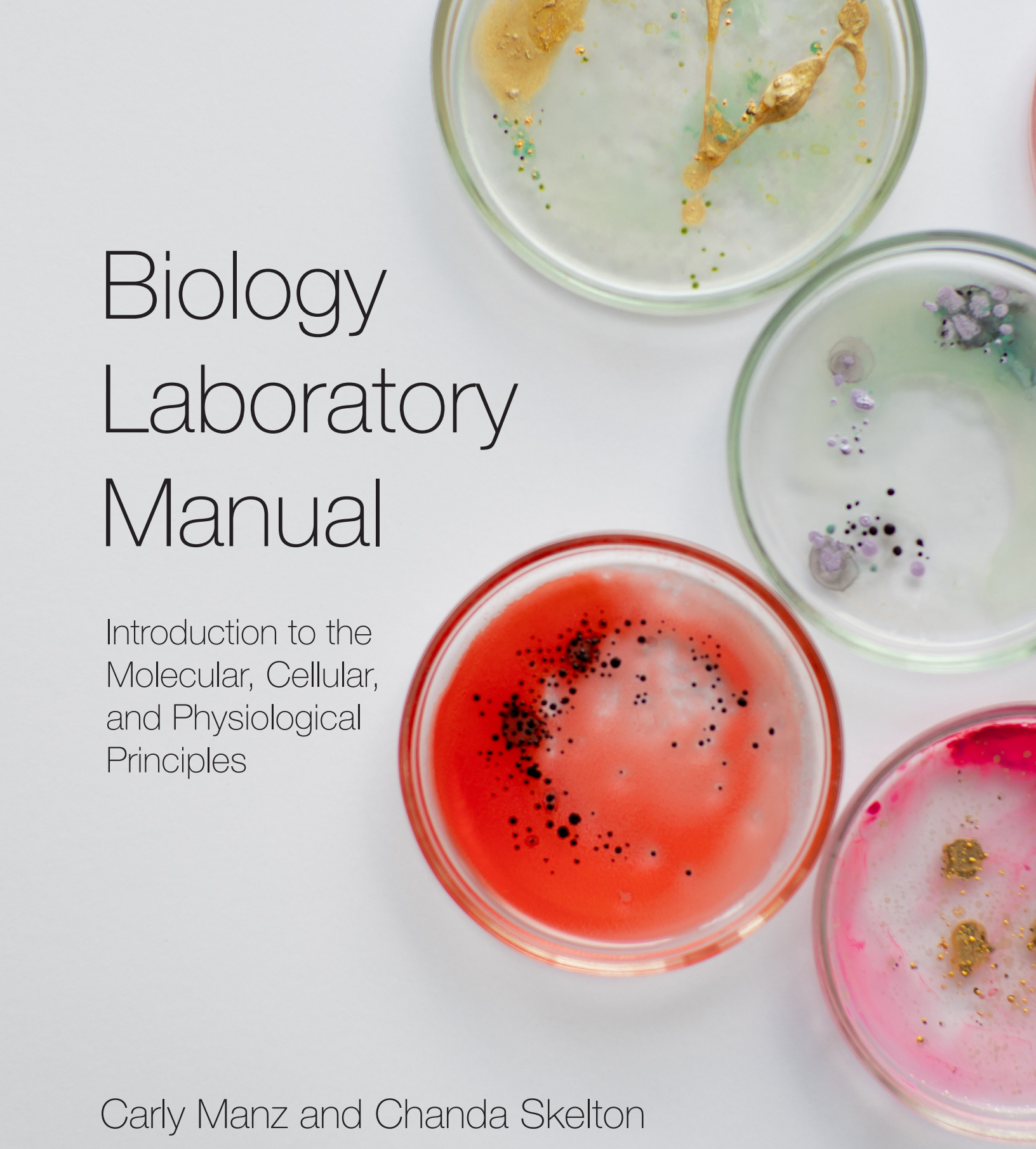

Biology Laboratory Manual

Introduction to the Molecular, Cellular, and Physiological Principles

Carly Manz and Chanda Skelton

IOWA STATE UNIVERSITY
Digital Press

Biology Laboratory Manual

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Introduction to Molecular, Cellular, and Physiological Principles

Carly Manz and Chanda Skelton

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Iowa State University is located on the ancestral lands and territory of the Baxoje (bah-kho-dzhe), or Ioway Nation. The United States obtained the land from the Meskwaki and Sauk nations in the Treaty of 1842. We wish to recognize our obligations to this land and to the people who took care of it, as well as to the 17,000 Native people who live in Iowa today.

Contents

INTER-CHAPTER 1.

ACADEMIC DISHONESTY

When you are submitting assignments for any of your courses, it is important that you are aware of the rules regarding academic dishonesty. Academic dishonesty is a serious matter that can result in dismissal from the university. **DON'T RISK IT**. Iowa State University outlines university policies in the Student Disciplinary Regulations #4.2.1 Academic and Research Misconduct.

4.2.1 Academic and Research Misconduct

Academic Misconduct. The academic work of all students must comply with all university policies on academic honesty. Examples of academic misconduct are:

1. Attempting to use unauthorized information in the completion of an exam or assignment;
2. Submitting as one's own work, themes, reports, drawings, laboratory notes, computer programs or other products prepared by another person;
3. Knowingly assisting another student in obtaining or using unauthorized information or materials; or
4. Posting past assignments and answers to internet study sites, such as quizlet, studyblue, chegg, course hero, etc. or using the answers posted to those sites.
5. Plagiarism.

Academic honesty policies are included in the policy on Academic Dishonesty, polices and procedures on Academic Misconduct Investigations, and the Acceptable Use of Information Technology Resources policy. Additional information on academic dishonesty is available in the Iowa State University Catalog (see Resources below). In addition, students are responsible for following ethical standards adopted by the various colleges and departments:

Student Code of Conduct. 2022. *Iowa State University Policy Library*. https://www.policy.iastate.edu/policy/SDR

Biology 2120L Expectation

All work submitted by you for grading in Biology 2120L is expected to be your own work, not something plagiarized from an outside source or from a colleague in Biology 2120L. Our goal is for you to demonstrate that

you have achieved the learning outcomes by being able to express your thoughts in your own words and figures.

In Biology 2120L, there are a number of potentially problematic areas where students could be tempted to violate university policies on academic responsibility, or through neglect or carelessness submit work that raises questions about whether the work submitted is the student's original work. We have outlined a number of the most common areas below and provided ways for students to avoid suspicions of academic misconduct. Students should note that instances of academic dishonesty are not limited to the categories listed below. These are just some of the more common examples.

Graph Submissions for Lab Experiments

Concern

Since students are working in groups to carry out experiments and collect data, they are often tempted to share electronic data files (including graphs and photos) when they work on their assignments and complete data analyses. One of the educational outcomes of Biology 2120L is that students will be able to produce graphs that effectively and clearly present their data to others. If the same graph is submitted by multiple students, there is no way for the TA or your instructors to determine whether or not all students have achieved this outcome.

Helpful Suggestions

How to prepare your submitted work to demonstrate that it is uniquely yours, and to avoid suspicion of plagiarism or academic misconduct.

- Do not share electronic data files with other students.
- Create your own graphs and figures using Excel (instructions are provided in the lab manual) along with video tutorials posted on Canvas).
- Change the default graphing settings in Excel so your graph is uniquely yours (change labels, data point symbols, colors, graphic representation format (line graph, bar graph, scatterplot), etc.). This has the added benefit of teaching you how to modify your graphs to more clearly represent your data.
- Make your graph titles very descriptive and add your name or initials to the title to help identify it as your own.
- If you are concerned that your graph may be too similar to your lab partner's graphs, ask your TA (BEFORE SUBMISSION) to look at your graphs and provide you with guidance. The TAs are here to help. **ASK BEFORE YOU SUBMIT YOUR WORK.**

Plagiarism

Concern

When students don't know the answer to a question, their first instinct is to Google the question to look for an answer. The internet is an incredible resource and can be very helpful for students looking to supplement their lab experience. However, students run into trouble when they copy and paste material directly from online (or printed) resources without citing them properly.

Helpful Suggestions

How to Avoid Suspicion

- **NEVER "Copy and Paste"** – any answers that you submit on assignments should be in your own words. Read the information, think about it, close the source, think about it more, and summarize the information using your own words.
    - Copying and Pasting and then changing a few words here and there is still plagiarism.
- To remove temptation to copy phrases from your resource, after reading the information close the book or internet browser and then write your answer. You are less likely to recreate the exact wording if you don't have it right in front of you.
- If you are concerned that your answer might be too close to the original source or someone else taking the lab, ask your TA (BEFORE SUBMISSION) to look at it and provide you with guidance.

Additional notes about online resources: The fact that information is present on the internet does not automatically mean that the information is correct. There is plenty of misinformation available online. Students must be mindful of the sources they access online for information. The best practice is to use multiple credible sources to confirm the information, always properly site your sources, and use your own words to summarize what you've read. Sources like Chegg, Quizlet, Course Hero, and Study Blue are often misleading or blatantly incorrect, so they are not reliable sources of information. Students providing any Biology 2120L course information to these (or similar) sites are committing academic misconduct.

The Biology 2120L instructional team does not post any course content on any site other than the course Canvas page. Students encountering any materials posted online that appear to be Biology 2120L course materials can assume they are online as a result of academic misconduct. These materials are often incorrect or misleading. Students should report any online content that appears to be from this course to the Biology 2120L teaching team (TA, Lab Coordinator, Faculty in Charge).

Submitting Identical Images for Study Guide Pages

Concern

For some lab topics, we ask students to submit study guide pages that they create using

images they've taken of the samples in lab. Our expectation is that each student will take their own photos in lab using the digital cameras available on the lab microscopes (or with other digital equipment provided in lab). While students are encouraged to work together in class, all photos should be your own, don't share electronic files and name your files using distinct labels.

Helpful Suggestions

How to Avoid Suspicion

- Take your own photos during lab.
- Edit your photos to include your name, section number, specimen identification, and magnification. You should also label any important structures seen in the photos.
- Save your photo files on Cybox so you can access them later. Make sure they upload successfully so you do not end up with corrupted (or missing) files.
- **DO NOT SHARE** your photo files with other students.
- Using another student's photo but cropping it to look a little bit different is not acceptable and can be easily detected by your TA.
- If you are concerned that your photo may look too similar to another student's photo, or you have trouble accessing your photo files from Cybox after lab, contact your TA for guidance BEFORE SUBMISSION.

Copying Another Student' s Work

Concern

Students are encouraged to work together in lab to think through problems, work on solutions, and brainstorm ideas as they work through lab exercises and assignments. It can be tempting when working together to approach a difficult question by brainstorming an answer and having multiple people write down the exact same answer on their assignment for submission. However, when the TA grades the assignments, they have no way of knowing if this answer was truly a group effort, or if one student came up with the answer and it was copied by others.

Helpful Suggestions

How to Avoid Suspicion

- Always write down your answers in your own words. Feel free to brainstorm with your lab partners and work together to find an answer, but when you write an answer for submission, make sure you use your own words and phrasing.
- If you are concerned that your written answers may be too similar to your lab partners', contact your TA (BEFORE SUBMISSION) to provide you with guidance.

Cheating on Quizzes or Exams

Concerns (include, but are not limited to, the following):

- Students may glance at another student's answer sheet during an in-class quiz or practical exam.
- Students may attempt to use books, study guides, or other (non-approved) resources during in-lab quizzes or practical exam.
- Students may attempt to take photos of in-lab quiz or practical exam materials to be shared with other students. Students may also attempt to use phones, tablets, or smart watches to send text messages containing questions or answers to other students.
- Students may attempt to alter an in-lab quiz or practical exam by moving a microscope stage or pointer, by moving a pin or marker in a specimen, or by damaging a specimen during the quiz or exam.

Helpful Suggestions

How to Avoid Suspicion

- Keep your eyes on your own work. Do not attempt to look at another student's answer sheet during an in-lab quiz or practical exam. Do not allow your eyes to wander during wait times, as it will look suspicious.
- Do not attempt to use any non-approved resources during an in-lab quiz or practical exam. During the post-lab quizzes and practical exam, students are allowed to use one hand-written page of notes. Students are not allowed to use printed photos, books, or any typed materials.
- Do not attempt to use any electronic devices during an in-lab quiz or practical exam. This includes (but is not limited to) phones, tablets, laptops, smart watches, etc. Your TA will ask you to put away all electronic devices before each in-lab quiz or practical exam. If you are a student who requires the use of an electronic device as part of a disability accommodation, please make sure that you discuss this accommodation with your TA and the lab coordinator in advance of any in-lab quizzes or practical exams.
- Do not in any way attempt to modify an in-lab quiz or practical exam by manipulating questions or specimens. If you think you may have accidentally changed something (for example, if you have accidentally moved the stage on the microscope and the pointer is no longer pointing at the original structure), please alert your TA immediately so the issue can be resolved before another student views the material.

How Academic Dishonesty is Handled in Biology 2120L

All Biology 2120L Teaching Assistants are given instructions to report any suspicions of academic dishonesty to the Lab Coordinator and not to handle the situation on their own. This is done to make sure that all similar cases within Biology 2120L are handled consistently, regardless of the TA.

- The Lab Coordinator and Faculty in Charge look at each reported case to determine if the suspicions are warranted.
- If the teaching team agrees that academic misconduct might be the cause of the suspicions, the Lab Coordinator will reach out to all of the students involved to alert them to a concern about one of their assignments and to request a meeting with the students.
- Students involved will meet with the Lab Coordinator and/or Faculty in Charge to discuss the concern. Students will be given an opportunity to share their perspective on the assignment in question.
- After the meeting, the teaching team will make a determination about how to handle the case, following University guidelines. The Lab Coordinator will contact the students involved to provide them with information about the decision and any instructions that are needed. Often cases are referred to the Dean of Students Office of Student Conduct for adjudication.

LABORATORY 1.

NATURE OF SCIENCE

Objectives

Following this week's lab, students will be able to:

- Describe and follow lab and safety protocols
- Ask scientific questions and create hypotheses
- Identify independent and dependent variables and controls in experiments
- Identify the major parts of a scientific article, describe the functions of each selection, and be able to properly cite a primary research article

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

1. Freeman *et al.* 2024. Biological Sciences (8th ed.), pp. 10–14 (Chapter 1.6).
2. Video and information links on course canvas page.
3. Voves, K.C., T.S. Mitchell, and F.J. Janzen. 2016. Does natural visual camouflage reduce turtle nest predation? *The American Midland Naturalist*. 176 (1): 166-172.
4. Citations Interchapter, pp. 17–20.

What is Science?

Inspiration for scientific discovery can come from a variety of places. Scientists are curious people who observe something they find interesting, ask questions about what they've observed, and then dive deeply into the subject to learn as much as they can.

Scientists follow a particular method for asking questions and designing experiments:

1. Ask a question.
2. Conduct background research (has someone else already answered this question?).
3. Use the initial questions along with what was learned from the background research to construct a hypothesis.
4. Design an experiment to test the hypothesis.
5. Carry out the experiment (often requires troubleshooting along the way).
6. Collect and analyze the data from the experiment.
7. Draw conclusions (do your data support the hypothesis?).
8. Compare experimental results to others published from similar research.
9. Communicate results to others, ideally in a peer-reviewed publication.
10. Very often, following this process, scientists come up with many new questions. What is learned in one experiment is often incorporated into the background information used to design new experiments to help the researcher answer new questions.

