Spring 2024 Sabbatical Report Dr. Barbara I. Evans

Advancing Student Centered Science at LSSU: Structured Labs, Experiential Learning and Professional Opportunities

Thank you for the award of 1 semester of sabbatical. The goals for the sabbatical proposal were based on a full year of sabbatical, but I still tried to complete as much as possible.

Project Abstract/Executive Summary:

There are several goals for my sabbatical proposal:

Goal 1) Revise the lab manuals for several classes with opportunities for hands-on activity.

Goal 2) Attract long-term funding for the Aquaculture Challenge competition developed by LSSU in 2016. Currently the competition is supported by sporadic grant funding, LSSU student labor and volunteer time, but we hope to attract ongoing funding to expand the scope of the competition and increase the visibility of LSSU in aquaculture.

Goal 3) Promote LSSU as a university of choice for aquaculture education, including cannabis aquaponics. This could include developing a BS in Aquaculture that includes both aquaponics and fin fish aquaculture, as well as working with chemistry to create a cannabis aquaponics concentration.

Goal 4) Complete and submit for publication @6 manuscripts with LSSU student co-authors. Peer reviewed publications reflect the quality of active student centered science at LSSU.

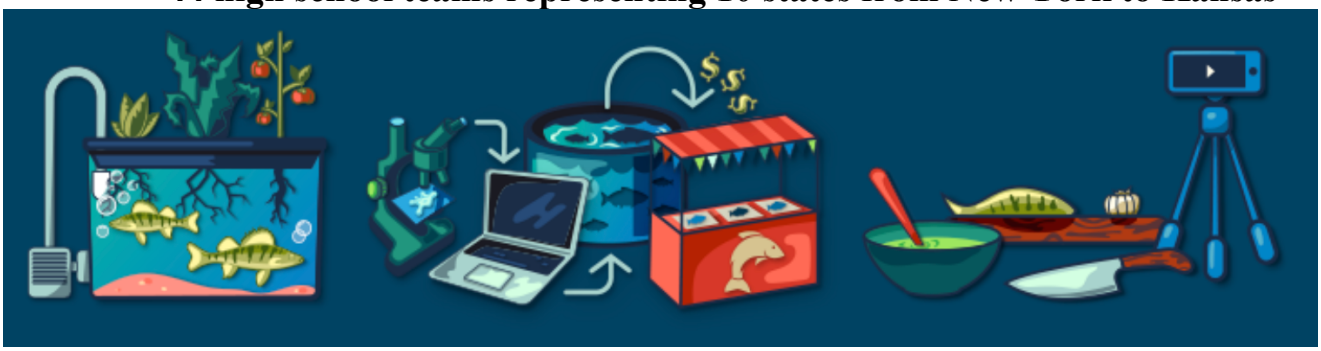
Goal 1) Revise the lab manual for BIOL131

The manual has been extensively revised for BIOL131 Introductory Biology I: Cells. In particular, the appendices have been included in the manual, and scholar's notebook expectations are made explicit. The revised manual is included in the appendix. Currently we are still making it available for free on Moodle, but there is the potential to publish.

Goal 2) Attract long term funding for the Aquaculture Challenge

The Great lakes Aquaculture Collaborative (GLAC) was interested in developing an aquaculture competition for high schools. They approached us about the possibility of taking over funding and administration of the Aquaculture Challenge. We are still involved, but are no longer solely responsible for running this competition.

The 10th anniversary of the Aquaculture Challenge.
44 high school teams representing 10 states from New York to Kansas



The Aquaculture Challenge is a high school competition initially proposed by students in the LSSU Aquaculture Club (also known as Superior AquaSystems). Initial funding for the competition was awarded to Dr. Christopher Smith (LSSU Computer Science and Mathematics) and Dr. Barbara I. Evans (LSSU Biological Sciences) by the Michigan STEM Partnership, followed up with several successful USDA-NIFA grants. In subsequent years, Michigan Sea Grant became a strong supporter of the competition with Elliot Nelson taking

on leadership of the competition.
The program challenges high school student teams to design and build an aquaponics system. They are required to monitor the system's physical parameters and water chemistry, ideally using sensors and microcontrollers. Other components of the challenge include aquaculture outreach, writing business plans to market their fish and plants, as well as a seafood cooking component to promote increased seafood consumption.
Recently the Great Lakes Aquaculture Collaborative (GLAC) asked if they could take on ownership of the competition. In their first year running the Aquaculture Challenge they have attracted 44 teams.
https://seagrant.umn.edu/programs/education-and-workforce-development-program/aqua-challenge
Although LSSU is no longer directly involved with running the competition, the program showcases the LSSU curricula that include aquaculture and aquaponics components.
The challenge has recently been highlighted by Great Lakes Sea Grant and Aquaculture North America
https://www.michiganseagrant.org/blog/2026/06/05/2026-aquaculture-challenge-champions/
https://www.aquaculturenorthamerica.com/minnesota-schools-showcase-aquaponics-systems-in-2026-aquaculture-challenge/

For more information contact Elliot Nelson at elliotne@msu.edu



April 2016 The first Aquaculture Challenge was won by a team from Sault Area High School

Goal 3) Promote LSSU as a university of choice in aquaculture.
We are still promoting aquaponics, and our students are recognized nationally for their contributions to aquaculture and aquaculture extension.

Goal 4) I am still working on getting my data published, and am working on implications of my Ph.D. dissertation that identified a search strategy used by many animals.

Biology 131 General Biology I: Cells
Lake Superior State University
Laboratory Manual

Barbara I. Evans, Jun Li and Nancy S. Kirpatrick

Table of Contents

Laboratory 1: The Microscope and Scientific Method

- **Read Appendix - The Microscope - before class today.**

Learning Outcomes

- To learn proper care and use of compound and dissecting microscopes
- To appreciate the contribution of the microscope to the study of biology
- To be familiar with different systems of measurement
- To appreciate the effect of scale on biological systems
- To begin using the scientific method

Materials Required

Dissection and compound microscopes
Seeds and Seed starter foam in Bento Box
Prepared slides
Cat grass and 4 oz cups with lids
Clear plastic rulers and drawing circles
Nutrient agar plates

Activities

I. Use of the microscope

1. Label the parts of the microscope on the diagram provided
2. Take the ruler provided and observe the lines on the ruler under the microscope.
3. Start at the lowest power, with the stage up as far as it will go.
4. Focus, by moving the stage down, away from the objective.
5. Using the ruler, measure the field diameter (D)
5. Switch to a higher power objective, and repeat

II. Magnification and Field Diameter

A. Dissection microscope

1. Begin using the dissection microscope, and measure the diameter of the field of view at 7X, low magnification using a clear ruler with millimeter scale.
2. Then, calculate the diameter of the field of view at medium and high power (30x) using the relationship $X_1D_1=X_2D_2$, where X is the total magnification and D is diameter of the field.
3. Measure the diameter of the field of view at medium and high power using a clear ruler.
4. How do these measurements compare with the calculated estimates?
5. What happens to the field of view as magnification increases?

B. Compound microscope

6. Working with the compound microscope, calculate total magnification (ocular lens x objective lens) at low, medium and high power.

7. Using the compound microscope, measure the diameter of the field of view at low magnification (4x objective) using a clear ruler with millimeter scale. Then, estimate the diameter of the field of view at medium and high power (40x objective) using the relationship $X_1D_1=X_2D_2$, where X is the total magnification and D is diameter of the field.

Magnification and Field Diameter Worksheet

Microscope	Total Magnification (X)	Measured Field Diameter (mm)	Calculated Field Diameter (mm)
Dissection (low)	7X		
Dissection (medium)	15X		
Dissection (high)	30X		
Compound (low)	40X		
Compound (medium)	100X		
Compound (high)	400X		
Compound (oil)	1000X		

III. Diagrams and Drawing Magnification

8. There are several prepared slides available for examination. Look at each available organism with both the compound microscope and the dissecting microscope at several different magnifications.

9. Choose one specimen, and using the compound microscope, sketch at three different magnifications.
10. Choose a seed from the available packets, and using the dissection microscope, sketch at three different magnifications.

11. In summary, you should draw each of your 2 specimens at 3 magnifications. This will be a total of 6 drawings. Label your drawings with: 1) the name of the organism, 2) the total magnification used and 3) the drawing magnification of your sketches. (see below)

12. For each of the organisms that you have observed, write a description, at each magnification, of exactly what you saw. As you increase magnification, your descriptions should become more detailed and more specific.

Drawing Magnification= Drawing Size/Actual Size

1. The field diameter will allow you to estimate the actual size of the sample you are viewing.
2. Read the next section on the basics of microscope drawing. Sketch a seed under the dissection scope at low, medium and high power. Use the plastic dish provided to make a circle.
3. After you have sketched your sample, measure the diameter of your drawing, and divide it by the actual size. This will tell you how much larger your drawing is than the actual size of the sample

The Basics of Microscope Drawing:

First of all, you are not expected to be an artist! Anyone can produce accurate and attractive drawings. Biological drawings should be simple, yet accurate, representations of what you see. They should not be busy work. If you have seen an object well enough to reproduce it clearly then you have seen it well. It is a fact that a well-done drawing rarely takes more time than a sloppy one. Here are several principles that should be followed in making drawings.

1. Use a reasonably hard-leaded **pencil.**

2. Make the drawing as large as the space provided. If drawing on a separate piece of paper, divide the paper into quarters and make four drawings per page.

