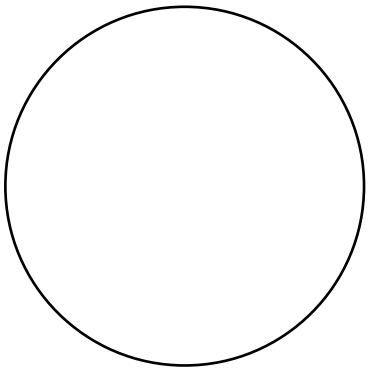
What is the color of the live Paramecia? _____

What does this suggest about whether these protists are autotrophic or heterotrophic? Why?

_____

Protist: Mixed Protist Culture
(Time permitting – check with your instructor)

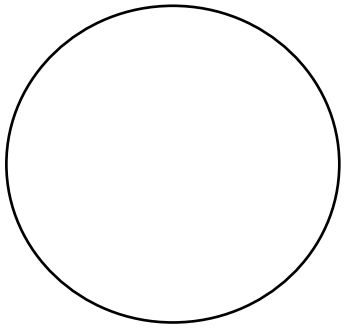
1. Make a wet mount slide of the **mixed protist culture** by taking a drop of sample near the **bottom** of the labeled jar with a pipette and placing it on a clean glass slide. Use **ONLY** the pipette next to the labeled jar to **AVOID** contaminating your sample with other organisms.
2. Place a clean plastic coverslip over your sample.
3. Observe the various organisms in your sample. Some organisms may be single-celled, having different shapes, while others will be clumps of cells called colonies.
4. Draw as many different kinds of organisms as you can see.



Total magnification: _____

Animal: Human cheek cell (squamous epithelial cell)
Animals are eukaryotic, multicellular, heterotrophic organisms that obtain their energy from feeding on other organisms or organic materials. We will use preserved, stained human cheek cells as a representative of animal cells.

1. Obtain the prepared slide labeled "squamous epithelial cell".
2. Locate and observe the cells under low magnification. Carefully switch to medium magnification to observe more detail of the cells.
3. Draw a couple of representative cells below and label the following structures: **cell membrane, cytoplasm and nucleus.**

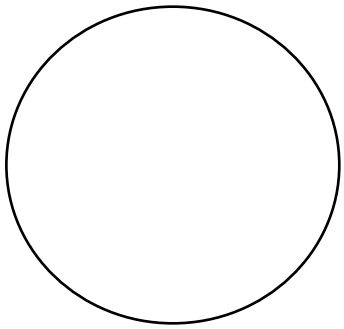


Total magnification: _____

*Can you see any organelles inside the cells?*_____

Plant: *Elodea*
Elodea is a photosynthetic aquatic plant. Plants are multicellular, autotrophic organisms and their cells contain three main structures that animal cells lack: a **cell wall** which provides the cell with structural support and gives the cell a rigid shape such as a cube or rectangle, **chloroplasts** which perform photosynthesis, and a large **central vacuole**, which stores water, pigments and toxins.

1. Obtain a **small** piece of a healthy (green) *Elodea* leaf and add a drop of water onto the leaf sample.
2. Add a coverslip over the sample. If the coverslip does not lay flat on the slide, then your sample is too thick, in which case remove the coverslip and place a smaller sample on the slide.
3. Using the lowest magnification, scan your specimen for the thinnest green section of the leaf.
4. Carefully switch to the medium magnification. Make sure the objective lens does not touch the slide.
5. You may need to adjust the condenser or diaphragm settings to get a clearer image with good contrast.
6. Draw several sample cells below and label the following structures: **cell wall, cell membrane, cytoplasm, chloroplasts, central vacuole and nucleus (if visible).**



Total magnification: _____

What color are the cells? _____

Which structures in the cell contain the green chlorophyll pigment? _____

Where are the chloroplasts located in the cell? _____

*Do you see any movement of the chloroplasts in the cell due to **cytoplasmic streaming**)?*

_____

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

_____

*List **three** ways in which you can differentiate between a plant and animal cell as viewed under the microscope.*

_____

5. Identifying Unknown Samples

1. Once you have completed all of the drawings and answered the questions in the previous sections, you will need to ask your instructor for three different unknown specimens that you will need to identify under the compound microscope.