In today's lab, we will be closely examining the process of science to learn how to ask good questions, develop a hypothesis, design an experiment, and read scientific literature. These steps are where the scientific process begins – and much like learning how to use scientific equipment properly, they are important components that need to be learned and practiced. In the next few labs, you will utilize these basic scientific skills repeatedly. Taking the time to learn the foundations now will set you up for a successful semester. Today in lab you will get an opportunity to practice some of these steps and to get some feedback from your classmates and your TA. This lab will require you to work closely with a lab partner to discuss questions, brainstorm ideas, and work through some parts of the scientific process. Don't be afraid to ask questions and show your curiosity!

Step 1 – Ask a Question

The scientific process starts when a scientist asks a question. Inspiration for these questions can come

from anywhere (wondering why different people like different foods, watching animals interact with each other, observing human behavior, wondering why leaves change color in the fall, etc.). To help you start thinking creatively, we have selected a few short videos for you to choose from. Watching these videos will lead you to come up with some interesting questions! Use the links on canvas or use one of the lab laptop computers and find the folder labeled Week 2 Videos inside the Biology 2120L folder on the desktop. This file contains four short videos. Select one of the four (described below) that you and your lab partner find most interesting and watch the video in its entirety. After watching the video, answer the questions below.

a. **Termite World** (a video clip about why termite mounds look the way they do)
b. **Butterfly Eggs and Caterpillar Survival** (a video clip about the interactions between a species of butterfly, ant, and wasp)
c. **Venus Fly Traps** (a video clip about how some carnivorous plants lure and trap their prey)
d. **Crab Shell Exchange** (a video clip showing how hermit crabs find new shell homes)

1. Which video did you choose?

2. What did you find most interesting about the video?

3. What is one new thing you learned from watching the video?

4. How could you learn more about the topic of this video?

5. Based on what you learned in the video, write down two research questions that you would be interested in learning more about.

6. Could the questions you asked above be investigated and tested (provided you had unlimited time and resources)? How would you test them?

Step 2 – Develop a Hypothesis

Now that you know how to ask a question, let's work on how to develop a hypothesis. Having a clear hypothesis is essential to designing an effective experiment. As is the case in many scientific endeavors, there is some important terminology to familiarize yourself with in order to successfully understand this portion of today's lab. Match the list of terms below with their definitions.

- Control Group/Treatment
- Experimental Protocol
- Hypothesis
- Dependent Variable
- Experimental Group/Treatment
- Independent variable

_____: A statement declaring a tentative answer to a question based on observations in nature. It often includes predictions based on stated assumptions so that it can be tested.

_____: A factor or condition that is changed by the scientist.

_____: A factor or condition that is observed and measured by the researcher, to see how it changes in response to a variable manipulated by the scientist (see above definition).

_____: A step-by-step description of how the experiment will be run so that other scientists could replicate it.

_____: Treatment in the experiment in which the scientist has changed the independent variable to measure an effect.

_____: Treatment in the experiment that the scientist intends to compare to the other treatments to test if there is a real effect of the independent variable. Determining this treatment is straightforward in some experiments and can be difficult in others, depending on the protocol and variables. Sometimes a reference standard is used.

When you have finished the matching activity, your TA will provide you with a question card that you can use to complete the rest of this activity. This activity is designed to help you learn about the structure and writing of hypotheses. For the purpose of this exercise, background information should be limited to what you already know about the subject of your question or a very brief Internet search on the subject. When you develop hypotheses in the future, you will be expected to complete background research to help educate yourself in the effort of writing an effective hypothesis. Read your question carefully, and then use it to complete the following activity:

1. Re-phrase the research question you've been provided into a hypothesis. Note that hypotheses are typically formatted in the following way:

   "If *[I do this]*, then *[this will happen]*."

   This allows you to pose a question and make a prediction. Your prediction should not be open-ended. If you expect something to change, it is important that you describe in your hypothesis how you expect it to change. Simply stating something will change is not sufficient.

2. If you were to carry out an experiment to test this hypothesis, you would need to develop a protocol. Prepare a brief protocol that you could use to carry out this experiment.

3. What would be your **independent** variable?

4. What would be your **dependent** variable?

5. What would be your **control**?

6. Why is a control necessary?

7. What kind of data would you need to collect in order to test your hypothesis?

8. How would you analyze your data in order to determine the results of your experiment?

9. What do you predict would be the outcome of your experiment?

Step 3 – Understanding Scientific Literature

This section of today's lab activity will help you to navigate reading and understanding primary scientific literature. One very critical step in the scientific process is to clearly communicate findings to other scientists and to the public. Scientists follow a rigorous process of publishing their work in peer evaluated scientific journals. That means that other scientists evaluate their work before the information is published and presented to a wider public audience. This evaluation helps determine if the methods used in the study are sound and makes sure there was not any bias inherent in the design of the experiment. Peer scientists are able to ask questions and request additional information about the study. Articles that do not pass the robust review process are not published. It is important to note that every journal has its own review process and its own set of standards required for publication.

We have selected a journal article for you to read and evaluate for this part of the lab activity. The article we selected was authored by researchers here at Iowa State University. This is one example of great research being conducted at your university! This particular article was selected because it is straight-forward and easy to read, which makes it a good way to begin learning how to read scientific literature. Learning to read primary literature for the first time can be challenging! It can take a lot of practice to feel comfortable. Take your time reading, make sure you follow the steps below, and ask your partner and TA any questions you have.

The article we've chosen is: "Does Natural Visual Camouflage Reduce Turtle Nest Predation?" by Kameron C. Voves, Timothy S. Mitchell, and Frederic J. Janzen.

The full citation for this article is:

Voves, K.C., T.S. Mitchell, and F.J. Janzen. 2016. Does natural visual camouflage reduce turtle nest predation? *The American Midland Naturalist*. 176 (1): 166-172.

This article has been placed onto the desktop of the laboratory laptop computers in the Nature of Science folder and can also be found on Canvas. Open the article to read and discuss it with your lab partner. Follow the activity below as you read through each section of the paper and answer the associated questions.

1. Read the Introduction

- Identify the hypothesis in this paper. Write it below.

- What organism was at the focus of this study?

- What is the meaning of the word *crypsis*?

2. Read the Methods

- What is the meaning of the word *depredated*?

- The authors of this study recruited a number of "observers" to rate photographs of nests. What criteria did they use to select these observers?

- Why is it important to have more than one observer?

- Why is it important for the scientists to include lots of detail in their methods section?

- What kinds of data did the scientists collect?

3. Read the Results

- How many total turtle nests were included in the study?

- Of those, how many remained intact?

- How many were depredated?

- What was the predation rate?

4. Read the Discussion

- Did the researchers' results show that camouflage was a predictor of nest survival? Were more camouflaged nests less likely to be predated?

- The authors of this study provided a list of factors beyond nest cues that may have had an effect on painted turtle nest predation. List 2 of these factors in the space below.

- Did the results of this study support the authors' predictions? Did the results support their hypothesis, or force them to reject their hypothesis?

- If the data collected by a researcher forces them to reject their initial hypothesis, does that mean their research was "bad"? "unnecessary"? "worthless"? Why or why not?

- Can valuable information be obtained from studies in which hypotheses are rejected?

5. Look over the References

- How many references did the authors include in their paper? Why is it important to include so many?

- If you wanted to learn more about a different study that the authors mention in the intro (like Spencer and Thompson, 2003), how would you do so?

- Why is it important to cite primary literature? What is primary literature?

6. Citations

The Literature Cited section at the end of a paper is very important. It provides you with all of the details you would need to find the sources referenced within the paper. If you wanted to find out more about one of the studies the authors referenced, you can look it up and read about the study yourself. For more information on citations, read the Citations Interchapter on pages 17-20.

7. Read the Abstract

- What is the purpose of having an abstract?

- Often scientists write the abstract last, why do you think that is?

8. Reflect on the Content

- What is one question that you still have after reading this paper?

- What was the most confusing part of this paper for you?

Step 4: Applying What You Learned

Now that you've learned a bit more about the process of science, it is time to start putting your new skills to work! One of the goals of Biology 2120L is to have students practice all steps of the scientific method over the course of the semester while conducting several experiments. These experiments will be done in a group, which is intended to help you learn yet another aspect of the scientific process – collaboration. Most scientists do not conduct their research on their own – science requires people to work together to come up with their best work. Sometimes scientists work with other researchers in their own laboratories, but often those collaborations are with researchers who may be hundreds or thousands of miles away at another university or research facility, perhaps even in another country. As a result, learning to navigate how to conduct a research experiment with others is an important skill.

In addition to learning how to conduct experiments with others, it is also important to learn how to examine the literature to determine what other researchers have learned from similar experiments. Before scientists begin their research, they spend time reading through scientific papers to determine what other scientists

have already done. They want to know if the experiment they want to conduct has already been done, and what the findings were. They want to know if there have been other studies with results that may contribute to the knowledge needed to design and conduct a new study to address a new question. Learning about what has already been done helps inform scientists as they form hypotheses and design new experiments.

It is important when gathering information that scientists use reliable sources they can trust. Scientists publish their work in scientific journals, which conduct a peer review before publishing any new work. That means that fellow scientists evaluate the paper, providing comments and feedback to the authors so they can make edits, provide clarification, or conduct additional studies before their work is approved for publication.

As part of a group project you will work on this semester, you will need to find peer-reviewed literature relating to your project that you will use as sources of background information. It will be a good idea to start on this early, so you have plenty of time to locate good sources of information that you can use later in the semester. Spend some time checking out the university library website and/ or Google Scholar to learn how to search for scientific papers.

1. What is a scientific journal? How do scientists publish in a scientific journal?

2. What is peer review?

3. Why should this process produce more reliable information than other information available on the Internet?

This semester, you will be assigned to a small group to work on a research and presentation project. For this project, you and your group will be assigned one of the topics of the class experiments in the next few weeks. Besides planning out and performing the experiment for the week that you are assigned, you will also research that topic in the scientific literature and present on it to the class. You will give a short class presentation on your experiment during Week 8. This will allow you to practice the scientific process from start to finish.

INTER-CHAPTER 2.

CITATIONS

Citations are important in that they give due credit to the original authors and also help to locate the article. Journals use their own preferred method for citations. It is important that authors learn the importance of each element of the citation and how it should be formatted for the specific journal for which they are contributing as it varies. There are many different citation formats, but they all contain similar components. The differences are in how each of the elements is presented. In this inter- chapter, we will be providing you with instructions for a citation format called Council of Science Editors (CSE) formatting that we want you to use for all citations in Biology 2120L. We've selected this format because it is fairly straight-forward and does not include any extra punctuation or formatting that could potentially complicate your citations. It is important that you learn the basics here so you become familiar with each element of the citation, paying attention to the subtle details of the formatting. If you ever submit a paper for publication, you'll need to be able to modify your citations to match the formatting for the journal in which you hope to publish.

Citations – A Version of CSE Format

To help familiarize you with the formatting that we expect you to use in Biology 2120L, we will use the journal article that you read during Week 2 of the lab to provide you with examples.

Literature Cited (Bibliography)

Note that **ALL** authors are listed in the bibliography.

Voves KC, Mitchell TS, Janzen FJ. 2016. Does natural visual camouflage reduce turtle nest predation? The American Midland Naturalist. 176(1): 166–172.

In the example above, we have color-coded each element of the citation to help you identify each important piece of information.

- **Green** – Author last name then first and second initials (may only use first initial if second isn't given). Commas only between authors with a period following the last author. No periods are used with the initials for the first and middle names.
- **Black** – Year published followed by a period.
- **Blue** – Name of the paper, article, etc. followed by a period or question mark (scientific names of organisms should be correctly formatted).
- **Orange** – The name of the journal where the paper was published followed by a period.
- **Purple** – Volume(edition) followed by a colon.

- **Red** – Page numbers followed by a period.

When the citation is included in your literature cited, references, or bibliography, it should appear as follows (without the color coding):

Voves KC, Mitchell TS, Janzen FJ. 2016. Does natural visual camouflage reduce turtle nest predation? The American Midland Naturalist. 176(1): 166-172.

In-Text Citations

When you reference your sources within the text of your paper, it is important that it is clearly identified so your readers can find the resource by looking at the literature cited section of the paper. In-text citations provide your readers with enough information to find the full citation in your literature cited section without taking up valuable space within the text of your paper. In this case, only the author and year are necessary. Follow the examples below to determine how to complete in- text citations based on the number of authors.

- If there is only **one author** on the paper, give the last name and year in parentheses, no punctuation. (Voves 2016)
- If there are **two authors,** give last name and last name and year in parentheses using the conjugate "and", no punctuation. (Voves and Mitchell 2016)
- If there are **more than two authors,** give the last name of the first author followed by et al. then the year, with the only punctuation being the period after et al. (Voves et al. 2016). The example article above would be correctly cited using this format.

Primary Sources

Many times you will find a journal article in a database that is online, such as PubMed.gov. This is not the article's original journal publication. You may need to search a bit further for the complete reference information. Usually all it takes is to open the full text.

- Found the article on: https://www.ncbi.nlm.nih.gov/pubmed/16492480
- Click on the full text link and it will take you to: https://www.sciencedirect.com/science/article/pii/S0083672905720148
- Here you can find all the information you need as well as the content of the entire article.

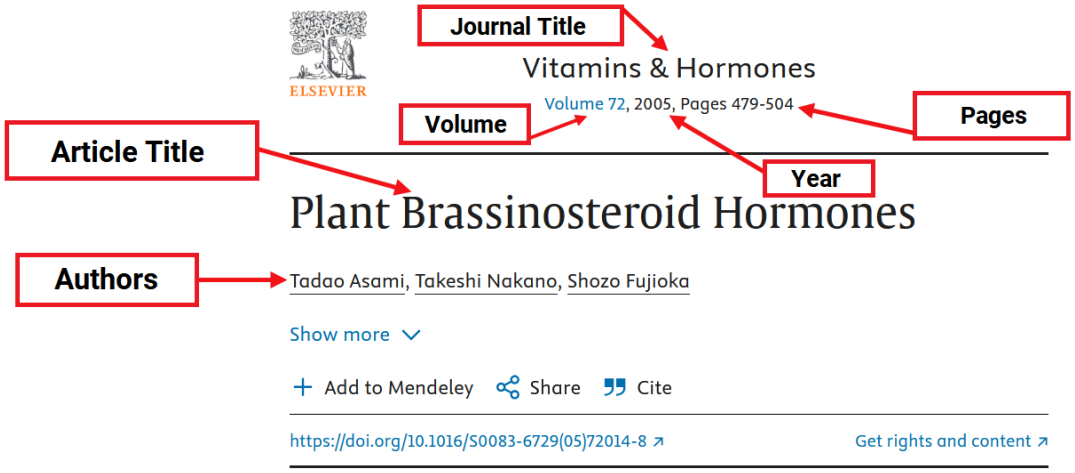

Figure IC2.1

How to Find Scientific References

It is easy to use an internet search engine to locate scientific references, but such a search can produce a lot of hits that are not part of the formal literature of science. For example, a google search on the plant hormone auxin provided links to more than 1.8 million sites; 16 of the first 20 were not part of the peer-reviewed, original scientific literature.