3. Avoid "sketchy" lines (``````). The boundaries of living things are generally sharp, not fuzzy.

4. Be sure to show the relative contrast in the specimen. This can best be done using stippling.

5. Place labels so that the lines to the objects are as parallel as possible.

6. Always add a drawing magnification (____ x). Note that this value is not the microscopic magnification, but the **ratio of picture size over actual size of the specimen.** To calculate drawing magnification:

    a) Measure one dimension (e.g., length or circle diameter) on your drawings
    b) Estimate the actual length of the same dimension on the specimen or field diameter at that magnification
    c) Put values "a" and "b" in the same units (e.g., mm)
    d) Calculate _____ x as drawing size (a) divided by actual size (b)

Activities for next lab:

1) Plant Growth

1) Choose seeds, seed starter foam and a Bento box.
2) Add water (rehydrate) seed foam
3) Add seeds to opening in foam and place in water in Bento box
4) Label lid of Bento box with your name and seed type

2) Bacterial growth

Have you ever wondered about the bacteria growing on a certain surface? Before you leave today:

1. Take one nutrient agar plate and one sterile swab.
2. Swab the surface you are curious about, and then gently rub the swab over the agar plate. You may need to dip the swab in sterile water before applying to plate.
3. With a sharpie label the *bottom* of the plate with your initials, date, and location.
4. Instructor will place all plates in an incubator and have them available for next week's lab.
5. Also, bring in any living or non-living specimens that you would like to examine with either the compound or dissecting microscope.

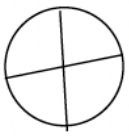
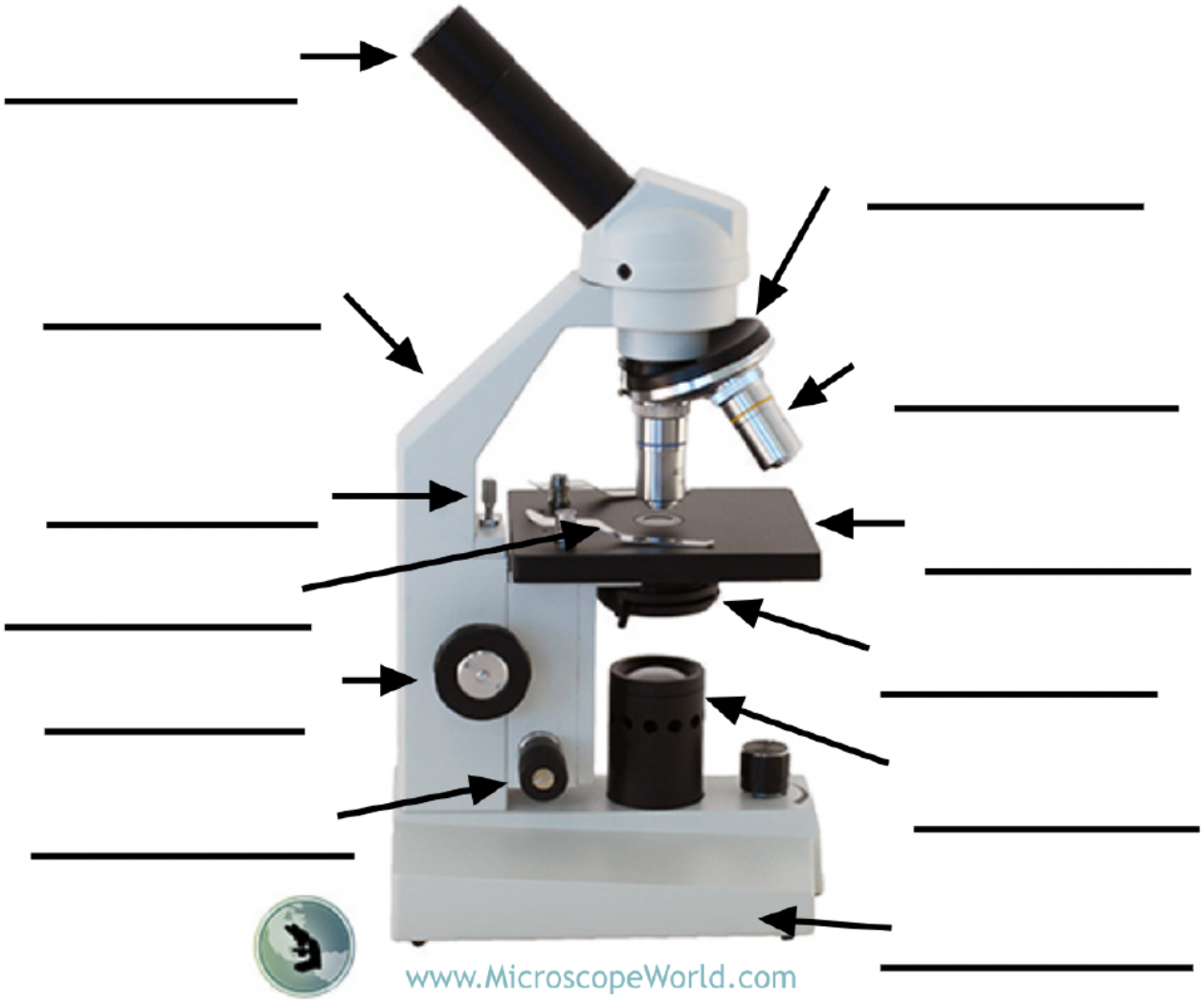


Figure 2.1 - By dividing agar plates into quadrants, you can sample four areas with one plate. Always label the bottom of the plate. This way if you drop a stack of plates, you don't have to worry about which lid goes with which plate.

Label parts of the Microscope:



SCHOLAR'S NOTEBOOK GRADING SHEET

Lab Week 1: The Microscope

Reflection-optional

Objective/Purpose

Methods

Vocabulary

Results/Observations (field diameter calculations table)

2 Questions

Conclusions

6 Drawings (with label and drawing magnification)

Appendix: The Microscope

The microscope is a very powerful tool for biologists. There are several varieties of microscopes available today, including the two most commonly used by students of biology, the compound light microscope and the stereoscopic dissecting microscope. Other types of microscope include the confocal, fluorescent, scanning electron, and transmission electron microscopes.

This appendix is meant to familiarize you with the care and operation of the compound light and the stereoscopic dissecting microscopes. There are several rules that you must adhere to each and every time you use a microscope, whether it is in a formal class, or you are doing independent research.

RULE #1: *Always carry the microscope with two hands. Use one hand to support the base of the microscope and the other to grasp the arm.* The ocular lens is removable and could fall out if the microscope is not held upright.

RULE #2: *Clean the lenses of the microscope with lens paper before and after use. Use only approved lens paper and cleaning solutions to clean lenses.* Kim wipes, Kleenex, handkerchiefs, and paper towels will scratch the lenses.

RULE #3: *Never use the coarse adjustment knob when focusing with the high-power or oil immersion objective lenses in place.* This could damage the lens or the slide.

RULE #4: *Always remove slides from the microscope with the low-power or scanning objective lens in place.* Removing a slide with the high-power or oil immersion lens in place could scrape these objectives.

RULE #5: *Never put a microscope away with the high-power or oil immersion objective lens in place. Always rotate the low-power or scanning objective into the viewing position before putting the microscope away.*

Parts of the Compound Light Microscope

Condenser - located under the stage, the condenser is a set of lenses that focuses light on the specimen. There is a control knob that raises and lowers the condenser. Normally it should be up as high as it will go towards the stage.

Focal adjustment knobs - used to focus the image. The **coarse adjustment knob** is used for initial focusing at low power. The **fine adjustment knob** allows for very slight changes in focus to create a precise image. Remember Rule #3 - *Never use the coarse adjustment knob when focusing with the high-power or oil immersion objective lenses in place.*

Iris diaphragm - used to adjust the amount of light striking the specimen. The diaphragm is adjusted by a lever or wheel on the side of the condenser. You can adjust the light coming

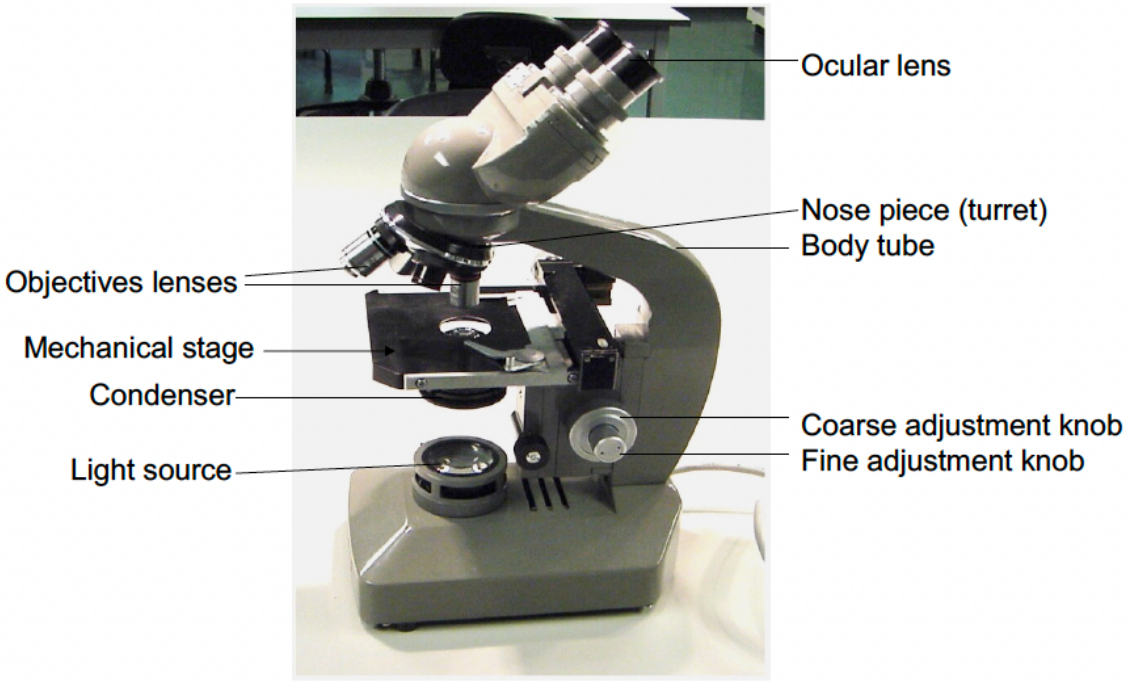


Figure VII.1- Olympus Compound Microscope

through the microscope either by adjusting the rheostat on the light switch or adjusting the iris diaphragm. Higher magnifications usually require more light.