2. Complete the table below and have your work checked when you are finished.

Note: This is an individual assignment – you must practice the proper technique for using the microscope and locating a specimen on the slide in order to be successful.

Unknown specimen #	Organism identification	Total magnification

6. Storage of the microscope

1. Remove the slide from the stage and return it to its proper place.
2. Make sure the stage is clean and dry.
3. Reposition the slide mount arm so that it does not extend out past the stage.
4. Turn the revolving nosepiece to the lowest objective.
5. Bring the stage all the way down (but don't force the coarse focus knob past its lowest point.)
6. Turn off the light switch and unplug the electrical cord.
7. Wrap the cord and secure it with the Velcro attachment.
8. Cover the microscope and tuck the cord **ON THE SIDE** of the microscope. **NEVER** tuck the cord in between the base and the stage as this damages the control knobs.
9. Return the microscope to the appropriate cabinet and numbered space with the microscope arm facing out.

VI. Post-Lab Questions

1. Complete the summary table below.

Organism	Identify group: bacteria, protist, animal or plant?	prokaryotic or eukaryotic?	single-celled, colonial, or multicellular?	autotrophic or heterotrophic?	Domain name
Anabaena					
Euglena					
Amoeba					
Paramecium					
Human					
Elodea					

2. Calculate the total magnification for the following:

Ocular lens magnification	Objective lens magnification	Total magnification
	4X	
	20X	
	50X	

3. When using the compound light microscope, it is important to use the _____ objective lens when locating a specimen on the slide for the first time because:

_____

4. Name three organelles or structures found in plant cells but not in animal cells.

5. Complete the following table to contrast prokaryotic and eukaryotic cells:

Characteristics	**Prokaryotic**	**Eukaryotic**
Membrane-bound nucleus present or absent?		
Membrane-bound organelles present or absent?		
Relative cell size		
Sample organisms		

Lab 7: Microbes

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Compare and contrast the characteristics of different types of microbes.
2. Identify the different Domains and Kingdoms that contain microorganisms.
3. Describe various microbes in your environment.
4. Compare the effectiveness of disinfectants and antiseptics on bacteria.
5. Identify and describe the characteristics of yeast.
6. Describe the characteristics and ecological importance of Fungi and lichen.
7. Conduct an experiment to determine which sugars best promote fermentation in yeast.
8. Identify the elements of the fermentation experiment and analyze the results.

II. Background Information:

Microorganisms (or microbes) are organisms that are microscopic (or too small to be seen with the eye without the use of a microscope). Microorganisms are very diverse; they include bacteria, fungi, protists and even animals.

A. Bacteria:

Bacteria are microscopic, single celled or colonial prokaryotic cells. Some cause disease, but most are actually helpful, if not essential to our daily lives. They help us digest our food and produce vitamins and important nutrients. The help out-compete pathogens and they run the biogeochemical cycles on earth. Bacterial cells come in a variety of shapes (even shaped like stars), but the most common are cocci (spheres), bacilli (rods), and spirilli (corkscrews). You observed these three cell shapes in the previous lab. In this lab, you will observe colonies of bacteria rather than individual cells.

B. Fungi:

Most fungi are multicellular and can be observed without the need for a microscope. The common pizza mushroom, *Agaricus*, is familiar to most of us, but the part that we eat is the reproductive body. This mushroom reproduces by producing sexual spores on the lamellae inside the button cap. When the spores mature they are released as the cap opens. The pattern of spores, called a **spore print**, can be observed by placing a closed mushroom cap on a piece of filter paper, wrapping it in foil, and placing it in the dark for several weeks. The cap will open and release the spores on the filter paper.