Searching scientific databases will filter out most non-scientific sites. To accurately look for a peer reviewed scientific article, you may want to look into Google Scholar, PubMed, Medline and Web of Science. For example, PubMed (http://www.ncbi.nlm.nih.gov/pubmed) is a US government database that indexes biomedical literature, including much biological research that is not immediately relevant to medicine. Searching for auxin on PubMed produced about 17,000 references, most from the peer-reviewed literature of science. One advantage of databases like PubMed or Biosis Previews (see below) is that they will often allow you to view the **abstract** (a short summary written by the authors) of each article, so you can get a good idea of the article's contents before you go further and read more of the article.

Using the ISU Library

Once you locate an article you want to read using Google Scholar or PubMed, you will often discover that you cannot get the whole article, or that a publisher asks you to pay for the privilege. Using the ISU library's links will allow you to access many of these articles for free. Working through ISU's library link (www.lib.iastate.edu) gives you free access to a great deal of scientific literature. Entering auxin into the library link's Quick Search box produced more than 30,000 references. Of those, 29, mostly books, are available in the library; 25,000 were from peer-reviewed journals, and full text for most was available on-line. Clicking a "Show only Peer-Reviewed Journals" button narrowed the list. Clicking on the title of one of those articles often takes you to the article or to a site where you can view and download the article for free.From ISU's library link page you can link to other pages that permit you to search a variety of biological databases.

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LABORATORY 2.

QUANTITATIVE TECHNIQUES AND ORGANIC MOLECULES

Objectives

After the week 2 lab, students will be able to:

- Demonstrate proper use of common laboratory equipment, including balances, pipettes, dispensers, and spectrophotometers
- Create a standard curve and use it to identify unknown concentrations of compounds
- Perform simple data analysis and data visualization techniques
- Identify bonds and functional groups of organic molecules and describe how they influence chemical behavior
- Make connections between the structure and function of monomers and the macromolecules they form

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks during this week's lab to earn Contribution Points. I am aware that point(s) may be deducted if my workspace is not appropriately clean at the conclusion of lab.

Introduction

During today's lab, your objective will be to learn how to use many of the common pieces of lab equipment that you will utilize repeatedly in this course including balances, micropipettes, repeating pipette dispensers, spectrophotometers, and some of the software available on the lab computers. There are 4 different stations set up in the classroom. Each one is designed to teach you how to use a particular piece of equipment.

There are **instructions** at each station that will provide you with the information that you need to complete the activity. There are also **video tutorials** on the laptop computers that provide you with instructions on how to use the equipment. Please **watch the video tutorial(s)** carefully before beginning each exercise; then follow the directions to complete the activity. Complete the data tables and answer questions in your lab manual as you complete the lab activity. You will utilize this information to complete the post-lab assignment on Canvas.

Each station is set up with multiple copies around the lab classroom. Feel free to start at any station you would like. You do not need to wait in line to complete these in a specific order.

NOTE

Data from Station D needs to be collected before the last hour of class so that class data can be compiled and posted for your data analysis.

Station A: Balance Station

Not all pieces of equipment (even if they are the same model) will work exactly the same with every use. Many factors (general usage, wear and tear, improper usage, etc.) can impact that equipment's ability to function properly. In order for a balance to work most effectively, it should be calibrated carefully. However, calibration takes time, skill, and isn't always possible if you don't have all the necessary tools available. The three balances in front of you have all been calibrated at some time in the past but may no longer be reliably accurate. They will all provide a mass for the objects you place on them, but the mass they report may have a degree of error, both within each balance and between balances. It will be your job to determine how much error is present in the readings you obtain from the three balances at this station.

1. Please watch the video tutorial in order to learn how to use the balances.
2. Choose two objects to weigh from the selection provided. You will need to choose one "heavy" object and one "light" object.
3. Record the identity of each of your objects at the top of ***Table A.1***.
4. Weigh each of your selected objects on each balance at this station 3 times and record your data in ***Table A.1***. Remember when you use a balance that you need to "tare" it with an empty weigh boat before taking EACH measurement. This removes any stored measurements from the balance so it will start its measurements from "0", making sure to remove as much error as possible.
5. After you have 3 measurements for each object from each balance, calculate the average mass for balances 1, 2, and 3 for both the "heavy" and "light" object in ***Table A.1*** and in ***Table A.3***.
6. When you finish weighing your objects, please return them to the appropriate tray.

Station A: Balance Station

Complete this worksheet as you work through the activities in the first week's lab. You can work in pairs but the answers to each question should be in your own words.

Light Object:

Heavy Object:

Table 2.A.1. LO = Light Object; HO = Heavy Object

	L.O. Mass 1 (g)	L.O. Mass 2 (g)	L.O. Mass 3 (g)	L.O. Avg Mass (g)	H.O. Mass 1 (g)	H.O. Mass 2 (g)	H.O. Mass 3 (g)	H.O. Avg Mass (g)
Balance 1								
Balance 2								
Balance 3								

Table2.A.2.

	Light Object	Heavy Object
Expected Mass		

Table 2.A.3. LO=Light Object; HO=Heavy Object

	L.O. Average Measured Mass (g)	L.O. % Error (absolute value)	H.O. Average Measured Mass (g)	H.O. % Error (absolute value)
Balance 1				
Balance 2				
Balance 3				

Analysis

Now that you have the mass of your objects, you will need to complete some simple statistics to determine if the mass you measured is what would be expected for each item. This will provide you with information about the accuracy of each of the balances you used. At the balance station there is a folder or flip card that contains the expected mass for each of the available items. Locate the information for the two items you selected and record their expected masses in ***Table A.2***.

To calculate the % error of your measurements from each balance, use the equation below. Fill out ***Table A.3*** to determine the percent error of each balance. **Note**: % error is an absolute value.

$$\%\text{error} = \frac{\text{measured value} - \text{expected value}}{\text{expected value}} \times 100$$

Which item (light or heavy), has a greater % Error? Why might this be the case?

Which balance has a greater % Error? Why might this be the case?

Station B: Pipette Station

Micropipettes are an important piece of lab equipment that you will utilize repeatedly during the course of the semester. They are also often used incorrectly by students, which can result in damage to the pipette and/or the dispensation of inaccurate volumes which can cause your experiment to fail. It is important that you learn how to effectively use a micropipette during today's lab so you can use them in future labs without error. Carefully watch the video tutorial on one of the lab laptop computers. Once you have watched the video, proceed with the activity below.

Volume Conversions
1 Liter (L) = 1000 milliliters (mL)
1 milliliter (mL) = 1000 microliters (µl)

Micropipettes come in a multitude of sizes. Each size of micropipette will dispense solution in a specific

range of volumes. It is important that you use the pipette correctly and do not attempt to dispense volumes that are outside its stated range. There are two different pipettes at this station, one that will dispense volumes under one milliliter (range dependent on model of micropipette) (small pipette) and one that will dispense volumes between 1 milliliter and 5 milliliters (large pipette).

One way to determine whether or not a pipette is dispensing the desired volume accurately is to use a balance to weigh the dispensed solution (we will use water), then use the expected weight of the volume of solution to check the accuracy.

We can figure out the expected weight of a specific volume of water because we know that the density of water at room temperature is 0.998g/mL. Since **Mass = Density × Volume**, 1.0 mL of water at room temperature would be expected to weigh 0.998g.

Your TA will have a cup on the TA bench that contains various cards with volumes written on them. Draw one paper from the Station B cup and record the volumes in the spaces provided on the assignment worksheet under Station B. Use these volumes for the activity at this station. Once you have recorded the volumes, please place the paper in the "Used Volumes" container.

DO NOT try to set the pipette to dispense a volume outside of its recommended range. Doing so may damage or destroy the pipette.

1. At this station, you will find two pipettes (one that dispenses volumes in a range of 200 µl to 1200 µl (0.2 mL to 1.2 mL) and another that dispenses volumes in a range of 1 mL to 5 mL, a balance, a small beaker, a large beaker of water, and pipette tips in two sizes.
2. Select the **small** (0.2 mL to 1.2 mL) pipette and set it to dispense the volume that you selected from the cup on the TA bench.
3. Place the small beaker onto the balance.
4. "Tare" the balance so it reads "0.0 grams". "Taring" the balance removes any stored measurements from the balance so it will start its measurements from "0", making sure to remove as much error as possible.
5. Place a **blue** pipette tip onto the pipette and carefully draw the designated volume of water into the tip.
6. Dispense the water into the beaker on the balance. Be careful not to apply too much pressure on the pipette when you draw and dispense the solution. Record the mass of the dispensed volume in first column in ***Table B.1*** as "measurement 1".
7. "Tare" the balance so it reads "0.0 grams". As long as you "tare" the balance between measurements, you do not need to dump the old water out.
8. Again, carefully draw the designated volume of water into the pipette tip and dispense the water into the beaker on the balance. Record the mass of the dispensed volume in ***Table B.1*** as "measurement 2".

9. Repeat steps 6 – 8 two additional times (making sure to "tare" the balance between each measurement) for measurements 3 and 4 so you have a total of 4 measurements. Make sure to record all measurements in ***Table B.1***.
10. Eject the pipette tip and return it to the correct location at your station.
11. **Repeat all of the above steps using the large (1 mL to 5 mL) pipette** so you have 4 measurements for each of your pipettes. Make sure you use the **natural** colored tips for this pipette.
12. Once you have all of your measurements, calculate the average mass dispensed for both the small and large pipettes in the shaded row of ***Table B.1*** and the second row of ***Table B.2***.

Station B: Pipette Station

Assigned Volume for 200 µl to 1200 µl (0.2 mL to 1.2 mL) Pipette:

Assigned Volume for 1 mL to 5 mL Pipette:

Table2.B.1.

Measure	Small Pipette Mass of dispensed volume (g)	Large Pipette Mass of dispensed volume (g)
Measurement 1		
Measurement 2		
Measurement 3		
Measurement 4		
Average		

Now that you have your measured values, do you notice any differences between them? Are all of your measured volumes for the small pipette the same?

Are all of your measured volumes for the large pipette the same?

Why might there be differences?

Analysis – % Error

To determine how accurate your pipetting is, you will use some simple statistics to calculate how much difference there is among your measured volumes. To do this, you should determine the expected mass for each of your volumes using the equation below. Record the expected mass for each volume in the first row of ***Table B.2***.

The expected mass of the volume of water you dispensed can be calculated using the equation below. The density of water at room temperature is 0.998g/mL.

Expected Mass = Density × Volume

Calculate the % Error for each of your measurements and record them in the last row of ***Table B.2***. **Note**: % error is an absolute value.

$$\%\text{error} = \frac{\text{measured value} - \text{expected value}}{\text{expected value}} \times 100$$

Table2.B.2.

Measure	Small Pipette	Large Pipette
Expected mass of assigned volume (g)		
Average measured mass (g)		
% Error		

Which pipette (small or large) had larger % Error? Why might this be the case?

Station C: Dispenser Station

During the course of the semester, you will find it necessary to dispense varying volumes of solutions in order to complete lab experiments. Our labs utilize a number of different dispensing options throughout the semester, so it is important that you gain some experience with each type of dispenser so you know how to use it when you encounter it in lab.

When having to dispense the same amount of solution multiple times (or by multiple people), it is often helpful to utilize a repeating pipette dispenser (especially if it is important that the solution amounts be exact each time). This dispenser consists of a bottle with a pipetting mechanism at the top which draws up the solution in the bottle to dispense it into the receptacle of your choice. We have varying types of repeating pipette dispensers in the lab, though they all function in the same manner.

Another dispenser you may encounter in lab is a squeeze bottle measuring dispenser. In this type of dispenser, a small measuring cup sits on top of a tube that runs inside a bottle. When you gently squeeze the bottle, solution is pushed up into the measuring cup. When you release the bottle, any excess solution drains back into the bottle and the desired volume of solution can be poured directly into the receptacle of your choice.

Please watch the video tutorial available on the laptop computers before doing the activity in order to learn how to use all dispensers correctly.

Each of the dispensers has been carefully set to dispense a specific volume for this exercise. In fact, each time you see a dispenser in lab, it will have been carefully set to a specific volume for your lab exercise. **Never change the settings on the dispensers!** If you notice that the dispenser is not dispensing the correct volume, consult with your TA so he or she can reset it for you.

All of the dispensers contain a solution dyed with red food coloring that will make it easier for you to see as you complete this exercise. One of the dispensers is set to dispense 1.0 mL of solution, another to dispense 5.0 mL of solution, and a third to dispense 9.0 mL of solution. In addition to the dispensers, there are several measuring devices available to you at this work station. You will find beakers, graduate cylinders, and #asks (in multiple sizes) for you to use as you complete the exercise.

To help you make comparisons in the lab, we have also placed a bottle of solution at the station which you will use to pour 7 mL of solution directly into a receptacle.

1. Select any three **different** measuring devices (beakers, graduate cylinders, #asks) from the choices available to you at this station. You will use the same three devices for all of your measurements.
2. Using the dispenser set to 1.0 mL, dispense 1.0 mL of solution into each of the 3 measuring devices.
3. Set them side by side and visually compare the volumes using the gradations on the device. The same amount of solution has been dispensed into each of the three devices (as assured by using the dis-

penser), but does it appear as though each device has the same amount of solution?

4. Dump the solution from the three measuring devices into the waste container at the station.
5. Dispense 5.0 mL of solution into the same three measuring devices using the dispenser set to 5.0mL at the station.
6. Place them side by side and compare them. Again, these measuring devices should all contain the same amount of solution. Do they appear as though they all contain the same amount?

7. Dump the solution from the three measuring devices into the waste container at the station.
8. Dispense 9.0 mL of solution into the same three measuring devices using the dispenser set to 9.0 mL at the station.
9. Place them side by side and compare them. Again, these measuring devices should all contain the same amount of solution. Do they appear as though they all contain the same amount?

10. Dump the solution from the three measuring devices into the waste container at the station.
11. Now you will try simply pouring out the solution into your measuring devices. Using the bottle of solution at the station, pour out 7.0 mL of solution into each of your three measuring devices. Did you have any difficulty pouring out the correct volume into each measuring device?

12. Place them side by side and compare them. Again, these measuring devices should all contain the same amount of solution, but it may have been more difficult for you to determine when you had poured out the correct amount, depending on the measuring device. Do they appear as though they all contain the same amount?

13. Dump the solution from the three measuring devices into the waste container at the station.

Station C: Repeating Pipette Dispenser Station

At this station, you dispensed 3 volumes (1.0 mL, 5.0 mL, and 9.0 mL) into 3 different measuring devices and made visual comparisons of each of these volumes.

> After dispensing the designated volumes from each type of dispenser into each of the measuring receptacles you made visual comparisons of the solutions in each receptacle. Did it appear as though they contained the same amount of solution? Why or why not?

After using the dispensers, you were asked to pour 7.0 mL of solution directly into your 3 measuring devices.

> When comparing the 3 devices side-by-side, do they appear as though they contain the same amount of solution? Why or why not?

> Based on your experience at this station in lab, which option for dispensing solution would you prefer to use in future labs? What reason(s) do you have for selecting this option?