Light Source - may be built into base of microscope with a lens to focus light onto the lower condenser lens; or it may be a separate light that is focused onto condenser lens by a mirror.

Objective lenses - (objectives) - Attached to the revolving nosepiece are four objectives containing a complex lens system producing magnification of the specimen. The magnification of the objectives is printed on the casing of each. The objectives by name and magnification are the **scanning (4X), low power (10X), high power (40X), and oil immersion (100X).** To calculate **total magnification** of the specimen, multiply the magnification of the ocular lens times the magnification of the objective lens.

Ocular lens or **eyepiece** - The lens you look through. The magnification of the ocular is printed on the casing. It is usually 10X although in some cases it is 12.5X. The microscopes we use have two oculars and are, therefore, called binocular microscopes.

Revolving nosepiece - This enables you to change the magnification of the image. The various objective lenses are mounted on this wheel and can be snapped into place for viewing at the different magnifications.

Stage - the flat surface on which the slide is placed. Notice the aperture (opening) on the stage through which light passes. Our microscopes are equipped with clips for holding the slide and control knobs for moving the slide into different positions. For this reason, it is called a mechanical stage.

Other important definitions

Depth of Field - is the plane of view that is in sharp focus. Depth of field is important when your specimen is more than a few millimeters thick. You can change the depth of field by focusing up and down through the specimen with the fine adjustment knob. By focusing up and down through the various planes of your specimen, you can almost get a 3-D effect.

Field of View - is the area that you can see when you look through the ocular and any of the objective lenses. There is an inverse relationship between the magnification of the objective and the field of view. As magnification increases, field of view decreases.

Parfocal - is the ability to focus an object with one lens, then change lenses without completely losing focus. One should be able to focus the specimen with the low power objective, change to the high power objective and get the specimen into sharp focus with a slight turn of the fine adjustment knob.

Resolving power or resolution - the ability to distinguish two closely spaced objects as being two distinct objects rather than one. The smaller the distance between the two objects that can be distinguished, the better the resolving power of the microscope. The smallest distance at which the human eye can resolve two objects is 0.1 mm. With the aid of a light microscope, the human eye is able to resolve two objects that are only 0.1 μm or 1,000 times closer than the unaided eye.

Resolving power can be expressed by the equation: $R = \frac{8}{2[n \sin (1/2\ 2)]}$

where:
R = resolving power
8 = wavelength of light used
n = refractive index of medium used (usually oil or water)
2 = angular aperture of light cone

The expression [n sin (1/2 2] is known as the **numerical aperture (NA)**, thus the above equation can be rewritten as: $R = \frac{8}{2NA}$

The numerical aperture is printed on all objectives next to magnification

The Stereoscopic Dissecting Microscope

This microscope is used for viewing small macroscopic organisms, e.g., insects, worms, plant parts. It is often used to aid in dissection of plants and animals, hence the name. The lens system of the stereoscopic dissecting microscope is different than that of the compound microscope. Each eyepiece contains both an ocular and an objective lens, thus each human eye is offered its own image. Because of this, the viewer is able to see the specimen in three dimensions. Magnification is changed by a dial located on the side or on the top of the microscope.

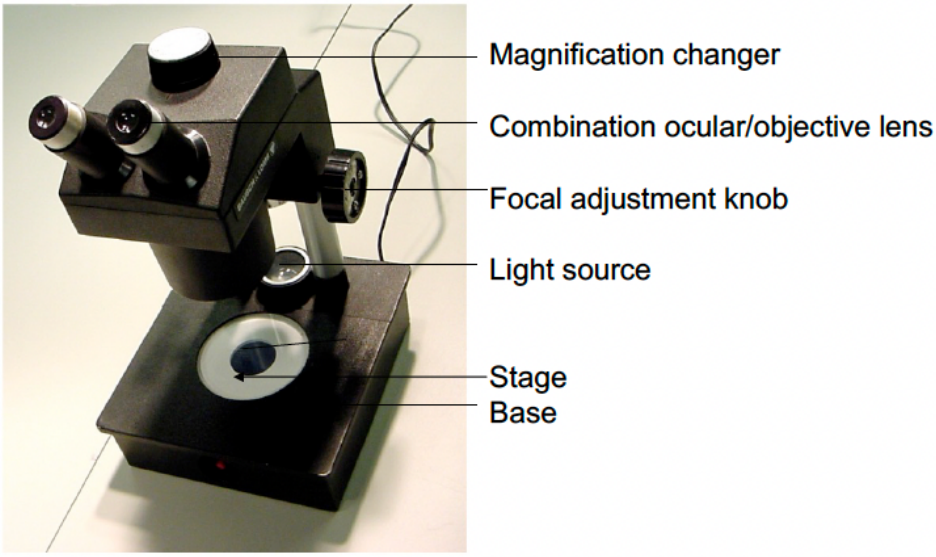


Figure VII.2 - Bausch & Lomb Dissecting Microscope

Microscope Lab Prep

12 dissections scopes
12 compound scopes
Lens paper
Seeds and seed starter foam
Bento boxes
Prepared slides
Cat grass and 4 oz cups with lids
Nutrient agar plates (1 per student)
Sterile swabs (1 per student)
Rulers
Circles

Laboratory 2: Observing the Microscopic World

- **Read Appendix– Microbiology Techniques - before class today.**

Learning Outcomes

- To appreciate the diversity of the biological world at the microscopic level
- To learn some tools of the microbiologist
- To strengthen your powers of observation

Materials required

- Microscope slides and cover slips
- Various living specimens
- Rulers, transfer pipettes
- Stains and staining supplies

Activities

A. Seed Germination

1. Retrieve your black plastic bin, and remove any excess water
2. Carefully take one of your plants and observe the roots and leaves.
3. In your scholar's notebook, record your observations.
For example: Species and number of seeds planted; number of seeds germinated.
4. Observe and sketch plants under the dissection scope
5. If some have not sprouted, sketch an ungerminated seed under the dissection scope.
6. Is there any sign it may germinate? How long do they typically take to germinate?
7. Remember to label your drawings and include both **total magnification** and **drawing magnification.**
8. Place the plant in a net pot and place in the aquaponics system
(or place the plant in the provided rack for the hydroponics system)

B. Living Organisms

1. Select several living specimens available in the classroom today.
2. Observe each specimen at <u>three</u> (3) different magnifications. You may use the compound scope, the dissecting scope, or a combination of the two.
    a. You may want to stain your specimens with one of the available stains for better visibility under the microscope
    b. You may also want to use Protoslo to slow down the organisms in order to better observe them
2. For the organisms that you have observed, describe, at each magnification what you saw. As you increase magnification, your descriptions should become more detailed and more specific.
3. Refer to last week's lab and make drawings to accompany each of the descriptions for **three** of your specimens. (3 specimens, 1 drawing of each at the "best" magnification.
4. Remember to label your drawings and include both **total magnification** and **drawing magnification.**

C. Bacteria

1. **Bacterial colony morphology** - A bacterial colony is a population of cells that arise from a single bacterial cell. One way to complete the above experiment is to describe how many and what types of colonies you observe. Figure VIII.2 shows examples of different colony morphologies.

    a. Remove plates from incubator and examine.
    b. Construct a table for each area sampled as illustrated in Table 1.
    c. Hypothesize as to how many different types of bacteria you have isolated.

Figure VIII.2 - Some examples of bacterial whole colony morphology

Colony Description				
Area Sampled	Appearance	Color	Diameter	# of this type
_____				
Incubated at:				
_____ °C for				
_____ hours				

Table 1 Data table for describing bacterial colony types

1. Obtain a clean microscope slide and prepare and fix a bacterial smear **(Use gloves)**

1. Slide Preparation

a. Obtain a sterile swab and microscope slide.
b. Place a drop of distilled water onto the center of a microscope slide.
c. Pick up a small quantity of your bacterial culture with the swab.
d. Stir the bacteria into the drop of water until it is a milky suspension.
e. Spread the drop until it is a dilute smear.
f. Let the smear air dry or use a hot plate.

2. **Gram staining** is extremely useful as a first step in bacterial identification.

Gram-positive bacteria: appear purple when viewed under oil immersion.
Ex. *Staphylococcus*, *Streptococcus, Enterococcus, Bacillus, Clostridium*

Gram-negative bacteria: appear pinkish red, the color of the safranin.
Ex. *Escherichia coli, Salmonella, Shigella, Cyanobacteria*

You will need four ingredients for this procedure:

a) Crystal violet stain (30 seconds, then rinse with distilled water)
b) Gram's iodine (10 seconds then rinse with distilled water)
c) 95% ethanol (decolorize to remove excess stain)
d) Safranin stain (30 seconds, then rinse with distilled water)
e) Blot edge of slide with a stack of paper towels or absorbent paper

2. Observe bacteria at 10X and 40X magnification.
    1. Before observing at 100X ask your lab instructor for assistance!
    Using the oil immersion lens - Because bacteria are so small, we must use the 100X objective lens to observe them. The 100X objective is also called the **oil immersion lens** because we must place a drop of oil between the slide and the lens. This is so the light from the microscope focuses directly on the object to be viewed and is not refracted away. The physical reasons for this will be explained in your microbiology class.

        a. Before using the oil immersion lens, focus your specimen under low power (10X).
        b. Move the turret so that the specimen is between 4x objective and the oil immersion lens.
        c. Place a drop of immersion oil in the center of the slide.
        d. Move the oil immersion lens into place.
        e. ***Never*** allow the high dry objective (40x) to touch the immersion oil. It will ruin the lens.
        f. ***Never*** use the coarse adjustment knob when focusing under oil immersion. The working distance between the lens and the specimen is too close and you may break the lens.