Yeast are an important type of fungi that are single-celled. Some of them cause disease, but many are important in food (and beverage) production, such as bread, beer and wine, kimchi, sauerkraut, yogurt, etc. Yeast ferment sugars (such as glucose) to produce ATP for energy, and produce alcohol and carbon dioxide (CO_2) as byproducts in this fermentation process.

$$C_6H_{12}O_6 + \text{yeast} \rightarrow 2\ CO_2 + 2\ C_2H_5OH + 2\ ATP$$

Because yeast perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process. In order to carry out fermentation however, the yeast need to possess the right enzymes to break down the sugars. Today you will test different sugars to determine which one(s) yeast can use for fermentation by observing the amount of CO_2 gas produced.

Fig. 7.1. The underside of a mushroom cap, showing the lamellae.[1]

C. Lichen:
Lichens are actually two organisms living together in a **mutualistic** symbiotic relationship between fungi and an algae (which is a photosynthetic protist) or cyanobacteria (photosynthetic bacteria). This relationship is mutually beneficial because the fungus provides structure, habitat, moisture retention and protection, while the algae or cyanobacteria provide nutrients via photosynthesis. Consequently, lichens are able to survive in extremely harsh environments such as the arctic tundra, hot deserts and rocky coasts. They are also abundant on leaves and branches in tropical rainforests and temperate woodlands as well as on bare rock, including walls and gravestones.

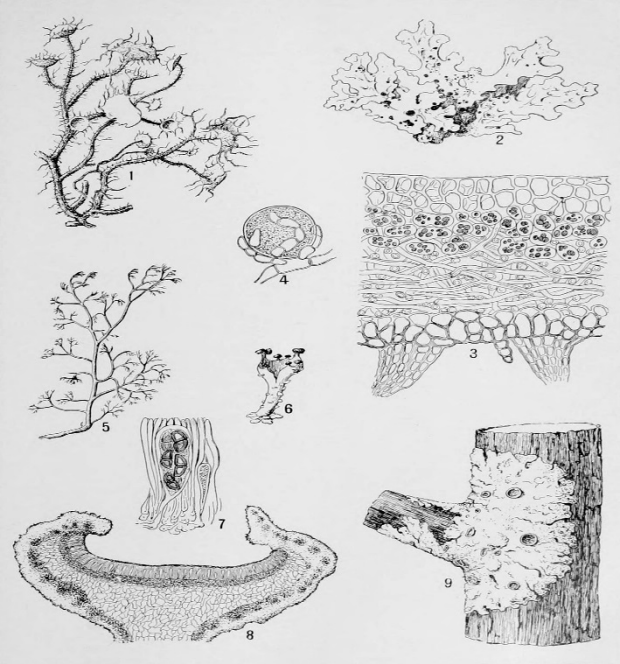


Fig. 7.2. Illustration of various lichen forms.[2]

[1] Wikimedia Commons: http://commons.wikimedia.org
[2] *The New International Encyclopædia*, v. 11, 1905, between pp. 210 and 211

Lichens are widespread and may be long-lived; however, many are also vulnerable to environmental disturbance, and are useful to scientists in assessing the effects of air pollution, ozone depletion, and metal contamination in soils. The three lichen growth forms that you will observe in lab are **crustose** (small and flat), **fruticose** (branched) and **foliose** (leafy). Watch the following video and see and hear more about this unusual partnertship: https://youtu.be/d167NrioW7c

D. Microbes in our Food and Environment:
Bacteria and fungi are everywhere in our environment. They are in the air, on surfaces and yes, all over our bodies - our "normal flora". Although many microbes cause serious diseases to plants and animals, most are not harmful and are actually necessary to the environment and to our health and well-being. Many places in your home provide the ideal conditions for bacterial and mold growth. Placing food products in the refrigerator might slow down the growth of bacteria and mold but it does not stop them altogether. Many processed foods contain preservatives to slow down or prevent bacterial and fungal growth. However, some people prefer to buy foods with as little chemical additives as possible. When buying and consuming preservative-free foods, it is important to use other methods (such as refrigeration, addition of salt, etc.) to retard microbial growth. Preservative-free foods usually have a much shorter shelf life than traditionally processed foods.