Based on your observations at this station, which of the available dispenser options provides you with the most accurate and consistent volume when dispensing liquids? What evidence do you have to support this observation?

Station D: Spectrophotometer Station

NOTE

Data from Station D needs to be collected before the last hour of class so that class data can be compiled and posted for your data analysis.

A spectrophotometer is a machine that uses light absorbing properties to identify and quantify colored solutions. You will use the spectrophotometer repeatedly throughout the semester in Biology 2120L. Having an understanding about how a spectrophotometer works will be beneficial in helping you interpret the data it produces.

To help you understand how a spectrophotometer works, watch the spectrophotometer video linked on the course Canvas page.

To help you understand how to use the specific model of spectrophotometer used in our labs, please watch the video tutorial on one of the lab laptop computers. Once you have watched the video, you can begin the activity designed for this station.

When using a spectrophotometer, you can collect data in either percent transmittance (which is reported on a scale from 0 to 100), or absorbance (which can range from 0 to 2.5). The relationship between light absorbance and transmittance is shown in the equation below.

$A = \log_{10}(1/T)$

A = Absorbance

T = Transmittance

Because light absorbance units are directly and linearly related to the concentration of the substance(s) in solution (the higher the concentration the higher the absorbance), we prefer to collect our lab data in absorbance units. This direct and linear relationship between solution concentration and light absorbance units is known as the Lambert-Beer Law.

For this exercise, we will be using a dye called bromophenol blue. This dye is often used as a color marker in agarose gels and as a pH indicator. For our purposes, it is a dye that changes the color of solution—the higher the concentration of bromophenol blue, the darker the color of the solution.

We have prepared a set of cuvettes (or tubes) for you to use in your investigation today in lab. These cuvettes contain bromophenol blue solutions in varying concentrations.

1. Each student in your lab section will be given a cuvette containing a bromophenol blue solution of a specific concentration.

2. Each cuvette is labeled only with a sample number. It will be your job to obtain the absorbance of that sample's solution and report it on the class data sheet.
3. Follow the instructions from the spectrophotometer video to collect your measurements.
4. Record your cuvette sample number and the absorbance of this sample at six wavelengths in ***Table D.1***.
5. Remember that you need to "blank" the spectrophotometer each time you change the wavelength.
6. At each wavelength, record the color of light used in the spectrophotometer. You can do this by inserting a narrow strip of white paper into the sampling chamber and looking at it with your hands cupped to block out light from the lab. Record the color you see at each wavelength in ***Table 1.D.1***. **Note:** If you are using the Genesys30 Spectrophotometers, you will need to blank the spectrophotometer at each wavelength **before** you will be able to view the light color.

Table 2.D.1

Wavelength	Light Color	Absorbance for Sample Cuvette #_____	Absorbance for Sample Cuvette #_____
440			
540			
580			
590			
600			
660			

7. After you are done, return the cuvettes to the box labeled USED CUVETTES on the TA bench. **DO NOT** dump these out.
8. We have already taken a careful look at the absorbance properties of bromophenol blue and have created an absorbance curve (below) that shows the absorbance of a solution of bromophenol blue (concentration of 0.02 mg/mL) at a series of light wavelengths. You can see from this graph that the wavelength that provides the greatest absorbance is 590 nm. In order to get the most reliable results for studying changes in bromophenol blue concentrations, it will be important to choose this wavelength for measuring your sample.

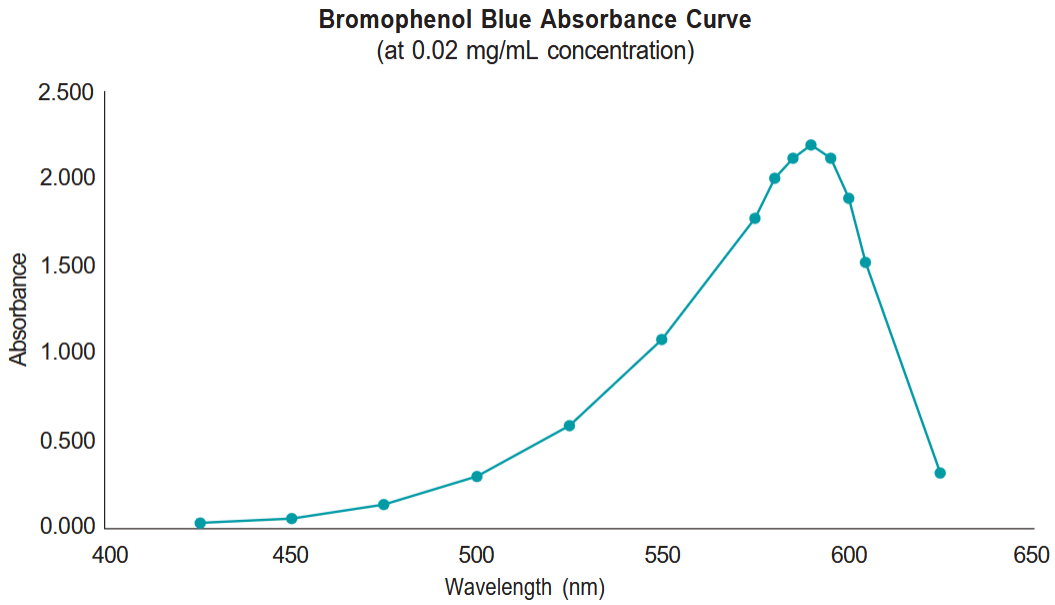


Figure 2.1. Bromophenol Blue Absorbance Curve(at 0.02 mg/mL concentration)

9. Report the measurement of your sample at 590 nm to your TA. They will compile a class data sheet with all of your classmate's measurements and you will use this data to create your graph.

Graph Your Data

Once all of the students in your lab section have obtained their sample's absorbance, your TA will compile the class data file and make it available to you via the projector. **YOU CAN NOT MAKE THE GRAPH UNTIL THE CLASS DATA IS PROVIDED BY YOUR TA**. Open the Excel template labeled "Week 2 Lab Template" on one of the lab laptop computers and use the data from the class data sheet to work through the Excel instructions in ***Appendix: Ch. 2*** to create a standard curve. Once the graph is complete, move on to the next section.

REPORT YOUR SAMPLE'S ABSORBANCE AT 590 NM ON THE CLASS DATA FILE.

Follow instructions in your lab manual to complete the Excel activity associated with this station.

Note: You will need to upload your Excel file containing your data table and graph on Canvas for credit.

When constructing your graph in Excel, you will add a trendline that is the best fit for your data. Write the equation for this line from your graph on the line below:

Using the equation calculated for you on your graph, calculate the concentration of your "unknown" using the instructions below.

"Unknown" Absorbance:

Calculated Bromophenol Blue Concentration:

Calculating an Unknown Concentration

After you have created your graph, you can use it to make conclusions about additional data points. The graph you created using the instructions in ***Appendix: Ch. 2*** should include an equation for the line that best fits the class data.

This equation is in the format:

$$y = mx + b$$

Where:

- ***y*** = absorbance
- ***m*** = slope of the line (Excel has calculated this for you)
- ***x*** = concentration of bromophenol blue in solution
- ***b*** = the location of the y-intercept of the line (Excel has calculated this for you)

On the TA bench, there will be a small bin labeled "Station D Unknowns" that contains pieces of paper with absorbance readings for bromophenol solutions with unknown concentrations written on them. Draw one piece of paper from this beaker and record your unknown absorbance on your assignment sheet. Return your paper to the "Used Absorbances" cup on the TA bench.

Since your unknown sample contains bromophenol blue, we know that it should fall along the same best fit line as your class data sample, so you can use the equation from your best fit line to calculate this unknown's bromophenol blue concentration by only knowing its absorbance! Record both your unknown absorbance reading and calculated bromophenol blue concentration.

Investigating Organic Molecules

Cells are made up of many organic molecules. These organic molecules have a **carbon backbone** and contain various functional groups. The functional groups influence the behavior of the molecule within the aqueous environment of an organism and the chemical reactions the molecule can participate in. The types of atoms and bonds found in a molecule determines whether it: can participate in hydrogen bonding with water (hydrophilic) or cannot (hydrophobic), can release protons into (act as an acid) or attract protons from (act as a base) solution, or can form bonds with other molecules through condensation reactions to create larger molecules.

When smaller molecular subunits are joined together, they can form **macromolecules**. There are four main categories of macromolecules in cells: **proteins**, **nucleic acids**, **carbohydrates**, and **lipids**. These compounds have different properties because of the arrangement of the carbon atoms and the functional groups in their subunits, which leads to their vastly different functions in the cell.

According to the textbook (Freeman 8th ed, p. 79):

"When you encounter an organic molecule that is new to you, it's important to do the following three things:

1. Examine the overall size and shape provided by the carbon framework
2. Identify the types of covalent bonds present based on the electronegativities of the atoms. Use this information to estimate the polarity of the molecule and the amount of potential energy stored in its chemical bonds.
3. Locate any functional groups and note the properties these groups give to the molecule.

Understanding these three features will help you predict the molecule's roles in the chemistry of life."

This activity is designed to give you practice doing this. It will also familiarize you with the properties of various organic compounds that will be used in future labs, such as proteins (Week 3), carbohydrates (Week 4), and nucleic acids (Week 6). You should observe the models of molecules (water and organic molecules) and fill out the table concerning each molecule's properties and predicted behavior.

To aid you with this, answer these questions first:

1. Which six elements account for 99% of the atoms in your body?

2. Which **four** elements in question 1 have **roughly equal electronegativity**, and therefore would form **nonpolar covalent bonds** with each other?

3. Which **<u>two</u>** elements from question 1 have **greater electronegativity**, and therefore would form **polar covalent bonds** with the elements in question 2?

4. **Nonpolar covalent bonds** make a molecule more (circle one) **hydrophobic / hydrophilic.**

5. **Polar covalent bonds** make a molecule more (circle one) **hydrophobic / hydrophilic.**

Table 2.D.2. Organic Molecules Exercise[1]

Properties	Water	Leucine	Serine	Stearic acid	Phospholipid	Glucose	AMP
Type of molecule	—	Amino acid	Amino acid	Fatty acid	—	Mono-saccharide	Nucleotide
Elements present **(circle all)**	**C H O N P S**	**C H O N P S**	**C H O N P S**	**C H O N P S**	**C H O N P S**	**C H O N P S**	**C H O N P S**
Number of carbon atoms	**0**						
Functional groups **(draw, then find each on the molecule model)**	**none**	**Amino:** **Carboxyl:**	**Amino:** **Carboxyl:** **Hydroxyl:**	**Carboxyl:**	**Phosphate:**	**Hydroxyl:** **Carbonyl:**[2]	**Hydroxyl:** **Phosphate:** **Amino:**
Types of bonds present **(circle all)**	**Polar covalent** **Nonpolar cov.**	Polar covalent Nonpolar cov.	Polar covalent Nonpolar cov.	Polar covalent Nonpolar cov.	Polar covalent Nonpolar cov.	Polar covalent Nonpolar cov.	Polar covalent Nonpolar cov.
Hydrophilic, hydrophobic, or amphipathic?	**hydrophilic**						
Why?[3]	Only polar covalent bonds present, so partial charges would interact with water						
Type of macromolecule it could contribute to? **(circle one)**	**N/A**	Carbohydrate Lipid Nucleic acid Protein	Carbohydrate Lipid Nucleic acid Protein	Carbohydrate Lipid Nucleic acid Protein	N/A (it *is* a lipid)	Carbohydrate Lipid Nucleic acid Protein	Carbohydrate Lipid Nucleic acid Protein

1. Shaded boxes have already been filled out as an example.

2. Looks different in ring.

3. Consider: how many polar vs. nonpolar bonds overall? Are exposed regions nonpolar or partially or fully charged?

Appendix: Ch.2

Using Excel for Data Analysis and Graphing

NOTE

These instructions are for the Excel version installed on the lab laptop computers. If you are using a Windows machine, the online version of Excel, or an older version of Office, these instructions may not be accurate.

Read the following and watch the Week 1 graphing tutorial on canvas to help you make your graph.

Excel is a spreadsheet program we'll use to manage and analyze data. You will be using this program repeatedly throughout Biology 2120L. It is important that you use Excel for the completion of your data tables and graphs in Biology 2120L. Other graphing programs will not be sufficient. If you need assistance locating Excel for your computer, consult with your TA. These instructions are written as a step-by-step guide to help you analyze the spectrophotometer data that you collected during this week's lab, but the instructions can be applied to any type of graphs you make in the future. Feel free to come back to this guide at any time to refresh your skills.

Get Familiar with Excel

1. Grab a lab laptop and turn it on. Locate the "Week 2 Lab Template" file in the Week 1 Folder on the desktop. Use this document to enter your data for this week's lab.
2. An Excel worksheet is arranged by columns, labeled with letters; and rows, labeled with numbers. Each box or cell on the worksheet corresponds to a column/row location. For example, the first box highlighted in the photo on the right, is cell A3. Going down the column the next box is A4. If you go to the right of cell A3, that cell is referred to as B3 and so on.
3. You enter data into Excel by clicking on a cell, then typing in that cell. You can enter a number or text within a cell. To move around in a worksheet, click on a cell. You can also use the arrow keys on your keyboard to move you one cell in the arrow direction.

Spectrophotometer Station: Bromophenol Blue Experiment

Tube	Concentration (mg/mL)	Absorbance @ 590nm
1	0.0025	
2	0.005	
3	0.0075	
4	0.01	
5	0.0125	
6	0.015	
7	0.0175	
8	0.02	

Enter Your Data

1. Take some time to get familiar with the template provided to you for this week's lab. There are columns provided for tube number, concentration, and absorbance. The tube numbers are already provided for you. Your TA will provide you with class values for the concentration and absorbance columns.
2. In cell A2 type your name.
3. In cell C2 type your lab section.
4. Your instructor will project the compiled class data with the values from the spectrophotometer station activity onto the projector screen. Use these numbers to fill in your template.
5. Enter the absorbance of each tube (using the projected class data) starting with tube 1 in cell C10, in the column "*Absorbance*". Repeat for the absorbance of tubes 2-8.
6. Before moving on, double check to make sure that each measurement was entered correctly.

Make a Standard Curve

We will be using the data you entered in the template to create a graph called a standard curve. A standard curve allows us to visualize the relationship between our two variables, concentration and absorbance. Once graphed, we can fit a trendline to the data.

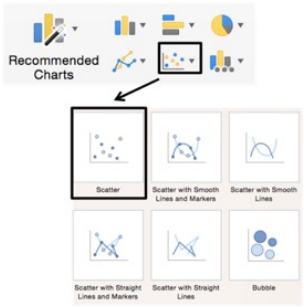


Figure 2.2.

1. Click and drag to highlight the columns "*Concentration (mg/mL)*" and "*Absorbance*". **Do not** include the column "*Tube*." Make sure you include the column titles when highlighting.
2. Click the **Insert** Tab in Excel's Ribbon. Find the section, in the middle of the ribbon, that has chart options. Move your cursor over the different chart types to see the chart descriptions. Choose the **X Y (Scatter)** option and **Scatter** from the drop-down menu as shown in the photo on the right.
3. A graph of your data should appear on your spreadsheet. It may not have a titles or axes labels. Before fixing this, check to make certain the dependent variable (absorbance) is on the Y-axis, and the independent variable (concentration) is on the X-axis.
4. If your graph does not show your axis labels or title, click on the graph. This will open the **Chart Design** tab in Excel's ribbon. Click on the icon called **Quick Layout,** as shown on the right, and choose the first option that includes a title, axis labels, and a legend.