3. Sketch your bacteria at high power; remember to label your drawings and include both **total magnification** and **drawing magnification.**

4. **Clean up your area**
    a. Place glass sides in the bleach solution
    b. Put agar plates, gloves and used swabs in the red hazardous waste container
    c. Wash your hands

Scholar's Notebook Entry

Week 2: Observing the Living World

Reflection

Objective/Purpose

Methods

Vocabulary

Results/Observations

2 Questions

Conclusions

6 Drawings (plant, ungerminated seed, bacteria, 3 other organisms)

Appendix: Microbiology Techniques

Many students choose to do open inquiry investigations on some aspect of bacterial growth. In this chapter we will describe several techniques used by microbiologists that you may find useful.

2. **Preparing a wet mount** – This technique is used to view living microscopic organisms under the compound microscope (see App VII).

    a. Use a transfer pipette to place a drop of water containing the organisms of interest in the center of a glass slide.
    b. Carefully place a cover slip over the drop, taking care not to create a bubble.

3. **Preparing a hanging drop slide** – This technique is used for viewing very small macroscopic organisms that may be crushed using the wet mount technique.

    a. Obtain a deep well slide and a cover slip.
    b. Put some petroleum jelly (Vaseline) on a toothpick.
    c. Rub a small quantity of the Vaseline one side of the cover slip.
    d. Place the cover slip on a paper towel, Vaseline side up
    e. Transfer a drop of water containing the organisms of interest to the center of the cover slip.
    f. Place the deep well slide on the cover slip so that the Vaseline forms a seal.
    g. Quickly invert the slide so that the cover slip is on top and the drop is suspended.
    h. The slide is now ready to be viewed under either the compound or dissecting microscope.

4. **Testing for microbes in the environment** - This is a very popular open inquiry experiment for first year biology students. There are many places where bacteria and fungi grow around campus; and it's fun to predict where you might find them.

    a. First, decide where you want to look for microbes and decide how many nutrient agar plates you will need.
    b. The lab assistant will make the plates for you.
    c. Obtain a bottle of sterile water and several sterile cotton swabs
    d. Using a Sharpie mark the bottom of your plates into four quadrants

Figure VIII.1 - By dividing agar plates into quadrants, you can sample four areas with one plate. Always label the bottom of the plate. This way if you drop a stack of plates, you don't have to worry which lid goes with which plate.

    e. Dip the swab into the sterile water, then swipe the surface you want to test
    f. Gently swipe the swab onto one quadrant of the nutrient agar plate.
        i. Identify the location of your swipe with a Sharpie on the bottom of the agar plate.
        ii. Also mark the plates with your initials, the date, and lab section.
    g. Place in 37° C incubator in CRW254B and let incubate for 24 - 48 hours.
        i. Incubate the plates upside down so if there is any condensation, it will be in the lid and not on your sample.

Laboratory 3: Use of Equipment

- **Read Appendix V & VI on the pH meter and spectrophotometer before class today.**

Learning Outcomes

- To learn how to use a pH meter, a spectrophotometer, and an electronic balance
- To learn the difference between a pH indicator and a pH buffer
- To understand an absorption spectrum
- To appreciate the limits of accuracy and precision

Materials

Spectrophotometers, electronic balances, pH meters
pH buffers & pH paper
Red cabbage juice
Spinach leaves (or other green leaves)
Test Tubes
Transfer pipets

Activities

Measuring pH, and using a pH Indicator
Making an Absorption Spectrum for Chlorophyll
Measuring Mass

Measuring pH

Every table should have one digital pH meter and litmus paper. Choose 4 pH buffers across the range of the pH buffers provided. After this exercise, you should have a sense of how reliable the digital probes are for accurate pH readings when compared to litmus paper and cabbage juice. The pigment anthocyanin, found in red cabbage juice, is a pH indicator. This means that as you change the pH of the cabbage juice, it will change colors. Chemists often use pH indicators to titrate chemicals.

1. Place 1-2 mLs of each pH buffer provided into a separate well in the spot plates on your table.

2. Carefully measure the pH of each of the four pH buffers using the digital pH meters, **rinsing the pH probe between measurements**.

3. Measure the pH of each of the buffers using pH litmus paper.

4. Compare the readings you obtain with the pH instrument and the pH paper. Is the digital model more accurate? Would you trust the hand-held model in a sensitive experiment? How about the pH paper?

5. Add 1-2 mLs of cabbage juice to each of the 4 pH buffers.

6. Compare the color to the chart provided. Is cabbage juice a good pH indicator?

	pH 4	pH 5	pH 6	pH 7	pH 9	pH 10	pH 12	pH 13
digital								
pH paper								

Table 3.1 Record your pH readings in a similar table in your Scholar's Notebook

Spectrophotometer Use (See Appendix VI)

In this exercise you will be plotting the absorption spectrum of chlorophyll, the green pigment found in all plants and algae. Spectrophotometers can identify pigments by their signature wavelengths which are measured by how much light they absorb or transmit.

1. Cut or pull off the stems of several leaves. Place a small piece of the leaves in a mortar.
2. Pour @10 ml of 80% acetone over the leaves and grind with pestle. This is your chlorophyll extract.
3. Filter the chlorophyll through a funnel lined with filter paper. Collect in a flask or test tube
4. Pour approximately 5 ml of your filtered chlorophyll extract into a spectrophotometer tube. In another tube place @5 ml of 80% acetone.
5. Using the 80% acetone as a blank, place in spectrophotometer and set at 400 nm. Zero or blank the spec as instructed.
6. Place the chlorophyll sample into the spec and take an **absorbance** reading at 400 nm.
7. Remove chlorophyll, put acetone "blank" back in spec and set at 450 nm. Zero spec.
8. Place the chlorophyll into the spec and take an **absorbance** reading at 450 nm.
9. Continue taking spec readings every 50 nm, making sure you zero the spec with the acetone blank before each chlorophyll reading. Take **absorbance** readings every 50 nm from 400-700 nm.
10. Record your **absorbance** readings at each wavelength in your Scholar's Notebook.
11. Plot the absorbance spectrum of chlorophyll vs wavelength (nm). Wavelength is plotted on the x-axis; Absorbance is plotted on the y-axis and will range from 0 to your highest value.

Wavelength (nm)	400	450	500	550	600	650	700
Absorbance							

Measuring Mass

You will measure a variety of known weights on two different balances and determine if the weights and balances are **accurate** and/or **precise**.

Accuracy is the extent that an observed value agrees with the true value.

Precision is the extent that measurements are repeatable when reproduced.

To illustrate let's use the targets below and assume that dart thrower wants to hit bull's eyes.
In Figure 3.1a the darts are all over the board; the thrower was neither accurate nor precise.
In figure 3.1b, the darts are clustered in one area of the outer ring; the thrower was precise, but not accurate.
In figure 3.1c the darts hit the edge of the bull's eye each time. The thrower was accurate, but not precise.
In figure 3.1d, all the darts hit near the exact center of the bull's eye; the thrower was both accurate and precise.

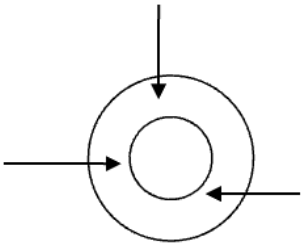


Figure 3.1a
Not accurate,
Not precise

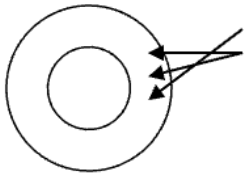


Figure 3.1b
Not accurate,
Precise

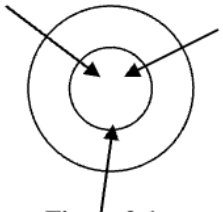


Figure 3.1c
Accurate
Not Precise

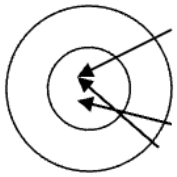


Figure 3.1d
Accurate &
Precise

Procedure

1. Select and weigh three (3) **equal** weights on **two electronic balances**.
2. Calculate the mean mass for the weights for each balance. Record your data in your Scholar's Notebook
3. Determine if the weights and balances are accurate and/or precise.
4. Randomly select five similar items from the plastic tubs.
5. Weigh each item on the **electronic** balance. Record your data and calculate the mean mass.
6. Return the items to the tub. Randomly select another five similar items and repeat step #5.

	Electronic 1	Electronic 2
Weight #1		
Weight #2		
Weight #3		
Mean		

Table 3.3 Record balance data using a similar table in your Scholar's Notebook.

Set #1	Mass (g)	Set #2	Mass (g)
Item #1		Item #6	
Item #2		Item #7	
Item #3		Item #8	
Item #4		Item #9	
Item #5		Item #10	
MEAN (Items 1-5)		MEAN (Items 6-10)	

Table 3.4 Record organisms weight data using a similar table in your Scholar's Notebook

Scholar's Notebook Entry

Week 3: Use of Equipment

Reflection

Objective/Purpose

Methods

Vocabulary

Results/Observations: cabbage juice color and pH

2 Questions

Conclusions

Graph of absorbance spectra

Appendix V: pH, pH Indicators, pH Buffers & the pH Meter

pH

One very important property of organic molecules is called **pH**, which literally means the "power of hydrogen." pH is a measure of the hydrogen ion concentration [H^+] of a solution. Water, known as the universal solvent, can disassociate into equal concentrations of hydrogen (H^+) and hydroxide ions (OH^-). The balanced equation for this reaction is:

$$2\ H_2O \leftrightarrow H3O^+ + OH^-.$$

By convention this is usually written as:

$$H_2O \leftrightarrow H^+ + OH^-.$$

In pure water the concentrations of hydrogen ions and hydroxide ions are equal at [1×10^{-7}] M. In terms of pH, water is considered to have a neutral pH. Any solution with a higher [H^+], e.g. [1×10^{-6}] is said to be acidic and any solution with a lower [H^+], e.g. [1×10^{-8}] is said to be basic.