III. Reading Assignments:

A. Required background reading:
Campbell Essential Biology (7^{th} ed.): pp.58-59 (Cells), pp. 101-103 (Fermentation) and pp. 303-304 (Ecology of Prokaryotes).

B. Recommended background reading (optional):
1. What are Microbes?
https://www.youtube.com/watch?v=_Vj0cIgwpQI

2. The Microbes We're Made of:
https://www.smithsonianmag.com/videos/category/smithsonian-channel/the-microbes-were-made-of/

3. A lichen ménage à trois
https://youtu.be/d167NrioW7c

IV. Pre-Lab Questions:

1. What is a microorganism?

2. List three different groups that contain microorganisms. Identify whether each of these groups is eukaryotic or prokaryotic.

i)

ii)

iii)

3. What Kingdom do yeast belong to? _____

4. a) Write the equation for fermentation in yeast.

b) Name the substrate, the main product and the by-products of fermentation.

5. In a lichen:

a) Which organism provides structure and protection? _____.

b) Which organism provides nutrients? _____.

c) Describe the three growth forms of lichen that you will be observing in lab.

6. Watch the following video to answer the questions: https://youtu.be/d167NrioW7c

a) The new discovery about lichens suggests that lichens are oftentimes a mutualism between how many different organisms?

b) How does this type of mutualism affect lichen diversity?

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• 5 large test tubes • test tube rack • compound microscope • DI water • plastic pipettes • agar plates (TSA) • sterile swabs in tubes • glass stir rod • ruler • small beaker Per Room: • agar plates (SDA) • swab disposal biohazard bucket • demo plates of bactericides	• hand cleansers (soap, alcohol wipes, hand sanitizer) • 45^{o}C water bath • dry yeast • sample sugars solutions (glucose, lactose, sucrose and starch) • medium beaker • glass stir rods • weigh boats • spatula • fresh mushroom for dissections • razor blades • glass slides and coverslips • fungi and lichen specimens for display • prepared lichen slides

B. Procedure

1. Testing for Microbes in the Environment:
You will label plates as shown by your instructor and then seal them tightly immediately after exposure. DO NOT OPEN THEM AGAIN. Plates will be incubated and stored until next week for observations.

a) Air demo:

1. ONE group in the class will expose an agar plate to the air for 15 min.
2. The group may choose to place the plate inside or outside but should AVOID the bathrooms. Choose a location where the plate will not be disturbed, remove the cover and place the plate facing up.
3. At the end of 15min, place the cover back on the plate, seal it closed and label the location of where the plate was exposed.
4. Place the plate in the appropriate area to be incubated (check with your instructor).

b) Soil demo:

1. ONE group in the class will sprinkle a small amount of soil onto an agar plate. The soil will likely contain many kinds of fungal spores that should grow on the agar medium.
2. After placing the soil on the agar, cover the plate, seal it closed and label the location of where the soil was obtained.
3. Place the plate in the appropriate area to be incubated (check with your instructor).

c) Surfaces:

1. Each group will conduct this experiment.
2. Choose 4 surfaces to test for microorganisms. These surfaces may be part of the lab room (door handle, table, floor, etc.) or your personal belongings (cell phone, bag, book, etc.) **Do NOT choose any surfaces on your body and do NOT swab any surfaces in the bathrooms.**
3. Obtain a Petri plate of TSA agar and divide it into 4 quadrants with a marking pen on the bottom of the plate.
4. Label the outer edge of each quadrant with the location name you wish to test.
5. Obtain a sterile swab and rub it on the surface you wish to test.
6. Streak the matching quadrant with the exposed swab by rubbing it gently across the surface of the agar. Do **NOT** puncture the agar with the swab.
7. Seal the plate closed and place it in the appropriate area to be incubated (check with your instructor).