Figure 2.3.

5. Click and drag on a corner of your graph window to adjust it to your desired size. To move the graph to a new location in the Excel window, click and hold anywhere in the white space and your graph outside your grid lines and move it to a new location.
6. Click and drag to select the text in the title box, then type your title. For this graph we can call it *"Bromophenol Blue Standard Curve."*
7. Scientific graphs rarely use gridlines within the graph. Click on one gridline to select all the gridlines, then hit the delete key to delete them. You should do this for every graph you create.
8. The legend Excel provides for this graph is not necessary or informative. Remove it by clicking on it and hitting the delete key.
9. Click on the X-axis title. An axis title box will appear on your graph – click in it and enter the title and units of measurement for your X-axis variable. In this case, *"Concentration (mg/mL)"*. Remember, entering units in your axis titles is extremely important!
10. Click on the Y-axis and enter the variable name and units of measurement for your Y-axis variable. In this case, "*Absorbance*" (which has no units).
11. If you want to personalize your graph by using different colors or changing design options, click on your graph to select it. The **Chart Design** tab will appear in Excel's ribbon. Choose the tab called **Format** to view your options. Feel free to experiment to make the graph your own!

Analyze Data for Best-Fit Line

Take a minute and look at your data points. They should look pretty linear. Use Excel to calculate and draw a best-"t straight line through your data set as follows.

1. Control-click on one of the data point symbols in your graph, then choose **Add Trendline** from the options. A **Format Trendline** window will open.
2. Click on **Trendline Options** and select **Linear**. Excel will use a statistical technique called linear regression to create a straight line that is the best possible fit to your data.
3. Now scroll down in the **Format Trendline** window and check the boxes marked **Display equation on Chart**, and **Display r-squared value on chart**. Those options will give you the equation for your regression line and a statistic called r^2. Display the equation and r^2 value each time you insert a trendline. An

example graph is shown on the right with sample data. This is not necessarily what your graph will look like.

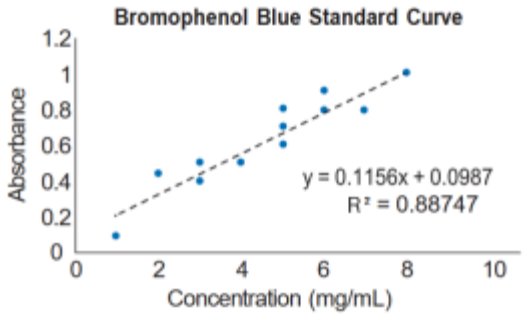


Figure 2.4.

4. The r^2 statistic measures how well the straight line describes your data. It is a number between zero and one that tells you what fraction of the variation in y-axis values you can account for if you know their x-axis values. An r^2 of one means that all the points lie right on the line – if you know concentration, you can predict absorbance perfectly. An r^2 of zero means that there is no linear trend to the data, and knowing concentration will not tell you anything about absorbance. The higher the r^2, the better the line fits the data points.
5. Click and drag to move the equation or r^2 value to the part of your graph that they can easily be seen.

Save Your Graph and Table

1. From the File menu, select Save As. Provide a file name and save the file to the desktop.
2. When the file appears on the desktop, make sure that you email it to yourself or save it on Cybox.
3. Confirm that the file is saved properly so you will have access to it later.

NOTE

You will be required to upload your table and graph file onto Canvas as part of your Week 2 post-lab assignment. The file posted on Canvas must be in Excel format.

LABORATORY 3.

PROPERTIES AND FUNCTION OF AN ENZYME

Objectives

Following this week's lab, students will be able to:

- Describe what an enzyme is and how it works
- Describe the effect of enzyme concentration on the rate of a chemical reaction
- Develop a hypothesis, perform an experiment, and analyze and display experimental data regarding enzyme activity
- Describe how one or more environmental conditions affects enzyme function

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

- Freeman *et al.* 2024. Biological Science (8th ed.), pp. 84–97 (Chapter 3)
- Enzyme videos (provided in lab and on Canvas).
- Canvas Resources

Background

Enzymes play an important role as biological catalysts, lowering the amount of activation energy necessary to carry out a reaction. They are utilized to control the chemical reactions that are carried out within cells, making sure that these reactions are not random events, but are instead carefully orchestrated to allow the cell to carry out its function.

Enzymes have specialized shapes determined by their amino acid sequences. The specific shape of each enzyme provides it with an active site – a location where only a specific molecule can bind. This specific molecule is called the substrate. When the enzyme binds to the substrate, the bonding process triggers a chemical change in the substrate that changes it into a new molecule (or molecules), called the product. While the substrate is changed during this reaction, the enzyme itself is not. This allows the enzyme to bind to another substrate molecule and continue the reaction cycle. This process is often demonstrated using a lock and key model, as illustrated in ***Figure 3.1***.

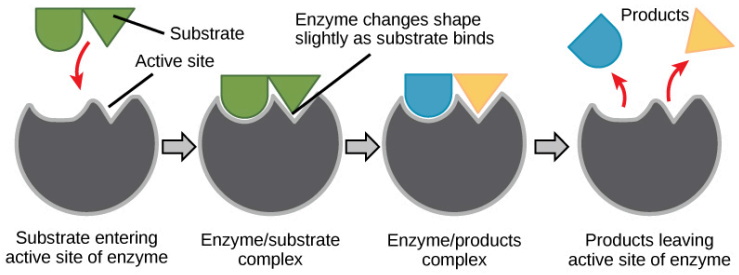


Figure 3.1. The lock and key model of an enzyme mediated reaction. Image Source: OpenStax Biology, Figure 6.16, CC BY 4.0.

There are a number of environmental factors that can affect the ability of the enzyme to catalyze reactions. Factors such as changes in temperature and pH can affect the rate at which an enzyme can function. Each enzyme has a set of "optimal" conditions under which it will function at its highest rate. Changes in those conditions will result in a change in the rate of its function.

For today's lab, we will be exploring enzyme function by utilizing peroxidase. Peroxidase, depicted in ***Figure 3.2*** is an enzyme that is found in a wide variety of organisms (from bacteria to humans). Hydrogen Peroxide (H_2O_2) is often one of the byproducts when an organism utilizes oxygen in redox reactions. However, hydrogen peroxide is toxic to cells, so a build-up of H_2O_2 can result in cell death. The function of peroxidase is to break down hydrogen peroxide into byproducts that are no longer toxic to the cell.

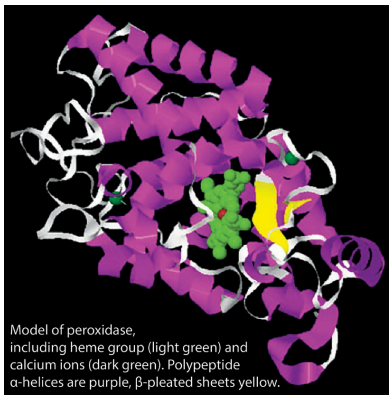


Figure 3.2. Model of peroxidase including heme groups (light green) and calcium ions (dark green).

In the natural world, peroxidase is utilized by plants to help them defend against pathogens. It can cause a color change and reduce the shelf life of frozen vegetables, which is why many of these vegetables are briefly submerged in boiling water (blanched) before they are frozen to denature the peroxidase and keep it from causing a color change. In animals, peroxidase provides an important function in the immune system – it can produce free radicals that are used by some immune

system cells to destroy invaders, damaged cells, and cancer cells. Peroxidase has also performed an important function in investigating neurobiology and developmental biology. Because it can be taken up by neurons and transported through the cytoplasm, it has been used to trace branches of these cells to visualize contacts with other cells. Horseradish peroxidase (the same enzyme utilized in lab today) is responsible for much of our knowledge about the nervous system architecture.

Peroxidase has found its way into manufacturing and industrial uses as well. We utilize peroxidase to help treat industrial wastewater (it aids in the removal of harmful phenols), it can be used to help convert toxic materials (like dyes) into less harmful substances and has been used in some manufacturing processes.

The normal function of peroxidase is to reduce toxic hydrogen peroxide, which can be produced in certain metabolic reactions, into water and another harmless compound shown in the equation below:

$$H_2O_2 + RH_2 \overset{\text{peroxidase}}{\leftrightarrow} 2H_20 + R$$

In this equation, R stands for another molecule that acts as an electron donor, even though it does not bind to the enzyme active site. Some molecules that can act as electron donors change color when they are oxidized in this reaction, and the color change can be used to follow the progress of the reaction. We use the dye guaiacol, which turns brown when oxidized. The reaction sequence is:

$$4H_2O_2 + 4\text{guaiacol (colorless)} \overset{\text{peroxidase}}{\leftrightarrow} 4H_20 + \text{tetraguaiacol (oxidized) (brown)}$$

In lab today, we will use the color change caused by the oxidation of guaiacol to determine the rate of the peroxidase reaction. The peroxidase we use in lab is a standardized horseradish peroxidase, which means it has been purified. We can reliably determine how much of the enzyme is in a given sample. However, as the video tutorial demonstrates, peroxidase can be extracted easily from turnips.

Experiment 1: Standardizing the Amount of Enzyme

NOTE

Every group should do this activity before moving on to create your own experiment.

Background

In this activity, you will adjust the concentration of peroxidase while keeping the concentration of the substrates and pH constant. Looking back to the peroxidase enzyme reaction equation above, what do you think will happen to the reaction rate (speed) as you increase the concentration of peroxidase? Why?

Following this protocol will not only help you understand the basics of enzyme function but will also give you practice in measuring enzyme activity which will aid in designing your own experiment later in lab.

Protocol

1. Choose a spectrophotometer station for your workstation. You will be using 7 test tubes for this experiment. Label each test tube with a piece of masking tape and number them from 1-7.
2. Fill each test tube with the reagents described in ***Table 3.1***. All of the reagents will be in repeating pipettes around the classroom. The repeating pipettes have already been set to a specific volume. **Do not change the pipette volumes**. You will notice that there are two test tubes for each treatment. This separates the reactants from the enzyme. The reaction will only occur once the test tubes are mixed.

Table 3.1. Mixing table for standardizing the amount of enzyme experiment. You will use Tube 1 as your blank.

Tube #	Buffer pH 5 (mL)	Hydrogen Peroxide (mL)	Peroxidase (mL)	Guaiacol (mL)	Total Volume (mL)
1(Blank)	5.0	2.0	0.0	1.0	8.0
2	0.0	2.0	0.0	1.0	3.0
3	4.5	0.0	0.5	0.0	5.0
4	0.0	2.0	0.0	1.0	3.0
5	4.0	0.0	1.0	0.0	5.0
6	0.0	2.0	0.0	1.0	3.0
7	3.0	0.0	2.0	0.0	5.0

3. Adjust your spectrophotometer to 470 nm and zero (or blank) it using the contents of Tube 1. If you need a refresher on how to do this, watch the Spectrophotometer video tutorial posted on the lab laptop computers from Week 1. Why do you need to use the contents of this tube and not another solution, such as water, to zero the spectrophotometer?

4. After you zero the spectrophotometer, assign one member of your group as a timekeeper, another as mixer, and the third as the recorder.
5. When ready, have your mixer combine the contents of tubes 2 and 3. Your timekeeper should start the timer immediately once the two solutions have touched. Mix the contents by pouring the solution from one tube to the other 2-3 times. As quickly as possible, pour the solution into a cuvette, cap it, wipe off the exterior, and place it in the spectrophotometer. Put the used test tubes in the wash bucket in the sink.
6. Your recorder should record the absorbance every 20 seconds for two minutes in ***Table 3.2***. Note: If you are getting negative values, you may need to set the blank again.
7. Once you have collected all the required measurements, discard the contents of the cuvette down the drain and rinse the cuvette out with distilled water from your squirt bottle.
8. Repeat steps 5–7 for tubes 4 and 5. You do not need to re-zero the spectrophotometer.
9. Repeat steps 5–7 for tubes 6 and 7.

Table 3.2. Absorbance readings for standardizing the amount of enzyme experiment. Measurements recorded at 470nm.

Treatments	40 seconds	60 seconds	80 seconds	100 seconds	120 seconds
Tubes 2 & 3: 0.5 mL Peroxidase					
Tubes 4 & 5: 1.0 mL Peroxidase					
Tubes 6 & 7: 2.0 mL Peroxidase					

10. When you have completed the experiment, remove the tape labels from the test tubes and wash the tubes thoroughly using the soap and brushes provided. Place the tubes upside down in the test tube rack to drain.

11. After you have cleaned up your station, return to your student desk. Your TA will discuss the class data and will analyze the class data during a presentation.

Experiment 2: Create Your Own Experiment

As discussed in the background information for this lab, a number of environmental factors may affect enzyme function and reaction rate. You have already determined how changing the amount of peroxidase enzyme effects the reaction rate. In this part of the lab, you will further test how enzyme function and reaction rates are affected by a variety of different factors. Each group should investigate **one** of the following 2 factors to test. Your TA may assign your group a factor to test:

- Changing the pH of buffer
- Changing temperature of the reaction

Once you have been assigned the factor that you will test, go to the TA bench to find the instruction and tip sheet folder that corresponds to your chosen factor. Read it over, and then answer the questions below. **Make sure you get approval from your TA before you start your experiment**.

1. **BEFORE STARTING,** work with your group to develop a hypothesis of how peroxidase is affected by your chosen (or assigned) variable and a method to test it. You must have **at least four experimental treatments or treatment levels** for approval. Receive approval from your instructor (**marked** on your assignment) for each of these steps before proceeding.
   a. State the **hypothesis** that your group will test. Explain your reasoning that supports your hypothesis.

Hypothesis and methods: Receive approval for your hypothesis and necessary tables from the instructor.

b. Define the **independent variables** you will manipulate and the **dependent variables** you are testing.

c. What will you use as a control or reference standard?

2. As you complete your experiment, record your data in ***Table 3.3*** below. Make sure you fill in the treatments for your individual experiment.

Table 3.3. Student worktable for create your own experiment data collection. Measurements recorded at 470nm.

Treatments	40 seconds	60 seconds	80 seconds	100 seconds	120 seconds
Tubes 2 & 3 Treatment: _____					
Tubes 4 & 5 Treatment: _____					
Tubes 6 & 7 Treatment: _____					
Tubes 8 & 9 Treatment: _____					

3. Once you have collected your data, you will need to input your measurements from ***Table 3.3*** into the Week 3 Excel template located on Canvas. Graph this data in a single graph that includes a scatter plot of data from each of your treatments. Your independent variable should be on the x-axis and dependent variable should be on the y-axis. Fit "best-fit lines" to each treatment using the information provided in **Interchapter 4: Using Excel for Data Analysis and Graphing** on page 51. The slopes of each line will represent the rate of enzyme activity for each treatment. This Excel file should be uploaded to Canvas and submitted as part of your Week 3 post-lab assignment.
4. Once you have your slopes (reaction rates) for each treatment, add them to the appropriate table in the template. Use this to create a derivative plot (rate comparison graph) comparing these treatments by following the appropriate instructions in **Interchapter 4: Using Excel for Data Analysis and Graphing** on pages 51–55. Your table and graph file will need to be uploaded to Canvas as part of your Week 3 post-lab assignment. The file submitted on Canvas will need to be in Excel format. Remember that each student must create and submit their own Excel data file. Sharing of electronic files would be considered academic dishonesty.