It is rather cumbersome to write out [1×10^{-6}], [1×10^{-7}], or [1×10^{-8}] so scientists came up with a shorthand method of stating pH values. pH is defined as the negative logarithm (log) of the hydrogen ion concentration in solution. The log of 1×10^{-7} is -7; the negative log is – (-7) = 7. Thus the pH of pure water is 7. The pH scale runs from 0 to 14 with 0 pH having the highest [H^+] and 14 the lowest [H^+].

[H^+] ion concentration (M)	Degree of Acidity	pH	Example
$1x10^0$		0	
1×10^{-1}	Very Acidic	1	Stomach acid
1×10^{-2}		2	Lemon juice
1×10^{-3}		3	Cola, Vinegar
1×10^{-4}		4	Tomatoes
1×10^{-5}	Weakly Acidic	5	Black coffee
1×10^{-6}		6	Urine
1×10^{-7}	Neutral	7	**Pure Water**/Blood
1×10^{-8}		8	Seawater
1×10^{-9}	Weakly Basic	9	Baking soda
1×10^{-10}		10	Great Salt Lake
1×10^{-11}		11	Milk of Magnesia
1×10^{-12}		12	Household ammonia
1×10^{-13}	Very Basic	13	Household bleach
1×10^{-14}		14	Drano

Table V.1 The pH Scale

Note that because the pH scale is logarithmic, each pH value has 10 times fewer H^+ ions than the pH value before it. For example, pure water at pH 7 has 10 times fewer H^+ ions than urine at pH 6 and 10 times more H^+ ions than seawater at pH 8.

pH Indicators

A **pH indicator** is a compound that changes color as the pH of the solution changes. A common indicator is litmus paper. It is made from an organic dye extracted from an organism called a lichen. It is naturally pink in color, but turns blue in basic solutions and red in acids. In biology lab we will be using a purple pigment called anthocyanin as a pH indicator. The anthocyanin has been extracted from red cabbage for use in lab.

Other common indicators, that you might see, are *p*-nitrophenol, which is colorless from *p*H 1 to 5 and yellow from *p*H 5 to 9; methyl orange which is yellow in basic and neutral solutions and reddish below *p*H 3.7; and phenolphthalein which is colorless in acid and neutral solutions, pink at about *p*H 8.5, and purplish at *p*H 10.

pH Buffers

It is critical that the pH of an organism remains constant, because most biochemical processes, e.g. enzyme catalyzed reactions, proceed only within a very narrow pH range. Constant pH is maintained in biological organisms by pH buffers. **pH buffers** are solutions that resist change in pH as small quantities of acid or base are added. They work by absorbing excess H^+ as the pH decreases or releasing H^+ as the pH increases. Human blood and milk are examples of biological fluids that are buffered.

pH Meter

A pH measurement system consists of three parts: a pH measuring electrode, a reference electrode, and a high input impedance meter. Think of the pH electrode as a battery, with a voltage that varies with the pH of the measured solution. The pH measuring electrode is a glass bulb that varies with the changes in the relative hydrogen ion concentration inside and outside of the bulb. The reference electrode output does not vary with the activity of the hydrogen ion. Often a combination electrode performs the functions of both the pH measuring and the reference electrode.

The digital pH meter used in this laboratory is a Model IQ150 pH meter from IQ Scientific Instruments (See Fig.V.1).

Figure V.1 Model IQ150 Hand held pH/ mV/ Temperature Meter. (Picture and instructions used by permission of IQ Scientific Instruments)

Calibrating the pH Meter

1. Connect probe to meter
2. Put probe in first calibrating buffer (pH 7)
3. Press **ON/OFF** button
4. Press **pH/mV** button until pH icon seen on display
5. Press **CAL** button
6. Press **SELECT BUFFER** button until display matches pH 7
7. Press **ENTER** button and wait until large number display stops flashing
8. When flashing stops, the next stored buffer value will appear on display (4.01 or 10.0)
9. Rinse probe in rinse solution and place in second calibrating buffer
10. Press **SELECT BUFFER** button until display matches second buffer value
11. Press **ENTER** button and wait until large number display stops flashing
12. Rinse probe in rinse solution and you are ready to measure pH of your sample

Appendix VI: The Spectrophotometer

Introduction

All molecules can absorb or transmit distinctive wavelengths of light. A spectrophotometer is an instrument that directs a specific wavelength of light into a solution and measures the amount of light absorbed or transmitted by that solution. Thus, a spectrophotometer can be used to identify molecules in a solution.

Visible light is a very small fraction of the electromagnetic spectrum. The spectrum is divided into wavelengths measured in nanometers. Each wavelength contains a specific amount of energy. The smaller the wavelength, the greater the amount of energy contained by that wavelength. Below is a diagram of the electromagnetic spectrum.

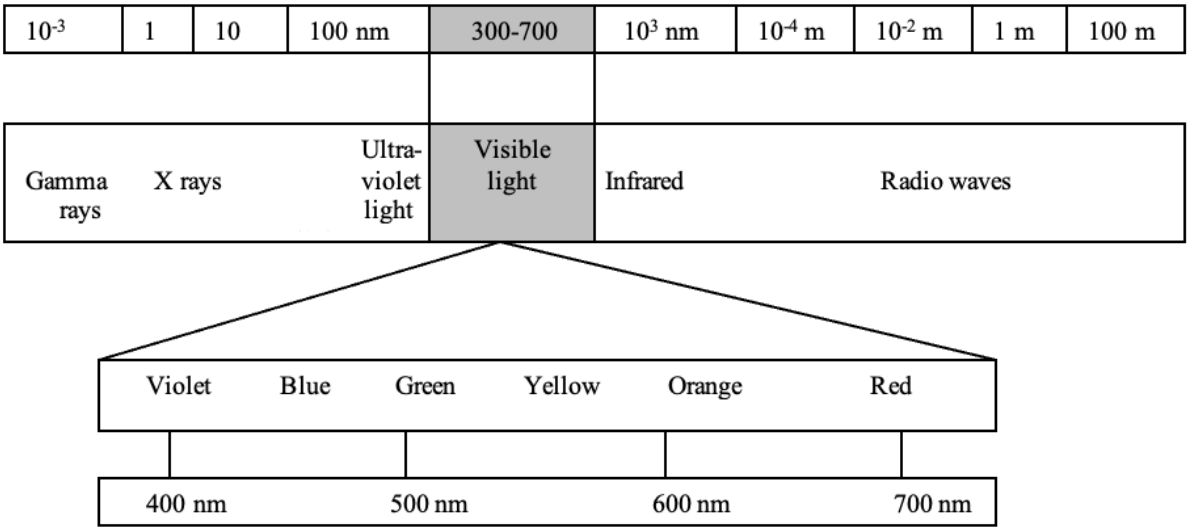


Figure VI.1 The electromagnetic spectrum

White light is the presence of all colors and can be separated into the wavelengths of the visible spectrum by a prism. A spectrophotometer is equipped with a white light source and a prism. Thus a spectrophotometer can separate white light into any wavelength in the visible spectrum. In some spectrophotometers a series of filters or colored glass plates are used instead of a prism to select a certain wavelength.

A spectrophotometer has many uses in the biology or chemistry lab. Since all molecules absorb or transmit light at specific wavelengths, it can be used to identify unknowns. It can be used to calculate the activity of a biological molecule such as an enzyme. By observing a decrease in absorbance, one can measure how much substrate was used in the enzyme catalyzed reaction. The spectrophotometer can also be used to measure the concentration of a substance in solution by using a standard curve. A **standard curve** is a graph depicting a series of known concentrations of a solution

versus their absorbances at a particular wavelength of light. Because absorbance is directly proportional to concentration, the relationship between the two (concentration and absorbance) is expressed as a straight line. This relationship is known as **Beer's Law**.

A common procedure used by scientists is determining the **absorption spectrum** of a molecule. In this procedure the absorption of a molecule in solution is measured at a series of wavelengths, e.g. 400, 450, 500 . . . 700 nm. This will determine at which wavelengths absorption occurs and will create a pattern unique to that molecule when plotted on graph paper. You will create an absorption spectrum for the pigment **anthocyanin** during BL131.

Operating the Spectrophotometer

The instrument we use in the biology and chemistry departments at LSSU is the Spectronic® 20 Genesys™ by Spectronic Instruments. It is pictured below.

Figure VI.2a
The Spectronic® 20 Genesys™ by Spectronic Instruments

Figure VI.2b Close-up of instrument panel of the Spectronic® 20 Genesys™

Instructions for use of the Spectronic® 20

1. Turn on instrument using toggle switch in back. Let warm up for at least 15 minutes.

2. Set wavelength using yellow up and down arrows in the half ovals with the nm abbreviation (not the big yellow arrows within the circles).

3. Push the **A/T/C** button until you see an **"A"** in the right hand corner of the digital display window.

4. Place a cuvette containing your "blank" into the cuvette holder and press the **0 ABS/100% T** button.

5 Remove the blank and insert your first sample. Read absorbance from digital readout and record in your Scholar's Notebook.

Appendix VIII: Microbiology Techniques

Many students choose to do open inquiry investigations on some aspect of bacterial growth. In this chapter we will describe several techniques used by microbiologists that you may find useful.

1. **Preparing a wet mount** – This technique is used to view living microscopic organisms under the compound microscope (see App VII).

    a. Use a transfer pipette to place a drop of water containing the organisms of interest in the center of a glass slide.
    b. Carefully place a cover slip over the drop, taking care not to create a bubble.

2. **Preparing a hanging drop slide** – This technique is used for viewing very small macroscopic organisms that may be crushed using the wet mount technique.

    a. Obtain a deep well slide and a cover slip.
    b. Put some petroleum jelly (Vaseline) on a toothpick.
    c. Rub a small quantity of the Vaseline one side of the cover slip.
    d. Place the cover slip on a paper towel, Vaseline side up
    e. Transfer a drop of water containing the organisms of interest to the center of the cover slip.
    f. Place the deep well slide on the cover slip so that the Vaseline forms a seal.
    g. Quickly invert the slide so that the cover slip is on top and the drop is suspended.
    h. The slide is now ready to be viewed under either the compound or dissecting microscope.