On which of the surfaces you are testing would you expect to find the greatest <u>quantity</u> of microbial growth? Why?

On which surface would you expect to find the least microbial growth? Why?

On which surface would you expect to find the greatest <u>diversity</u> of microbial growth? Why?

d) Hand cleansers:

1. Each group will conduct this experiment.
2. Obtain a Petri plate of TSA agar and divide it into 4 quadrants with a marking pen on the bottom of the plate.
3. You are provided three different types of chemicals (alcohol wipes, soap and hand sanitizer) to test the effectiveness of the cleansers/antiseptics in removing microorganisms from fingers.
4. Label the outer edge of each quadrant with the hand cleanser type. The 4th quadrant should be labeled "control." For the control treatment, you can either use water or not apply any substance to the finger.
5. One person in the group should clean each finger with a different cleanser and gently rub the cleaned finger across the surface of the agar in the appropriate quadrant. Do **NOT** puncture the agar.
6. Seal the plate closed and place it in the appropriate area to be incubated (check with your instructor).

In which quadrant would you expect to see the most microbial growth? Why?

In which quadrant would you expect to see the least microbial growth? Why?

e) Household cleaners:
Observe the demo plate(s) of bacteria and chemicals that kill or inhibit bacteria (disinfectants). There may be multiple plates divided into quadrants, each with a different species of bacteria. Each quadrant has a different chemical applied to it on a circular paper. If the chemical killed the bacteria growing on the plate, there will be a clear ring around the paper. This is called the **zone of inhibition.** Measure the **radius** of the zone of inhibition for each quadrant on the demo plate(s) to determine the effectiveness of each disinfectant.

Bacteria used: *Staphylococcus saprophyticus*

Chemical	Zone of inhibition (mm)	Chemical	Zone of inhibition (mm)
Water		Hydrogen Peroxide	
Formaldehyde		Isopropanol	
Lysol		Listerine	
Bactine		Antibacterial Soap	

Which household cleaner is most effective in killing/inhibiting this bacteria according to these results?

Explain how the quadrant containing water is a negative control.

Explain how the quadrant containing formaldehyde is a positive control.

2. Fungi:
a) Fermentation by Yeast:

In this experiment, you will compare the fermentation rate of yeast in the presence of different sugars. The ability of the yeast to metabolize a particular sugar will depend on whether it has the appropriate enzyme to break down the sugar molecule. The sugars that you will test are **glucose**, **lactose**, **sucrose** and **starch**.

Glucose, **fructose** and **galactose** are simple 6-carbon sugars (monosaccharides).
Lactose is a disaccharide made up of a **glucose** joined to a **galactose** molecule.
Sucrose is a disaccharide made up of a **glucose** joined to a **fructose** molecule.
Starch is a very large molecule (polysaccharide) made up of many **glucose** molecules.
Complete the following table before proceeding with your experiment.

Question:	
Hypothesis:	
Independent variable:	
Dependent variable:	
Control Treatment:	
Experimental Treatment(s):	

Preparing the Yeast Solution
(Check with your instructor first to see if the yeast solution has already been prepared)

1. One group should prepare the stock solution of yeast for the whole class.
2. Add 200ml of warm water to the large empty beaker provided (You may use warm tap water or heat up the water in the microwave for 30-60 seconds). The water temperature should be close to 45^{o}C.
3. Weigh out 14g of yeast and add it to the beaker of warm water. If using individual yeast packets, use two packets.
4. Using a clean glass stir rod, thoroughly mix the yeast until it dissolves.