Week 3 Post-Lab Assignment

An Excel file containing the following tables and graphs will need to be submitted on Canvas as part of your Week 3 post-lab assignment.

Tables showing raw data for Experiment 2 (create your own).

Graph showing the raw data (with best-fit lines) for your Experiment 2.

Derivative graph for your Experiment 2.

1. Look at your data and briefly summarize your results. Did your results support your hypothesis? If not, why?

2. Based on your findings, how does the variable you tested affect the reaction rate of peroxidase? Which of your conditions produced the fastest reaction rate? Which of your conditions limited the reaction rate the most?

3. Based on your findings, how could you improve your experiment or what additional experiments could you perform to better understand your results? Why? Answers such as "collect more data" or "use better equipment" will generally be considered insufficient for full credit.

INTER-CHAPTER 3.

USING EXCEL FOR DATA ANALYSIS AND GRAPHING

Notes

- These instructions are for Office 365 installed on the lab laptop computers. If you are using a Windows machine, or an older version of Office, these instructions may not be accurate.
- Excel is a spreadsheet program we'll use to manage and analyze data. You will be using this program repeatedly throughout Biology 2120L. Other graphing programs will not be sufficient. If you need assistance locating Excel for your computer, consult with your TA. These instructions are written generally to help you choose the best way to analyze your data throughout the semester. Feel free to come back to this guide at any time to refresh your skills.
- Video tutorials are provided on Canvas to supplement these instructions. Read these instructions and view the video tutorials to complete the Excel assignments. Consult with your TA if you have additional questions.

This inter-chapter includes information on how to complete data analysis and graphing that you will use for various labs throughout the semester. Refer back to this information as frequently as you need throughout the semester.

Get Familiar with Excel

- Grab a lab laptop and turn it on. Once it has booted up, locate Excel on the computer you are using. Open Excel by clicking on the Excel icon on the dock and select "new document" or open a saved file if you have it.
- An Excel worksheet is arranged by columns, labeled with letters; and rows, labeled with numbers. Each box or cell on the worksheet corresponds to a column/row location. For example, when you open Excel, the highlighted cell is A1. Going down the column the next cell is A2. If you go to the right of cell A1, that cell is B1, and so on.
- You enter data into Excel by clicking on a cell, then typing in that cell. You can enter a number or text within a cell. To move around in a worksheet click on the desired cell. You can also use the arrow keys on your keyboard to move you from cell to cell.

- Always include your name and section number on your Excel file.

Graphing Your Data

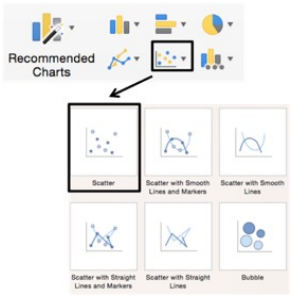


Figure IC3.1

1. Click and drag to highlight the columns of data that you want to graph. Make sure you include the column titles when highlighting.
2. Click the **Insert** Tab in Excel's Ribbon. Find the section, in the middle of the ribbon, that has chart options. Move your cursor over the different chart types to see the chart descriptions. In this lab we most commonly use the **X Y (Scatter)** option and **Scatter** from the drop-down menu as shown in the photo on the right. This is the right option to use if you want to create a best fit line. You can also choose other options for graphing as you need.
3. A graph of your data should appear on your spreadsheet. It may not have titles or axes labels. Before fixing this, check to make certain the dependent variable is on the Y-axis, and the independent variable is on the X-axis.

Figure IC3.2

4. If your graph does not show your axis labels or title, click on the graph. This will open the **Chart Design** tab in Excel's ribbon. Click on the icon called **Quick Layout,** as shown on the right, and choose the first option that includes a title, axis labels, and a legend.
5. Click and drag on a corner of your graph window to adjust it to your desired size. To move the graph to a new location in the Excel window, click and hold anywhere in the white space and your graph outside your grid lines and move it to a new location.
6. Click and drag to select the text in the title box, then type your title. Make sure your title has details and is specific.
7. Scientific graphs rarely use gridlines within the graph. Click on one gridline to select all the gridlines, then hit the delete key to delete them. You should do this for every graph you create.
8. The legend Excel provides for this graph might not be necessary or informative. If so, remove it by clicking on it and hitting the delete key.
9. Click on the X-axis title. An axis title box will appear on your graph – click in it and enter the title and units of measurement for your X-axis variable. Entering units in your axis titles is extremely important!

10. Click on the Y-axis and enter the variable name and units of measurement for your Y-axis variable.
11. If you want to personalize your graph by using different colors or changing design options, click on your graph to select it. The **Chart Design** tab will appear in Excel's ribbon. Choose the tab called **Format** to view your options. Feel free to experiment to make the graph your own!

Analyzing Data for Best- Fit Line

Take a minute and look at your data points. If they fall in a relatively straight line, you can use Excel to calculate and draw a best-fit straight line through your data set as follows.

1. Control-click on one of the data point symbols in your graph, then choose **Add Trendline** from the options. A **Format Trendline** window will open.
2. Click on **Trendline Options**, and select **Linear**. Excel will use a statistical technique called linear regression to create a straight line that is the best possible fit to your data.
3. Now scroll down in the **Format Trendline** window and check the boxes marked **Display equation on Chart**, and **Display r-squared value on chart**. Those options will give you the equation for your regression line and a statistic called $\mathbf{r^2}$. Display the equation and $\mathbf{r^2}$ value each time you insert a trendline. An example graph is shown on the right with sample data. This is not necessarily what you graph will look like.

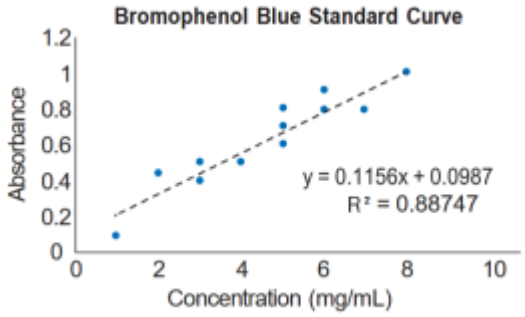


Figure IC3.3

4. The $\mathbf{r^2}$ statistic measures how well the straight line describes your data. It is a number between zero and one that tells you what fraction of the variation in y-axis values you can account for if you know their x-axis values. An $\mathbf{r^2}$ of one means that all the points lie right on the line – if you know concentration, you can predict absorbance perfectly. An $\mathbf{r^2}$ of zero means that there is no linear trend to the data, and knowing concentration will not tell you anything about absorbance. The higher the $\mathbf{r^2}$, the better the line fits the data points.
5. Click and drag to move the equation or $\mathbf{r^2}$ value to the part of your graph that they can easily be seen.

Creating a Derivative (rate of change) Plot

A derivative graph is a figure that is used to describe the rate of change of the variable that we measured in our experiment. The higher on the Y axis our values are, the faster the rate of change is occurring at that given X axis value. In the example below, the rate of activity for peroxidase peaks at around 5.5 pH. Before you can create a derivative plot, you need to have the slopes for each treatment. That can be done by creating best fit lines and using the equation provided for each treatment to determine the slope (rate). The slope is the number before the "x" in the equation.

1. In a new table, create a column for treatment and a column for rate/slope.
2. Enter your slopes for each treatment.
3. Once you have all of your data entered, Click and drag to highlight the columns of data that you want to graph. Make sure you include the column titles when highlighting.
4. Click the **Insert** Tab in Excel's Ribbon. Find the section, in the middle of the ribbon, that has chart options. For a derivative plot, a best fit line is often not the best representation of your data. Instead, we want to use a smooth line to connect the points and visualize the data. Move your cursor over the different chart types to see the chart descriptions. Choose the **X Y (Scatter)** option and **Scatter with Smooth Lines and Markers** from the drop-down menu. An example of what this may look like is shown below.

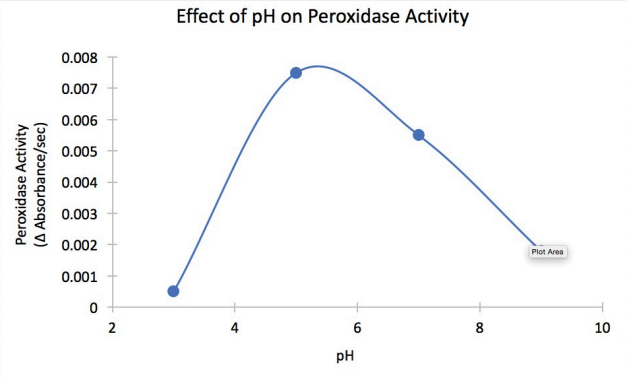


Figure IC3.4.

5. If you want to edit your graph, add axis labels, add a title etc., follow the instructions in the previous "Graphing your data" section.

Calculating Mean and Standard Deviation

1. If you have multiple measurements per treatment and want to know the average or mean value of each group, you can calculate that in Excel. First click in an empty cell and label it with the word "average"

and the name of your treatment. Make sure you label everything clearly, so you know which means goes with which treatment.

2. In the cell next to it you will enter the prompts necessary for Excel to calculate your average. Begin by writing "=average". As soon as you start typing the word average, a drop-down menu will show up next to your text.
3. Choose "average" from the menu. This will auto-populate the cell with "=average()". Select the cells/values that you want to calculate the average of. Do this by clicking on the first value and dragging to select all of the values you want to include in the calculation.
4. Then hit the **Return** or **Enter** key. Always end a formula by hitting the **Return/Enter** key. When you hit the **Return** or **Enter** key, the average measurement for your treatment should appear. If you click anywhere else, Excel will add the cell in which you clicked to your formula. You will need to do this same process for each treatment.
5. If you want to calculate the standard deviation of your treatments, you can do this in the same way. Begin by choosing an empty cell and label it with the words "Standard deviation" and the name of your treatment.
6. Now enter a formula to get the standard deviation. In the cell next to your label, begin writing "=stdev.s". As soon as you start typing the word average, a drop-down menu will show up next to your text. Choose "STDEV.S" from the menu. This will auto-populate the cell with "=stdev.s()".
7. Select the cells/values that you want to calculate the standard deviation of. Do this by clicking on the first value and dragging to select all of the values you want to include in the calculation. Hit the **Return/Enter** key.
8. Your calculated standard deviation should appear. Remember to round this value to the appropriate number of significant figures. You will need to repeat the process for each treatment.

Save your Tables and Graphs

1. From the File menu, select Save As. Name your file and select the save location.
2. Make sure to save the file to Cybox or send it to yourself through email. Make sure that your file uploads completely to Cybox before closing. Lab laptops will delete all student files when they are shut down, so it is important that you save your file carefully before leaving lab.
3. **You will need to upload your completed file to Canvas for submission of your weekly assignments requiring graphs and tables.**

LABORATORY 4.

FERMENTATION

Objectives

After this week's lab, students will be able to:

- Develop a hypothesis and design an experiment to test how environmental conditions affect the rate of fermentation
- Present the results of an experiment on fermentation rates appropriately and accurately in a graph
- Describe how one or more environmental conditions affect fermentation rates

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

- Freeman *et al*. 2024. Biological Sciences (8th ed.) recommended reading sections 9.1, 9.2, and 9.6 for overview of cellular respiration and fermentation (for a more complete look, read the whole chapter, pp. 201–220).
- Canvas resources.

Background

Cellular respiration and fermentation involve the oxidation of organic molecules, usually carbohydrates or lipids, releasing energy that allows cellular function. Cellular respiration includes many reactions catalyzed by many enzymes, but we can measure the rate of cellular respiration using the following principles:

- identify the inputs (reactant molecules) and outputs (product molecules) of the entire set of reactions
- measure the rate at which one reactant is used or one product is produced

The respiration rate of all the cells in an organism is that organism's **metabolic rate**.

Oxidation and Reduction

In chemistry, oxidation refers to the transfer of electrons from one molecule to another. The molecule that accepts electrons is **reduced**, and the molecule that gives up electrons is **oxidized**. A general name for such reactions is **redox** reactions. When electrons are transferred from a higher to a lower energy state in a redox reaction, energy is released that can be used to drive other reactions, like

the synthesis of ATP. Oxidation does not necessarily require oxygen to proceed; oxidation reactions were named because oxygen is a powerful and convenient oxidizing agent, so redox reactions with oxygen (like fire) were the first to be studied.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 29\ ATP$$

Cellular respiration can be either **anaerobic** (molecular oxygen is not involved) or **aerobic** (molecular oxygen is used). These processes are shown in ***Figure4.1***.

Anaerobic respiration and fermentation involves 10 – 11 (depending on the organism and the end products) enzyme-catalyzed steps—the reactions of glycolysis. End products of anaerobic respiration vary between organisms, but can include ethanol, organic acids (lactate, fatty acids), methane, and CO_2. In yeast, the principal end products are ethanol and CO_2, so we can use ethanol or CO_2 production to estimate anaerobic respiration. The reactions of anaerobic respiration occur in a cell's cytoplasm, so anaerobic ATP production is immediately available to drive cytoplasmic reactions (like muscle contraction), even though much of the energy in a sugar molecule cannot be used.

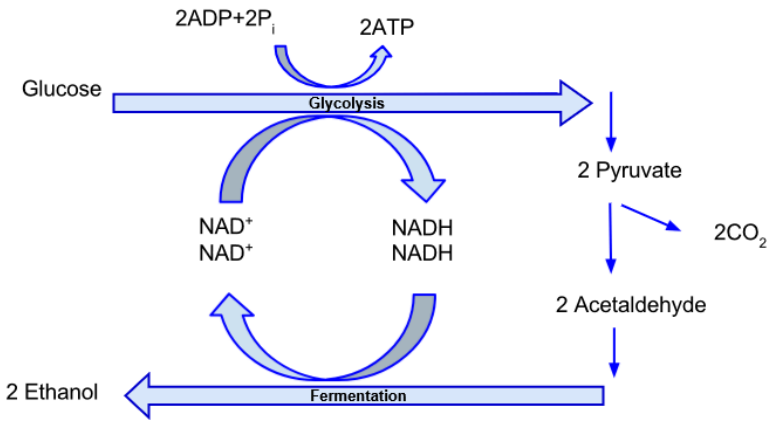


Figure 4.1. Overview of fermentation. Adapted from "Ethanol fermentation" by David Carmack on Wikimedia Commons, CC BY SA 3.0.

In ethanol fermentation, ~95% of the energy in the fuel molecules (sugars) remains in the ethanol end products, so the ATP yield is low (2 ATP/glucose). That is why we can feed yeast with corn sugar and extract ethanol containing enough energy for biofuels. Currently, a single bushel of corn (25.4kg of shelled kernels) can produce 10.6L of ethanol; however, researchers are investigating methods to increase this yield to 11.4 or more liters per bushel in response to the Renewable Fuel Standard enacted by congress in 2005 and updated in 2007. The standard requires that fuel companies in the U.S. increase the amount of biofuels they mix with non-renewable fuels each year until 2022, at which point the original goal was to use approximately 136 billion liters of biofuels a year. That goal is unlikely to be met, however. In 2019, only ~62 billion liters/year were used. In 2020, due to COVID-19, the amount consumed is estimated to be even lower.