3. **Testing for microbes in the environment -** This is a very popular open inquiry experiment for first year biology students. There are many places where bacteria and fungi grow around campus; and it's fun to predict where you might find them.

    a. First, decide where you want to look for microbes and decide how many nutrient agar plates you will need.
    b. The lab assistant will make the plates for you.
    c. Obtain a bottle of sterile water and several sterile cotton swabs
    d. Using a Sharpie mark the bottom of your plates into four quadrants

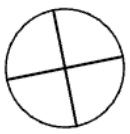


Figure VIII.1 - By dividing agar plates into quadrants, you can sample four areas with one plate. Always label the bottom of the plate. This way if you drop a stack of plates, you don't have to worry which lid goes with which plate.

e. Dip the swab into the sterile water, then swipe the surface you want to test
f. Gently swipe the swab onto one quadrant of the nutrient agar plate.
    i. Identify the location of your swipe with a Sharpie on the bottom of the agar plate.
    ii. Also mark the plates with your initials, the date, and lab section.
g. Place in 37° C incubator in CRW254B and let incubate for 24 - 48 hours.
    i. Incubate the plates upside down so if there is any condensation, it will be in the lid and not on your sample.

4. **Bacterial colony morphology -** A bacterial colony is a population of cells that arise from a single bacterial cell. One way to complete the above experiment is to describe how many and what types of colonies you observe. Figure VIII.2 shows examples of different colony morphologies.

    a. Remove plates from incubator and examine.
    b. Construct a table for each area sampled as illustrated in Table VIII.1.
    c. Hypothesize as to how many different types of bacteria you have isolated.

Figure VIII.2 - Some examples of bacterial whole colony morphology

Colony Description				
Area Sampled	Appearance	Color	Diameter	# of this type
_____				
Incubated at:				
_____ °C for				
_____ hours				

Table VIII.1 Data table for describing bacterial colony types

5. **Streak plate method for isolating a single bacterial colony -** Often, after observing bacterial colonies, we want to isolate one or two specific colonies in order to identify them or to examine their growth patterns. See Figure VIII.3.

    a. Obtain an inoculating loop.
    b. Sterilize it by holding it under the hottest part of a Bunsen burner flame
        i. the blue area
    c. Let the loop cool in the air - **Don't** blow on it. Why?
    d. Touch the loop to one colony from your mixed culture plate.
        i. Be sure to touch just one colony
    e. Lift one edge of the Petri plate cover and streak the first sector by making as many streaks as possible without overlapping previous streaks. See Figure VIII.3.
    f. Flame loop and let it cool.
    g. Turn plate so next sector is at the top.
    h. Streak through first sector, then make several streaks in sector two.
    i. Flame loop and turn plate again.
    j. Streak through second sector and make several streaks in third sector.
    k. Flame loop, let cool, streak through third sector. Streak the remaining area of agar surface (sector four). Be careful not to touch any of the streaks in sectors one, two, or three.
    l. Single, isolated colonies will be found in sector four.

6. **Inoculating a broth culture -** A **broth culture** is a liquid nutrient medium in which a large number of bacteria can grow in a limited space. Broth cultures are usually contained in screw top test tubes and are easily transported in test tube racks.
    a. You will need:
        i. An inoculating loop.
        ii. A Bunsen burner
        iii. A bacterial culture (Petri plate or slant)
        iv. Test tube with 10 ml sterile nutrient broth
    b. Sterilize inoculating loop by holding it under the hottest part of a Bunsen burner flame.
    c. Let the loop cool in the air - **Don't** blow on it. Why?
    d. Open lid of Petri plate and, using the loop, obtain a small quantity of bacteria from a single colony.
    e. Remove cap from sterile nutrient broth tube while still holding inoculating loop.
        i. Hold cap between your pinkie and your palm in the same hand that is holding the loop.
    f. Hold tube containing sterile broth at an angle and pass the mouth of the tube through the gas flame.
    g. Insert loop containing bacteria into sterile nutrient broth
        i. Twirl the loop around a few times in the broth to make sure some bacteria are transferred. This is call "twinkling."

Laboratory 4: Cell Division and Statistics

Purpose:
To observe the process of mitosis and meiosis in plant and animal cells using light microscopy
To observe statistical differences in the cell cycle across with different sample sizes.

Objectives:
1) Identify and draw the stages of mitosis in onion root tip cells.
2) Compare and contrast the mitotic process in plant and animal (whitefish blastula) cells.
3) Reconstruct the cell cycle by estimating the percentage of cells in each phase in onion root tip cells
4) Observe the stages of meiosis in animal reproductive organs
5) Calculate "standard error" and determine changes in "confidence" with sample size (n)

Introduction

Mitosis is a process of replication that produces identical copies of a parent cell. It provides a means of reproduction for unicellular eukaryotes, and is involved in the growth, repair and maintenance of multicellular eukaryotic organisms. Because of this, mitosis is most readily observed in tissues that are rapidly growing, such as the cells of a developing embryo or the roots and shoots of a growing plant.
Each cell produced by mitosis contains an identical complement of chromosomes and genes, (except for the rare event where a mutation during DNA replication causes one of the daughter cells to be genetically altered). To achieve this, each chromosome is duplicated, and the sister chromatids are separated to create the nuclei of the daughter cells. While mitosis is a continuous, smooth-flowing process, it is convenient to consider it as a five-stage process. These stages are usually considered to be Prophase, Prometaphase, Metaphase, Anaphase, and Telophase, with the daughter cells then returning to Interphase, which is not considered to be a part of mitosis.

Meiosis is a special type of cell division of germ cells in sexually-reproducing organisms that produces the gametes, such as sperm or egg cells. It involves two rounds of division that ultimately result in four cells with only one copy of each chromosome. Wikipedia

Instructions:

Mitosis in Onion Root Tip Cells: The onion root tip is an excellent material to study mitosis in plant tissues. Mitosis in an onion root tip is a relatively synchronous process, with many cells dividing at the same time (usually in the early hours of the morning). Onion root tips can be collected during periods of active division and prepared by thin-sectioning and staining to show the chromosomes. The slide before you is a snapshot of the tissue at a particular moment in time, which is likely to include many cells captured in various stages of division. (**Note:** because the cells were killed during the slide-preparation process, you will NOT be able to observe motion and watch the cells divide in real time).

1) Prepared slides of onion root tips are available on the back bench.

2) You will need to locate an area of the root containing a high percentage of actively dividing cells. In general, the greatest amount of mitotic activity is located close to the rounded tip of the root, just above the root cap. Focus on this area using the low power objective lens and work your way up to the highest power lens.

3) Scan the tissue and locate a cell that is in **interphase**, then locate cells in each of the 5 stages of mitosis.

4) **In Figure. 4.1, draw all five phases in the order** they would occur. Draw only what you see through the microscope. Do not add detail that might be visualized with an electron microscope—focus on what you can see with the tools you have.

Figure 4.1 Sketch the phases of the cell cycle as seen through the light microscope

Onion Root Tip Cells

Interphase
Prophase
Prometaphase
Metaphase
Anaphase
Telophase

5) Once you are confident in your ability to correctly identify interphase and each of the 5 stages of mitosis, focus your microscope on a field of view that includes several actively dividing cells while using the high power lens.

6) **Count the cells that are visible in your field of view, identifying the phase of each cell**. Record your data in **Table 4.1**. Pool your data with the classmates at your table and record your table total in **Table 4.1**.Record your table total on the board to generate a class total for each phase. Record your class total in **Table 4.1.**

Table 4.1 Number of cells in each phase of cell cycle in one field of view

Onion Root Tip Cells

	Your total	Table total	Class total
Interphase			
Prophase			
Prometaphase			
Metaphase			
Anaphase			
Telophase			
Total Cells			

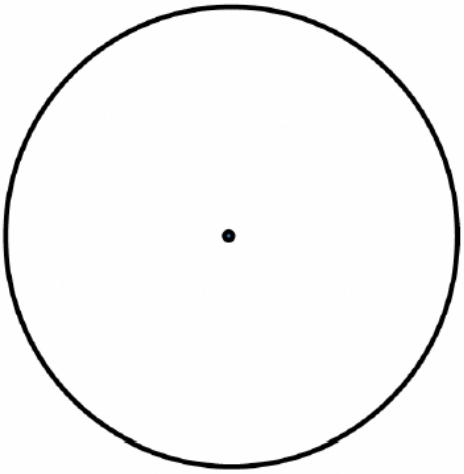
1) Calculate the percentage of cells in each phase for each species for your own samples and for the table and class totals and record these percentages in Table 4.2.

Table 4.2 Percentage of cells in each phase of the cell cycle in one field of view

Onion Root Tip Cells

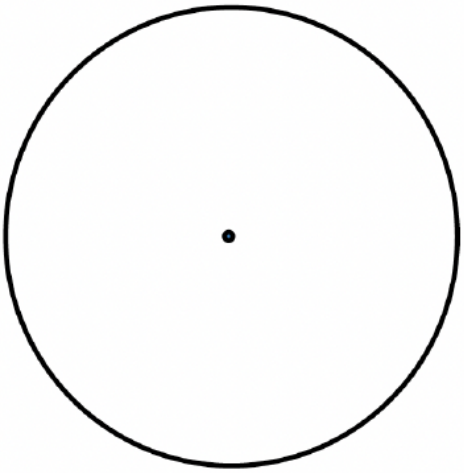
	Your %	Table %	Class %
Interphase			
Prophase			
Prometaphase			
Metaphase			
Anaphase			
Telophase			

2) Illustrate the cell cycle for each species by creating two pie charts, one showing the class totals and one showing your own results. Each chart should show the stages of the cell cycle in order, beginning with interphase and including all 5 stages of mitosis. These should be drawn roughly to scale, and each section should be labeled with the name of the phase and the percentage of the cycle it represents (e.g., "Anaphase, 2%"). Label each chart clearly.