Fermentation with Different Sugars:

1. Obtain 5 clean test tubes and label them with your group name and also in the following order: 1-Water, 2-Glucose, 3-Sucrose, 4-Lactose, 5-Starch
2. Add 5ml of DI water to test tube 1.
3. Add 5ml of the labeled sugar to each of the other 4 test tubes.
4. Gently mix the stock solution of yeast on the counter, pour 25ml of the solution into a small, clean beaker and bring it to your lab bench.
5. Add 5ml of the yeast solution to each of the 5 test tubes. Be sure to gently mix the yeast solution in the beaker just before transferring it to each tube.
6. Mix the sugar and yeast solution in each tube by gently swirling the test tube or using a clean stirring rod.
7. Place your test tubes in the 45°C water bath making sure that they are clearly labeled.
8. Record the experiment start time and check on your test tubes every 2 min. If bubbles do not form in a test tube by the end of the experiment, write 0.
9. End your experiment either after 30 min, or when the bubbles in one of your test tubes reaches the top of the test tube, or when the gas bubbles have peaked and dissipated (whichever comes first). Do NOT let the solution in your test tubes overflow.
10. Before you clean up the experiment, measure the amount of gas bubbles formed by using a ruler and recording the **height** of the gas bubbles in millimeters. This a relative measure of the amount of carbon dioxide that was formed due to fermentation.
11. Wash, dry and return all your experiment materials to their appropriate places.

Fermentation Experiment Results:

Complete the following table with class data after all the groups have completed their experiment so that you can compare the results.

	Maximum height of CO_2 gas bubbles formed (mm)				
Group	Water	Glucose	Sucrose	Lactose	Starch

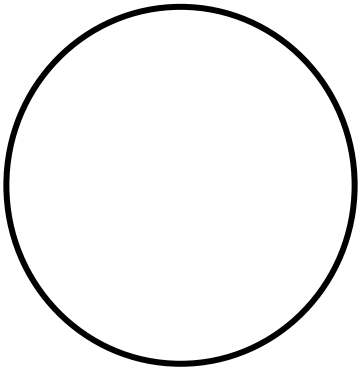
Were there any discrepancies in the experimental data among the different groups?

Were there any unexpected or surprising results? If so, how might you explain them?

Fermentation Experiment Conclusion:
Was your hypothesis supported by the experimental data? Provide a biological explanation for the results that you observed.

b) Mushroom Dissection:

1. Dissect the mushroom cap by removing the stem and cutting the cap in half using a razor blade (this may already be done for you). **Use caution with the sharp razor blade.**
2. Carefully remove one single "gill" (lamella) from under the cap. The spores will be on the lamella.
3. Place the gill on a slide with a drop of water and add a coverslip over your sample.
4. Observe the sample under the **compound microscope** under low magnification. Once you have located your specimen, switch to the medium magnification. Look for small dark dots, which are the spores on the surface of the lamella.
5. Draw the lamella and spores of the mushroom in the space below. Include the total magnification of your drawing.

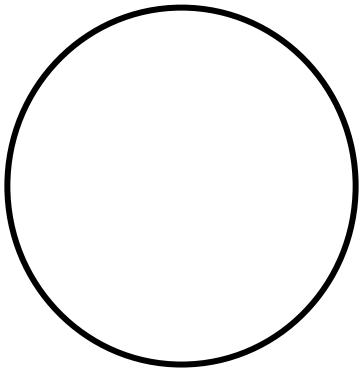


Mushroom spores (total magnification: _____)

c) Spore Print Demo Drawing:
Observe the spore print and draw it below.

d) Lichen observations:

1. Observe the prepared lichen slide using a **compound microscope** and draw your observations below.
2. Label the fungus (which may be stained green) and the algae cells (which may be stained red).