Aerobic respiration involves about 30 enzyme-catalyzed steps—the reactions of glycolysis, the citric acid cycle, and oxidative phosphorylation. In aerobic respiration, a much higher proportion of available energy can be captured in ATP (about 25–36 ATP/glucose) than in anaerobic respiration. Aerobic respiration requires the presence of O_2 to serve as the final electron acceptor so we could measure the rate of aerobic respiration by measuring O_2 consumption. The end products of aerobic respiration are CO_2 and H_2O, so we could also measure its rate by measuring CO_2 or water production; however, water production is difficult to measure as respiration water is a miniscule fraction of the total water in a cell. The reactions of aerobic respiration take place not in the cytoplasm, but in mitochondria, so it takes more time to increase rates of aerobic ATP production than anaerobic ATP production.

In the following lab you will compare ethanol fermentation by yeast under different environmental conditions. As ethanol fermentation produces CO_2 without consuming O_2, you can measure fermentation based on the net change in gas within an enclosed space, such as a respirometer.

MEASURING FERMENTATION LAB GUIDE

Materials

- Respirometers (***Figure 4.2***)
- Different yeast strains
  - Baker's yeast (*Saccharomyces cerevisiae*)
  - Champagne yeast (*S. bayanus*)
  - White wine yeast 1 (*S. cerevisiae*)
  - White wine yeast 2 (*S. cerevisiae*)
- Sugar stock solutions available for dilution (***Figure 4.3***)
- pH buffers (pH 3, pH 5, pH 7, pH 9)
- Salt (NaCl) solution available for dilution

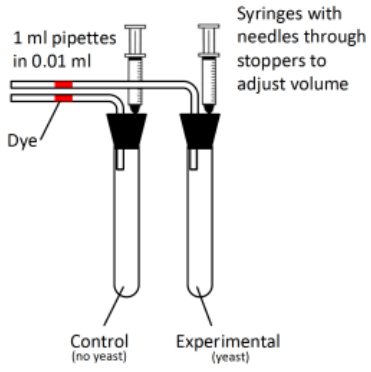


Figure 4.2. Respirometer setup for measuring yeast fermentation

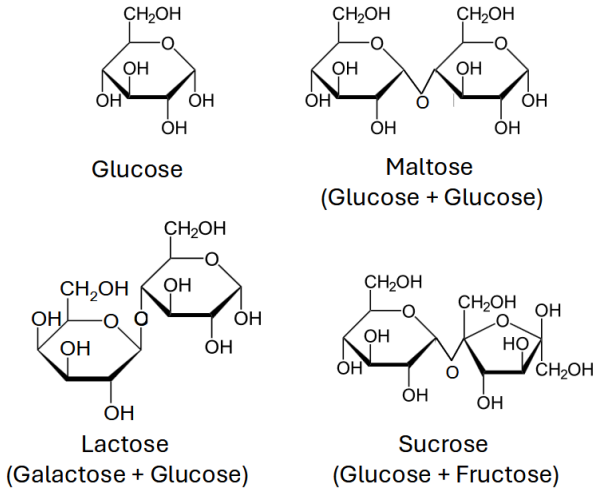


Figure 4.3. Sugars available for fermentation experiment and their monosaccharide subunits.

NOTE

Glycolysis, which is the first step of both cellular respiration and fermentation, is a complex series of reactions that breaks sugar down. These reactions are each catalyzed by a specific enzyme acting on a specific substrate. For disaccharide sugars to be used in glycolysis, they need to be broken down into monosaccharides. And depending on the monosaccharide, it may need to be further transformed before it can be used in glycolysis.

Procedure

1. **BEFORE STARTING**, work with your group to develop a hypothesis of how ethanol production via fermentation is affected by a variable of your choice and a method to test it. You must have **at least four experimental treatments or treatment levels** for approval. Receive approval from your instructor (marked on your assignment) for each of these steps before proceeding.
   a. State the **hypothesis** that your group will test and explain your reasoning behind it:

   b. Define the **independent variable** you will manipulate and the **dependent variable** you are testing for your experiment with. Is the variable you are testing continuous or discrete? Think about how that will affect the way you display your results.

c. What will you use as a control or reference standard?

d. Outline a **BRIEF** protocol for how you will test your hypothesis in lab:

2. Fill each of your experimental test tubes with the following solutions for a total of 35 mL of solution per test tube and fill in ***Table 4.1*** accordingly:
   a. 10 mL yeast solution (**required** for all tubes)
   b. 10 mL sugar solution (**required** for all tubes)
      i. Unless you are manipulating the type of sugar used as part of your experiment, use glucose as your default sugar solution.
   c. 15 mL (total) of the following solutions (you may, but are not required to, mix these solutions as necessary for your experiment; for example: 5 mL of pH buffer, 5 mL of salt solution, and 5 mL water):
      i. More sugar solution (for a total of ≤ 25 mL)
      ii. pH buffer
      iii. Salt solution
      iv. Water

Table 4.1. Worktable to set up your experiment.

Test tube	Yeast strain (10 mL)	Sugar type (10 mL)	Additional Solution (list what is used and how much) (15 mL)	Total volume (35 mL)	Notes
1				35 mL	
2				35 mL	
3				35 mL	
4				35 mL	
5				35 mL	

3. Mix the solutions in each test tube thoroughly with the wooden stick provided at your station before

capping with a rubber stopper and sealing with a Parafilm wax strip. Make sure the plunger in the syringe is set around the half-way mark so you have room to adjust your dye as your experiment progresses. Set your test tubes standing upright in your test tube rack.

4. Two factors will change the air pressure within your test tubes: CO_2 produced through fermentation will increase the total amount of gas in the test tube and changes in external air pressure due to weather or temperature will change the density of this gas. To control for changes in external air pressure, you will need to construct a **thermobarometer**, a device used to measure such changes. To do this, prepare an additional tube as in step 2, but using 35 mL of water instead of the various solutions listed. Any change in air pressure within the thermobarometer will be due to changes unrelated to your experiment and can be subtracted from your measurements to calculate the actual amount of CO_2 produced in your experimental test tubes. Note: The thermobarometer is a way for you to monitor changes in air pressure that may happen during your experiment. It is not an experimental control, which would be based on the independent variable you are testing.
5. After setting up your experiment, **wait 10 minutes** before starting to allow conditions to reach equilibrium. If you attempt to collect data before this time, you may not get meaningful results.
6. As your measurements will consist of a volume of gas, you must adjust for factors that affect gas volume: air pressure and temperature. These adjustments are critical to compare results collected at different times or places. For example, consider how the volume of air in a balloon would change moving from Ames to Mt. Everest, or as a low pressure weather system approaches. You will adjust your data for these factors later, but first **record** the room temperature and pressure in the space below **before** you begin collecting data.
    a. Lab temperature (T_{meas}): _____°C
    b. Lab air pressure (P_{meas}): _____ inHg
7. Use a long-tipped pipette to place a small bead of dye in the pipette extending from the stopper in each test tube (***Figure 4.2***). You do not need to place the dye exactly at 0 mL at this point; you will be able to adjust the location at the start of your experiment later using the syringe in the stopper.
8. Set the dye in the pipette to 0 mL using the syringe: pull up on the plunger to move the dye toward the test tube and press down to move it away from the test tube.
9. Read the position of the dye in the pipettes every two minutes for at least 14 minutes and record these values in ***Table 4.2***. If you do not see any change in this time, or if you see a change of less than 0.05 mL, consult with your TA before proceeding.
    a. **NOTE:** The pipettes are marked in major units of 0.1 mL (large marks) and minor units of 0.01 mL (small marks). Estimate the location of the dye bead to the nearest 0.005 mL.
    b. If you are concerned about the dye in your pipettes falling out, use the syringe to move the dye back to 0 and add its previous progress to its new position. For example, if you moved the dye to 0 when it reached 0.6 mL and at the next time point the dye is at 0.1 mL, then the total CO_2 production up to that point is 0.6 mL + 0.1 mL = 0.7 mL.

10. The changes you observed in the test tubes containing yeast are the result of two factors: the production of CO_2 by the yeast and changes in air pressure and temperature. To control for changes in air pressure and temperature, subtract your thermobarometer readings from your treatment readings in ***Table 4.2***.

Table 4.2. **Carbon dioxide (CO_2) production (in mL) and thermobarometer corrected data. Note that the thermobarometer (TB) readings will be the same for each treatment if run simultaneously.**

Treatment		0 min	2 min	4 min	6 min	8 min	10 min	12 min	14 min
	Yeast (Y)								
	Thermobarometer (TB)								
	Corrected (Y-TB)								
	Yeast (Y)								
	Thermobarometer (TB)								
	Corrected (Y-TB)								
	Yeast (Y)								
	Thermobarometer (TB)								
	Corrected (Y-TB)								
	Yeast (Y)								
	Thermobarometer (TB)								
	Corrected (Y-TB)								
	Yeast (Y)								
	Thermobarometer (TB)								
	Corrected (Y-TB)								

Clean-Up

1. Dump the contents of your test tubes down the drain, wash them with soap and water, and set them upside down in your test tube rack to dry.
2. Rinse the dye out of the pipettes with tap water. Dry the inside of your pipettes using the compressed air hose at designated stations in the lab while keeping the pipette pointed into the trash receptacle at the drying station. Please wear the safety goggles available in lab while completing this task. Make sure you wipe up any dye that you spill.
3. Return any additional supplies to their correct location and throw away any garbage generated.

Analysis

1. Utilize the instructions in the Excel Inter-Chapter (pages 51–55) to complete the graphs required for this week's assignment.
2. Use Excel to **plot** your data from ***Table 4.2*** in a single graph that includes scatter plots of CO_2 produced as a function of time for each of your treatments and fit straight lines (in Excel, use the 'Trendline' tool) to each dataset. The slopes of these trendlines are your Vmeas values and they represent the fermentation rates (mL of CO_2 produced/min) in each treatment. Attach your graph to the assignment.

Table 4.3. Rate of change of Carbon Dioxide production.

Treatment	V_{meas} (mL CO2/min)

3. As mentioned previously, the temperature and air pressure in the room can affect your results, which is why a thermombarometer is used to detect any changes in these values over the course of the experiment and to be able to correct for them. The specific values of temperature and external air pressure during your experiment would also make it difficult to compare your results to those of other experiments unless the values are standardized. Before you can fully analyze your results, you must standardize your data so that researchers at different times and locations can compare their results to your own (see step 6 in the procedure above). To do this, you must convert your measurements to **standard temperature and pressure** (STP), or the volume that the gas would occupy at 760 Torr (the average atmospheric pressure at sea level) and 273 degrees Kelvin (0 °C). If you have not already done so, record the temperature and air pressure in lab in the space provided above. Once you have these values, convert the fermentation rates (mL of CO_2 produced/min) you calculated in your raw data plot

(V_{meas}) to standard temperature and pressure (V_{STP}, see equation below). Fill out ***Table 4.3*** in the assignment.

NOTE: Before you use this equation, T_{meas} and P_{meas} **must be in units of degrees Kelvin (K) and torr (Torr)**, respectively. If you need to convert your measurements you can do so with the following equations:

Tmeas = K = ____ °C + 273.15

torr = 25.40 × ____ in Hg

$$V_{meas} \times \frac{273K}{T_{meas}} \times \frac{P_{meas}}{760Torr} = V_{stp}$$

A tutorial video is posted on Canvas to help you understand how to complete this conversion.

4. Use Excel to **plot a derivative** (rate of change) **graph** illustrating how the rate of CO_2 production (STP-adjusted values) changes with treatment. **You should use the V_{STP} values (last column of Table 4.4) in your derivative graph.** If you used a continuous variable for your treatment, include a trendline that best describes the relationship between the net rate of CO_2 production and your treatment variable. **<u>You will need to upload your Excel file to Canvas for submission.</u>**

RESULTS

a. Upload your graph plotting the raw data from ***Table 4.2*** with associated trendlines to the Week 4 post-lab assignment on Canvas.

b. Fill in ***Table 4.4*** below, converting the raw slopes for each of your treatments to standard temperature and pressure using the formula in excel.

Table 4.4. Worktable to adjust measured volumes/min to STP.

Treatment	Vmeas (mL/min)	273 K/Tmeas	Pmeas/760 Torr	VSTP (mL@STP/min)

c. **Upload your derivative (rate of change) plot to the Week 4 post-lab assignment on Canvas.**

What was your hypothesis? Was it supported or refuted? Why? What evidence can you show from your experiment to support your statement?

LABORATORY 5.

INVESTIGATING PHOTOSYNTHESIS

Objectives

Following this week's lab, students will be able to:

- Describe the function and absorption spectrum of chlorophyll
- Develop a hypothesis and design an experiment to test how the rate of photosynthesis varies under different conditions
- Present the results of an experiment on photosynthetic rates appropriately and accurately in a graph
- Describe how one or more environmental conditions or plant characteristics affect the rate of photosynthesis

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

- Overview of the anatomy, physiology, and types of photosynthesis: Freeman *et al*. 2024. Biological Science (8th Ed.). Chapter 10, pp. 222–241.
- Canvas resources.

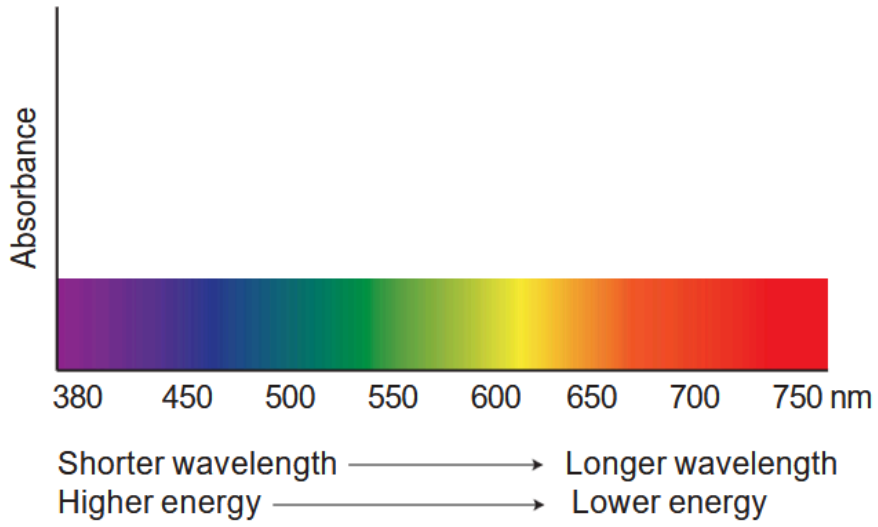
PART I: Absorption Spectrum

Background

In this exercise you will determine the light absorption properties of chlorophyll. The porphyrin head of a chlorophyll molecule absorbs some wavelengths of light for use in photosynthesis while reflecting others. Using a spectrophotometer to measure absorbance of chlorophyll at different wavelengths, you will determine the absorption spectrum for this compound. Before you begin, you should make some predictions about the absorbance properties of chlorophyll.