A. Onion Root Tip Cells

Figure 4.2 Cell cycle in onion root tip from individual data



A. Onion Root Tip Cells

Figure 4.3 Cell cycle in onion root tip from class data

3) You should have two separate pie charts (Figs. 4.2 and 4.3):
a. Your personal data, table data and the class's data for the onion (Table 4.2)

Mitosis in Whitefish Blastula Cells: The whitefish blastula is often used to study mitosis in animal cells. The blastula is an early stage in embryonic development in which the cells are rapidly dividing but have not yet begun to differentiate into distinct tissues. The blastula therefore often resembles a raspberry—a ball of nearly identical cells with few distinct features. Whole mounts of the whitefish blastula can be prepared and stained for microscopic examination.

Meiosis in Ovary and Seminiferous Tubules

1) Observe the slides of reproductive tissue

2 a) In your scholar's notebook, sketch the cells you observe

b) What stage of meiosis are these cells in?

Statistics

Confidence Intervals: When a sample, rather than the entire population, is surveyed, estimates differ from the true population values. The component of this difference that occurs because samples differ by chance is known as sampling error, and its variability is measured by the standard error of the estimate. A sample estimate and its estimated standard error can be used to construct confidence intervals. A confidence interval is a range of values centered around the sample estimate that is likely to include the true population value with a degree of confidence. https://www.youtube.com/watch?v=UuHqq09nTAk

Standard Error: To compare samples, calculate the **mean, standard deviation, and confidence intervals** of your data and enter results in Table 4.3.
The **mean** is the average of all data points for a sample set.
The standard deviation measures how different the values are from each other.
The **confidence interval** is a measure of how confident we are that our mean is correct.

Calculating the Standard Error (SE)

$$SE = \frac{S}{\sqrt{n}}$$

where S = standard deviation, X = mean, and n = sample size

Table 4.3 Confidence Intervals (SE)

	Table %	n (table)	Table SE	Class %	n (class)	Class SE
Interphase						
Prophase						
Prometaphase						
Metaphase						
Anaphase						
Telophase						

Activities

1. Use Excel functions to calculate mean, standard deviation, and standard error
2. Graph the means and CIs for the phase of mitosis using data from your table.
3. Is there a significant difference between the phases?
4. Does sample size make a difference?
5. Graph the means and CIs for the phases of mitosis using data from the whole class
6. Is there a significant difference between the means of the two groups?

Week #4: Scholar's Notebook Entry

Reflection

Objective/Purpose

Methods

Vocabulary

Results/Observations: Data on cell cycle

2 Questions

Conclusions

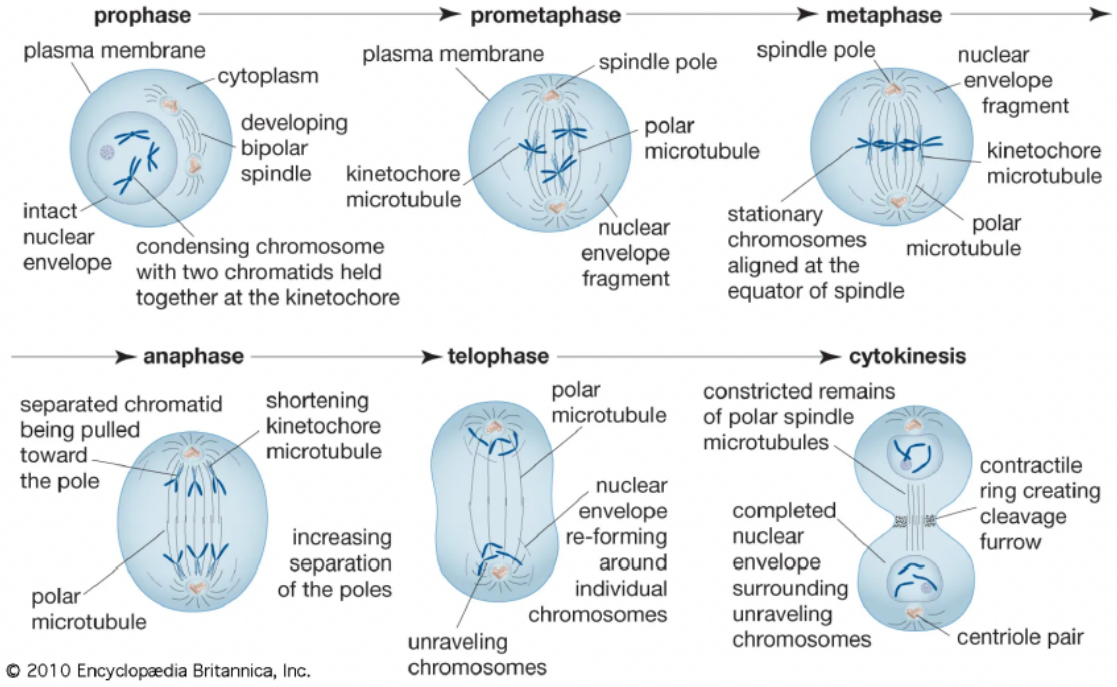
Drawings (mitotic figures)

Lab Report

After completing your Scholar's notebook entry for this week, you will write a laboratory report using the methods and results of lab week 4.

For guidance, read Appendix III - Writing a Lab Report.

Mitosis, or somatic cell division



Laboratory 5: DNA Extraction Techniques

Learning Outcomes

- To gain skill in laboratory techniques
- To compare two methods of DNA extraction
- To consider further experimentation using DNA

Activities

Learn micro-pipetting techniques
Extract DNA from human cheek cells
Extract DNA from strawberries

I) Micro pipetting technique

Procedure

1) Place a 3 ul sample of the basic dye colors (red, green, blue & yellow on the filter paper and label the spots.
2) Use the chart below to mix the required ul of basic colors onto the square of parafilm.
3) Mix all each dye mixture in a singlet droplet on the square of parafilm.
4) Place a 3 ul sample of the mixed colors on the filter paper with the basic colors
5) Attach the filter paper to your lab report

	Red	Green	Blue	Yellow
Teal		5ul	15ul	
Gothic Rose	15ul		5ul	
Orange	6ul			14ul
Chartreuse		2ul		18ul

II) Chelex Method of DNA Extraction from Human Cheek Cells

Materials

10% sterile Chelex
sterile toothpicks
1.5 ml microcentrifuge tubes
95° C heat block
Microcentrifuge

Procedure

1. Place 300 µl of sterile 10% Chelex into a 1.5 ml microcentrifuge tube.
2. Gently scrape the inside of your cheek with a sterile round-ended toothpick
3. Swirl toothpick in the Chelex to transfer cheek cells.
4. Label your tube, and place in the 95°C heat block.
5. Let incubate for 20 minutes. (Start strawberry DNA extraction)
6. Let cool for 2 minutes and place in freezer rack.

III) DNA Extraction from Strawberries

DNA can be isolated from plants and animals using several different techniques. The procedure described below is interesting because it uses ingredients found in almost every household.

Materials

Strawberries	10 mls extraction buffer
Cheesecloth	10-25 ml graduated cylinder
Isopropyl alcohol	Funnels
NaCl	6% meat tenderizer solution
50 ml test tubes	Ziploc® freezer bag
spooling pipettes	

Procedure

1. Measure @10 mls of extraction buffer
2. Obtain @ two (2) strawberries and place in Ziploc® bag. Remove air and seal bag.
3. Mash strawberries inside bag with your hands (being careful not to puncture the bag).
4. Pour the extraction buffer into the plastic bag. Remove air, seal bag, and mix mashed strawberries and extraction buffer together.
5. Obtain a funnel, a 50 ml test tube, and a 10 x 20 cm piece of cheesecloth.
6. Put the funnel in the test tube and line the funnel with the cheesecloth.
7. Cut the bottom corner of the bag, and pour the strawberry mixture into the cheesecloth and let strain into test tube.
8. Remove funnel from test tube and discard cheesecloth.
9. Add 5 ml of a 6% meat tenderizer solution. Mix gently.
10. With a pipette slowly drizzle @10 ml of ice cold isopropyl alcohol down the sides of the test tube.
    a. The alcohol should form a layer on top of the strawberry mixture (@ 1 inch).
    b. A white stringy precipitate should be visible at the interface between the alcohol and extraction buffer. This is your DNA.
11. Place a spooling pipette into the test tube and slowly rotate (*not stir*) in one direction. The DNA should spool onto the pipette.
12. Record your observations in your Scholar's Notebook with a sketch of the test tube.
13. Rinse all glassware and use sponge to wipe counter tops.

Scholar's Notebook Entry

Reflection
Objectives
Methods
Results (color wheel)
2 questions
Vocabulary
Conclusions
Activity: Sketch of strawberry DNA in test tube

Laboratory 6: PCR and Gel Electrophoresis DNA Analysis Techniques

Learning Outcomes

- To gain skill in DNA laboratory techniques
- To understand gene amplification and fragment analysis using gel electrophoresis

Activities

Set up polymerase chain reactions (PCR) using DNA extracted from human cells
Go through a virtual PCR setup
Prepare PCR products for electrophoresis

I) Polymerase Chain Reaction (PCR) reaction setup

The presence of mutations in the HFe gene, can lead to hereditary hemochromatosis. Persons with these mutations are prone to excess iron absorption, which causes a variety of potentially serious health conditions. Once diagnosed, patients can easily be treated, by therapeutic phlebotomy (blood letting). When the body makes new red blood cells, it takes the excess iron from the body, and reduces the iron overload. Symptoms of iron overload begin with fatigue, but it can also cause diabetes (pancreas), cirrhosis (liver) and many other diseases depending on which organs are receiving excess iron deposits. Unfortunately, many physicians are not aware of this condition, so it is under-diagnosed, and often patients are treated for the symptoms, but not the underlying cause. C282Y and H63D are the most common mutations in Caucasian populations. In this lab, you will use primers to amplify these regions of the HFe gene using PCR. Then you will load your PCR products on to the electrophoresis gel, to see if you carry the mutation. This information will be kept confidential.