Lichen (total magnification: _____)

3. Observe the lichen specimens on display and draw the three growth forms below.

Crustose (small and flat)	Foliose (leafy)	Fruticose (branched)

VI. Post-Lab Questions

1. Draw a bar graph below that illustrates the results of your fermentation experiment. Include the results for your control experiment as well as all your experimental treatments. Be sure to label the axes.

2. When baking bread, what product of fermentation causes the dough to rise?

3. a) Based on your experiment, which sugars (glucose, sucrose, lactose or starch) could you successfully use to make the dough rise when baking bread? Why?

b) Provide a biological reason for why yeast cannot use certain sugars for fermentation.

4. Lichens can often survive in very harsh environments that most other organisms cannot tolerate. What characteristics of lichens allow them to survive in such environments?

Lab 8: Microbe Analysis

I. Learning Objectives:

By the end of this lab activity, you should be able to:

1. Identify the presence and type of bacterial and fungal colonies on exposure plates.
2. Identify which exposure plates contain a greater diversity of microorganisms.
3. Analyze the amount of bacterial growth for different hand cleansers.
4. Explain the various ways in which bacteria communicate with each other and how to develop new antibiotics.
5. Discuss the ecological implications of antibiotic resistance.

II. Background Information:

A. Environmental Sampling Plates:

Remember that last week your class did environmental sampling to see what kinds of microbes (fungi and bacteria) exist in the soil, in the air and on surfaces. This week we want you to take a close look at your plates and observe the characteristics of the organisms on them.

B. Colony Morphology:

Bacteria have distinctive characteristic growth patterns on solid media. A colony essentially starts with one bacterium, which then multiplies logarithmically via binary fission. Within hours a colony becomes visible on the surface of the media. Colony morphology (shape, size, pigmentation, etc.) is used as starting point in the identification of bacteria. While these characteristics can't completely identify bacteria, (additional biochemical tests or genetic analysis are necessary), today you will use colony morphology to describe the growth on your plates and take a few guesses as to what some of the bacteria could be. Here are the characteristics most often used in describing the colony morphology of bacteria:

- Overall shape
- Elevation-height of the colony up from the plate
- Margin –outline of the edge of the colony
- Surface- is the surface dull or glossy? Smooth or wrinkled?
- Optical properties - transparent (clear), opaque, translucent (almost clear)
- Pigmentation—is the colony white, cream, golden, yellow, pink, red, purple, etc.

Colony Morphology Characteristics:

1. Overall shape:

Punctiform Circular Irregular Rhizoid

2. Elevation:

Flat Raised Convex Umbonate

3. Margin:

Entire Undulate Lobate Erose Curled

C. Organism Identification:

The following microorganisms are commonly found on skin, soil, and surfaces (remember that on surfaces like desks most organisms will not be actively growing, but there may be spores and cells carried on dust, aerosols, etc.).

Staphylococcus epidermidis (skin): circular, entire, convex, pinpoint, smooth, shiny, white, opaque

Staphylococcus aureus (skin): circular, entire, convex, moderate, smooth, shiny, cream colored with yellow or golden tint, opaque

Micrococcus spp. (skin and soil): circular, entire, convex, small, smooth, shiny, yellow, pink, or red, opaque

Actinomycetes spp. (soil): white powdery, moderate, threadlike filaments (look fungal like, but filaments are much smaller than hyphae, and colonies are not a robust in growth)

Bacillus spp. (soil, surfaces): Irregular, undulate, raised, moderate-spreading, smooth, dull, cream, sometimes gray-white, opaque

Yeasts: Often large colonies, creamy, pinkish, pasty, smooth, sometimes slightly glossy.

Fungi: Typically fuzzy colonies that might be white, gray, black, yellow, red, green, etc. The fuzzy structures are microscopic hyphae that the fungi extend out in order to absorb nutrients from the surrounding medium.

III. Reading Assignments:

A. Required background reading
Campbell Essential Biology (7th ed.): pg. 265 (Antibiotic Resistance), pp.58-59 (Cells) and pp. 303-304 (Ecology of Prokaryotes).

IV. Pre-Lab Questions:

1. What is a bacterial colony?

2. Describe one method of determining whether a colony growing on your exposure plate is a fungus or bacteria.

3. a) Last week your group used different hand cleansers to determine whether it would inhibit growth of microorganisms. What was the control treatment for that experiment?

b) Briefly describe your experimental design (which finger(s) did you sample, was there more than one participant's fingers being sampled in the experiment, etc.). How might your experimental design have affected the outcome of your experiment in unexpected ways?

4. TED Conference Video of Dr. Bonnie Bassler: How Bacteria "Talk" (April 2009) http://www.ted.com/talks/bonnie_bassler_on_how_bacteria_communicate.html

Watch Dr. Bonnie Bassler's TED presentation and answer the following questions:

a. In terms of the number of cells and amount of genetic information, approximately what percent of your body is "bacterial" vs. human?

b. What is special about *Vibrio fischeri* bacteria?

c. What benefit does the Hawaiian Bobtail squid get from the bacteria?

d. How does the squid prevent bacteria build-up in its body?

e. What is "Bacterial Quorum Sensing" and how does it work?

f. Which bacteria use quorum sensing to communicate?

g. How can bacteria control their pathogenicity (virulence)?

h. How can bacteria distinguish between members of their own species and other species?

i. What kinds of antibiotics is Dr. Bassler proposing developing and how would these new kinds of antibiotics be better than the kinds currently being used?

V. Lab Exercise:

A. Materials

Per Group:	Per Room:
• Incubated hand cleanser and surface exposure plates	• Dissecting microscope • Incubated air and soil exposure plates

B. Procedure

Do NOT open any of the exposure plates at any point.

1. Observe your group's exposure plate for **surfaces**. Sketch your exposure plate below. Label which colonies are most likely fungi and which are bacterial. Be sure to label the locations that were swabbed for each quadrant.

In which quadrant do you see the most microbial growth?

In which quadrant do you see the least microbial growth?

Which quadrant contains the greatest diversity of microbial growth?

Do these results match your hypotheses from last week? If not, what might be the reason for the discrepancy?

2. Observe your group's exposure plate for the **hand cleansers**. Sketch your exposure plate below and label the treatment applied in each quadrant.

In which quadrant do you see the most microbial growth?

In which quadrant do you see the least microbial growth?

Do these results match your hypotheses from last week? If not, what might be the reason for the discrepancy?

3. Using your group's exposure plate for **surfaces and/or hand cleansers**, complete the following data table for four of the bacterial colonies on your plate (each row is for a single colony, not an entire quadrant on your plate). Use a dissecting microscope to see more detail if possible.

Origin of Sample (location)	Shape	Elevation	Margin	Surface	Opaque?	Pigmentation (color)	Possible Organism
1.							
2.							
3.							
4.							

4. Compare your plate with other groups in the class.
Did you sample similar things? If so, do you have similar or different looking colonies growing? Why might that be?

5. Observe the exposure plates for the soil and air demo.

What location did the organisms on the soil and air demo plates come from? Which plate has more microorganisms?

What similarities and differences are there between the colonies growing on the soil and air exposure plates?

VI. Additional Activity (time permitting) & Post-Lab Questions

TED Talk by Tal Danino:
Programming Bacteria to Detect Cancer (and Maybe Treat it)

https://www.ted.com/talks/tal_danino_we_can_use_bacteria_to_detect_cancer_and_maybe_treat_it#t-383825

Watch Tal Danino's TED talk and answer the following questions. (You should have already watched Bonnie Bassler's video from the pre-lab activity to provide you with the necessary background information.) You may be asked to discuss your answers with your classmates.

1. What are the main ideas that Tal Danino presents in his talk regarding bacterial communication?

2. What are the beneficial medical applications that Tal Danino is proposing using bacterial quorum sensing?