Materials

Extracted DNA
PCR Master Mix
Thermocycler
Micropipettes and tips, and reaction tubes

Procedure

1) Take your extracted DNA out of the freezer, and allow to come to room temperature.

2) Place your sample in a balanced configuration, in the microcentrifuge. Spin for 1 minute.

3) Your DNA will be in the supernatant, the top layer above the Chelex beads

3) Label two PCR tubes with your name (salmon-normal; green-mutation)

4) Measure 12 ul of each Master Mix and add it to each PCR tube.

5) Take 3 ul of your DNA (from the very top of the supernatant) and add to each PCR tube.

6) Place the labeled PCR tubes in the rack provided; return your DNA into the rack.

7) Your lab instructor will run the PCR reactions on the thermocycler.

8) Once the PCR reactions are complete, you will load your PCR products into a gel.

II DNA Analysis Virtual Lab

Materials

Computer with internet connection
https://learn.genetics.utah.edu/content/labs/extraction/
http://learn.genetics.utah.edu/content/labs/pcr/
https://learn.genetics.utah.edu/content/labs/gel/

1) DNA Extraction Procedure (optional)

Try extracting DNA at home:
https://learn.genetics.utah.edu/content/labs/extraction/howto/
Go through the virtual extraction lab.
a) how did this method differ from what we did in lab using Chelex?

2) Virtual PCR
https://learn.genetics.utah.edu/content/labs/pcr/

1) In your scholar's notebook:
I: write down the components of the PCR reaction as you add them to the reaction tube.

II: Answer the following questions:
a) During the thermocycler cycle 1 what was:
- i) the first temperature and what happened?
- ii) the second temperature and what happened?
- iii) the third temperature and what happened?

b) How many copies have been made after 30 cycles?
c) what was the size of the target DNA fragment in the virtual lab?

III Gel Electrophoresis (Fragment Analysis of PCR-products)

Materials

Electrophoresis gel set up
size standard ladder
Micropipettes and tips
Reaction tubes

Procedure

1) The thermocycler will take @90 minutes to go through 30 cycles of the reaction.
2) With a clean pipette tip, add 1ul of your PCR products into the gel well
3) Note the position of your samples on the sheet provided
4) Results will be available after lab. Record these result in your scholar's notebook.

Scholar's Notebook Entry

Reflection Objectives
Methods
Results: Virtual PCR responses (see above)
2 questions
Vocabulary
Conclusions

BIOL131 Electrophoresis Lab

Step 1: Wear gloves

Step 2: Obtain gel box with agarose gel and carefully remove black rubber ends

Step 3: Place in chamber

Step 4: Fill chamber with 1XTBE running buffer to cover gel

Step 5: Carefully remove both combs from gel

Step 6: Using a fresh tip, add 15 ul of your green (mutation) PCR product to the **upper** well.
The well you will use is up to you

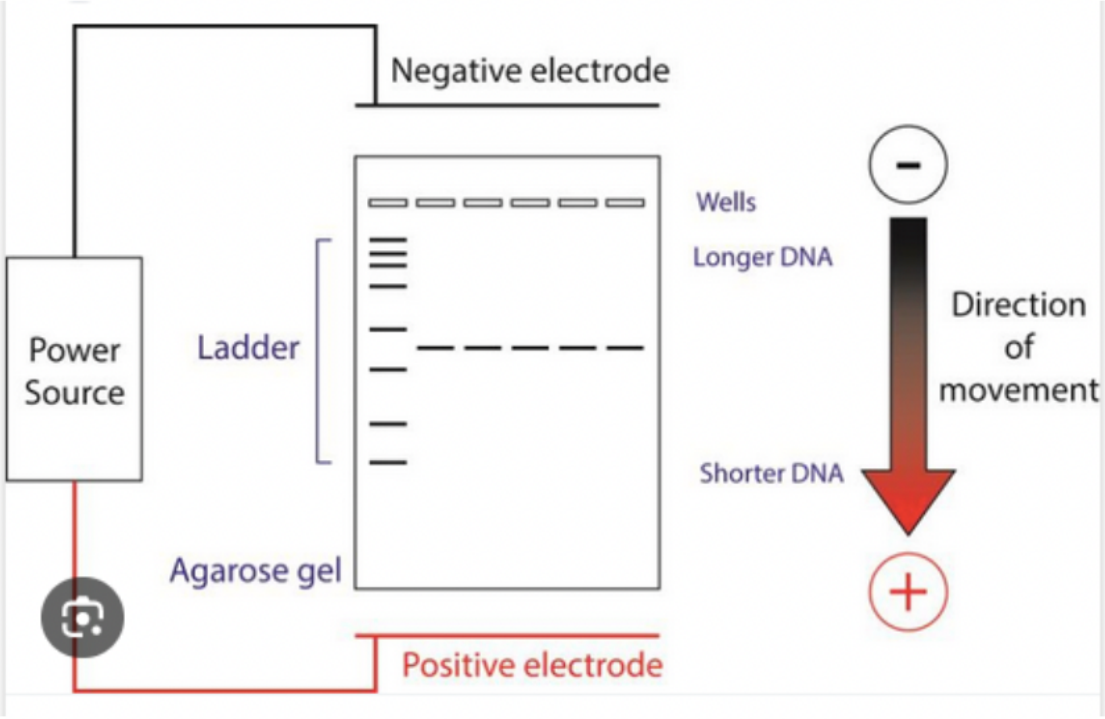
Step 7: Using a fresh tip, add 15 ul of your salmon (wild type) PCR product to the **lower** well.
The well you will use should be the same as above

Step 8: Your instructor will add a size ladder to each gel in well #1

Step 9: Cover the gel box, plug in the power supply, and set to 150 V

Step 10: Let the gel run until the dye front is 2/3 down the gel (@30 minute)

Step 11: Turn off the power supply, and remove the gel from the box.
Place gel on the UV gel illuminator, and record the results



Lab #7 Guided Inquiry Experiment

Learning Outcomes

- **To understand the meaning of scientific inquiry**
- **To learn how to properly design an experiment**
- **Put into practice the scientific skills you have learned this semester**

Structured inquiry is how most general biology and general chemistry laboratory manuals are set up. They are often called "cookbook" manuals because everything is laid out for you. Guided inquiry and open inquiry experiments allow a student to use his/her own imagination and resources to answer scientific questions. Our goals for this laboratory are to teach you a few of the tools that a biologist uses, how to think for yourselves and to discover that science is fun.

A good researcher always has a well thought out plan, called an experimental design. The success of an experiment often depends on its design. Our first activity is a guided inquiry that will help you design an experiment to answer a question that is given to you.

Activities:

1. Use the scientific method to design an experiment for the following question:

Which brand of paper towel is most absorbent?

2. Perform the experiment.
3. Graph your results with statistics.
4. Perform a cost analysis (which brand is most cost effective?)
5. Turn in a scholar's notebook entry and also complete a formal lab report

Guided Inquiry Scholar's Notebook

Reflection
Objectives
Hypothesis
Methods (what you did)
Results
Conclusions
Vocabulary
2 Questions

Laboratory 8: Scientific Inquiry & Experimental Design

Learning Outcomes

- To understand the meaning of scientific inquiry
- To learn how to properly design an experiment
- To learn how to apply the scientific method

Introduction

One of the goals of this course is to teach you how to design experiments. You will soon learn some of the tools of the trade, how to use equipment such as the microscope and spectrophotometer, how to analyze data, and different methods of DNA extraction.

Next we will introduce you to inquiry based science. What is inquiry? Inquiry is the way scientists study the natural world and propose explanations based on evidence. The four basic types of inquiry-based experiments are[1]:

1. **Structured Inquiry**
   Student presented with question
   Procedures given
   Results given

2. **Directed Inquiry**
   Student presented with question
   Procedures given
   Results discovered

3. **Guided Inquiry**
   Student presented with question
   Student designs procedure
   Results discovered

4. **Open Inquiry**
   Student formulates question
   Student designs experiment
   Results discovered

Structured inquiry is how most general biology and general chemistry laboratory manuals are set up. They are often called "cookbook" manuals because everything is laid out for you. Guided inquiry and open inquiry experiments allow a student to use his/her own imagination and resources to answer scientific questions. Our goals for this laboratory are to teach you a few of the tools that a biologist uses, how to think for yourselves and to discover that science is fun.

Experimental Design

Experimental design or the scientific method is the steps that a scientist takes when planning an experiment. Every science textbook and laboratory manual presents the scientific method just a little differently; but they all really say just about the same thing. Our version, which we prefer to call experimental design, is below.

1. **Ask a question**.

2. Formulate a **hypothesis** and make a **prediction**.

3. Identify your **variables**.
   a. The **independent variable** is the trait you choose to alter
      e.g. amount of certain chemical used.
   b. The **dependent variable** is the feature that shows a response to the alterations of the independent variable, e.g., change in growth rate.

4. Formulate your **procedure**.
   a. These are the instructions as to how the experiment will be performed.

5. Include **controls** (variables that are held constant between the treatments).
   a. Many experiments have a positive control and a negative control
      i. The **positive control** tells you that the procedure is working.
      ii. The **negative control** lets you know that there are no contaminants in your experiment.

6. Use at least three **replicates** (3 examples of the same independent variable).
   a. It is OK to work with another group and combine replicates.

7. Decide how you will **analyze** your data
   a. Be sure to include a statistical test

[1]Herron. 1971. The Nature of Scientific Inquiry. Scholastic Review. 79(7):171-212.