Pigments in Photosynthesis

- What should the absorption spectrum (plot of absorbance vs. wavelength) of chlorophyll look like?
- Make a sketch to illustrate your answer below.



Absorbance

Procedure

1. Zero the spectrophotometer using petroleum ether as a blank.
2. Work with your group to measure the absorbance of the chlorophyll extract at 20 nm intervals beginning at 400nm, recording your observations in ***Table 5.1***. Each group member should take a turn to measure and record a subset of the values. If there are three members of your group, each student should measure and record a third of the values in ***Table 5.1***. You should then pool your results so that everyone has a complete table. Remember to zero the spectrophotometer with the petroleum ether blank for each new wavelength before measuring the absorbance of chlorophyll.
3. At each wavelength, record the color of light used in the spectrophotometer. You can do this by insert-

ing a narrow strip of white paper into the sampling chamber and looking at it with your hands cupped to block out light from the lab. Record the color you see at each wavelength in ***Table 5.1***. **NOTE**: If you are using the Genesys30 Spectrophotometers, you will need to blank the spectrophotometer at each wavelength **before** you will be able to view the light color.

Analysis

1. Use Excel to plot your light absorption spectrum for chlorophyll (include title, units of measurement and axis labels). **Attach your graph to the assignment**. Follow the instructions for "standard curve" that you utilized in the Week 2 Quantitative Techniques chapter.
2. Compare your graph of the absorbance spectrum for chlorophyll with the prediction you made earlier in lab. Was your prediction close? Why or why not?

3. At what wavelengths do you predict photosynthesis would be more productive? Now look at the action spectrum of photosynthesis in the Freeman textbook (page 219). Does the action spectrum support your prediction?

Table 5.1. Light absorption characteristics of chlorophyll.

Wavelength (nm)	Light color	Absorbance
400		
420		
440		
460		
480		
500		
520		
540		
560		
580		
600		
620		
640		
660		
680		

Part II: Photosynthetic Rate

Materials

- Respirometers
- Plants (***Figure 5.1***)
- $NaHCO_3$ solution available for dilution
- Portable lights
- Measuring tape/meter sticks
- Photometers

Background

$$6CO_2 + 12H_20 \xrightarrow[\text{Chlorophyll}]{\text{Light} +} C_6H_{12}O_6 + 6H_20 + 6O_2$$

Carbon dioxide + Water → Light + Chlorophyll → Glucose (sugar) + Water + Oxygen

You will test how photosynthetic rates change under different conditions using one or more of the aquatic plants available in lab. All of these plants use CO_2 that has dissolved into the water, emitting O_2 as a by-product of photosynthesis. As O_2 is less soluble than CO_2, small bubbles of O_2 will form on the plants. When enclosed in a container like the respirometers you used in the respiration lab, these bubbles displace water and force it to move through a pipette, allowing you to measure O_2 production (see ***Figure 5.2***).

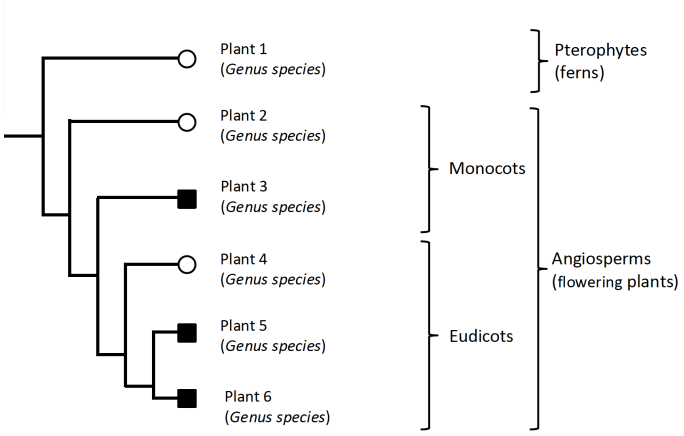


Figure 5.1.Generic phylogenetic tree. A phylogenetic tree of the aquatic plant species available this semester will be provided in lab. Mode of photosynthesis is represented by open circles (C3 photosynthesis) or closed squares (C4 photosynthesis). Actual phylogenetic distances are not represented to scale in this tree. *Note that the species used in class are subject to change due to availability.*

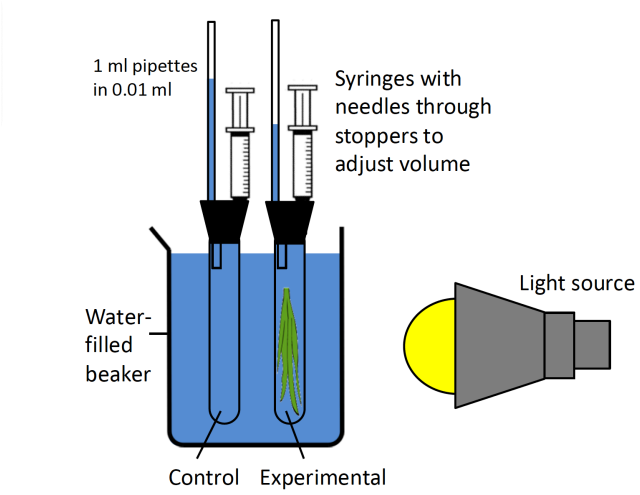


Figure 5.2. Experimental setup for measuring photosynthetic rate.

Procedure

1. **BEFORE STARTING**, work with your group to develop a hypothesis of how photosynthesis is affected by a variable that is available in lab and a method to test it. You must have at least four experimental treatments or treatment levels for approval. Receive approval from your instructor for each of these steps before proceeding.
   a. State the hypothesis that your group will test. Explain your reasoning for this hypothesis:

   b. Define the **independent variable** you will manipulate and the **dependent variable** you are testing for your experiment with. Is the variable you are testing continuous or discrete? Think about how that will affect the way you display your results.

   c. Outline a BRIEF protocol for how you will test your hypothesis in lab:

2. Obtain a piece of an aquatic plant 5–6 inches long, cutting the basal end and placing the shoot in a test tube, **cut end up**. If the shoot is so large that it must be forced into the test tube it will shade itself and reduce the rate of photosynthesis.
3. **Fill the test tube with sodium bicarbonate solution** (the source of CO_2 for the plants) and cap the tube

with a rubber stopper holding a curved pipette. Do not use water to fill your tubes, unless explicitly addressed in your experimental design. The end of the pipette in the test tube should be submerged in the solution or else you will not be able to measure O_2 production. Dry the outside of the tube and seal the top of the tube with Parafilm. If the tube is not sealed or if the stopper moves, you will not get good measurements. Make sure the plunger in the syringe is set at about the half-way point so it can be used.

4. Prepare a second tube as in step 2–3, but without a plant, to serve as the thermobarometer (a control tube used to detect changes in temperature and pressure). Fill this tube with sodium bicarbonate unless you change the type of solution used in the test tube with the plant. If that is the case, you will need to adjust your solution in the thermobarometer so as to maintain an appropriate control. Note: The thermobarometer is a way for you to monitor changes in air pressure that may happen during your experiment. It is not an experimental control, which would be based on the independent variable you are testing.
5. Suspend both tubes in a beaker filled with water from a clamp rod. As this beaker will buffer the test tubes from changes in temperature during your experiment, it is very important that you submerse the tubes as far as possible. If there are any markings on your test tubes, turn them so the markings face away from the light source to avoid shading.
6. After setting up your experiment, **wait ten minutes** before starting to allow conditions to reach equilibrium. If the fluid in the pipettes moves during this time, you can reset it to zero using the syringes. **NOTE**: You should keep your light o! during the equilibration period. You can place any tubes that you have set up but are not currently using at the equilibration station while you are waiting.
7. After reaching equilibrium, record the room temperature and pressure below.
   a. Room Temp: _____°C
   b. Pressure: _____inHg
8. Turn the light on and read the position of the fluid in the pipettes every two minutes for at least ten minutes and record these values in ***Table 5.2***. If you do not see any change, or if you see a change of less than 0.05 mL, consult with your TA before proceeding.
9. The changes you observed in the test tube containing the plant are the result of two factors: the production of O_2 by the plant and changes in air pressure and temperature. To control for changes in air pressure and temperature, subtract your thermobarometer readings from your plant readings in ***Table 5.2***.
10. Repeat steps 2–9 for your other treatments.
11. When you are finished with a plant, gently pat it dry and measure its mass before returning it to the appropriate tank. **DO NOT RUB PLANTS DRY** as this may damage or kill the plant. Record plant mass below and in ***Table 5.3***.

Table 5.2. Planning your experiment.

Test tube*	Treatment**	Plant Species	$NaHCO_3$ concentration	Notes
1				
2				
3				
4				
5				

***Note:** Depending on your independent variable, it may be better to use the same test tube/plant for each treatment. If that is the case for your variable, make a note of it.

****Depending on your independent variable, this column may be duplicated by one of the other columns in this table.

Table5.3. Oxygen production (in mL) and thermobarometer corrected data.

Treatment		0 min	2 mins	4 mins	6 mins	8 mins	10 mins
	Plant (P)						
	Thermobarometer (TB)						
	Corrected (P – TB)						
	Plant (P)						
	Thermobarometer (TB)						
	Corrected (P – TB)						
	Plant (P)						
	Thermobarometer (TB)						
	Corrected (P – TB)						
	Plant (P)						
	Thermobarometer (TB)						
	Corrected (P – TB)						
	Plant (P)						
	Thermobarometer (TB)						
	Corrected (P – TB)						

Table5.4. Plant mass.

Plant	Mass (g)

Measuring Light Intensity (Optional)

You may need to measure light intensity as an experimental variable for your experiment. If so, the following procedure will help you through this process.

1. Measure light intensity using a Quantum light meter (photometer). These meters measure light intensity as photosynthetic photon flux (PPF), or the number of photons of photosynthetically active light, per unit area, per unit time. The units of this measurement are micromoles of photons per square meter per second (µmol m-2*s-1) for photosynthetically active radiation (PAR) in the range of 400 to 700 nm.
2. To make a reading, turn the meter on. The sensor is the white dome at the top end of the meter. Position the sensor dome at the spot where the plant will be located during the measurement, and point it directly at the light source. Due to variation in lights and reflections from the desktop and sink, light intensity is not uniform at all heights. Therefore, measure light intensity at three different heights above the tabletop—about 5 cm, 9 cm, and 13 cm above the table (***Figure 5.3***), and take the average of those three measurements to represent light intensity. You may use the wood blocks to set the light meter at the appropriate height.
3. The light meters are very sensitive to the direction of the incoming light and need to be pointed directly at the light source. Aiming them a few degrees away will underestimate light intensity from your lamp.
4. **Do not put the photometer in water.** Mark the position where the plant will be during the photosynthesis measurements using a piece of tape, move the water bath and plant, measure the light intensity, and move the plant back into position. Record your data in ***Table 5.5***, and use the average at each distance for your analysis. When you finish measuring light intensity, move the water bath and plant back into the light path.

Table5.5. Light intensity at different treatments (optional). Light meter readings (µmol photons per meter squared per sec)

Treatment	Height = 5 cm	Height = 9 cm	Height = 13 cm	Average

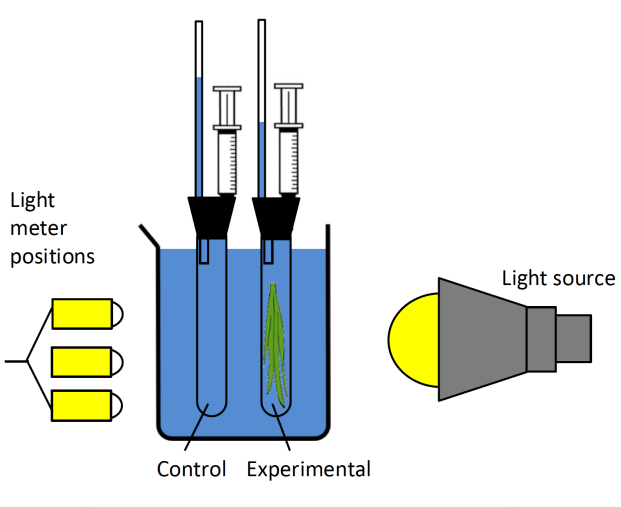


Figure 5.3. Measuring light intensity.

NOTE: Do not place your light meter in the water! The illustration above shows where you should take light intensity readings.

Analysis

1. To complete this part of the lab activity, please read through the Using Excel for Data Analysis and Graphing Interchapter for instructions (pages 51–55).
2. Use Excel to **plot** your data from ***Table5.3*** in a single graph that includes scatter plots of oxygen production as a function of time for each of your treatments and fit straight lines (use the 'Trendline' tool) to each dataset. Attach your graph to the assignment. The slopes of these lines provide you with Vmeas values.

Table5.6. Rate of change of Oxygen production.

Treatment	Vmeas (mL O2/min)

3. **Record** the temperature and air pressure in lab and **convert** the raw slopes from the previous step (rates of oxygen production, V_{meas}) for each of your treatments to standard temperature and pressure ($V_{STP,}$ see equation below). Divide the STP slope by the plant mass to find the mass- specific STP

slope. Fill out ***Table 5.6*** in the assignment for this lab for full credit.

NOTE: You may need to convert some of your values before using this equation.

$$V_{meas} \times \frac{273K}{T_{meas}} \times \frac{P_{meas}}{760Torr} = V_{STP}$$

Lab temperature (T_{meas}):

Lab air pressure (P_{meas}):

NOTE: Before you use this equation, T_{meas} and P_{meas} **must be in units of degrees Kelvin (K) and torr (Torr)**, respectively. If you need to convert your measurements you can do so with the following equations:

K = _____ °C + 273.15

torr = 25.40 × ____ inHg

4. Use Excel to **plot a derivative** (rate of change) **graph** illustrating how the rate of oxygen production (mass-specific, STP'adjusted values) changes with treatment. **You should use mass-specific V_{STP} values (last column of Table 5.7) in the derivative graph.** If you used a continuous variable for your treatment, include a trendline that best describes the relationship between the net rate of oxygen production and your treatment variable. **You will need to upload your Excel file to Canvas for submission.** Read the Excel Inter-Chapter for more information on how to construct a derivative plot.

Results: Part I: Absorption Spectrum

1. Upload your graph of light absorption spectrum for chlorophyll to the Week 5 post-lab assignment on Canvas.
2. Use this plot to **predict** the color(s) of light that is/are most active in stimulating photosynthesis. **Write** your prediction, and **explain** in writing how your data justify your prediction below.

Results: Part II: Photosynthetic Rate

a. Upload your graph plotting data from ***Table 5.2*** with associated trendlines to the Week 5 post-lab assignment on Canvas.

b. Fill ***Table 5.7*** below, **converting** the raw slopes for each of your treatments to standard temperature and pressure. Divide the STP slope by the plant mass to find the mass-specific STP slope.

Table5.7. Worktable to adjust measured volumes/min to STP.

Treatment	Vmeas (mL/ min)	273 K/ Tmeas	Pmeas/760 Torr	VSTP(mL@STP/ min)	Plant mass (g)	Mass- specific VSTP (mL@STP/ min/g)

c. **Upload your derivative (rate of change) plot to the Week 5 post-lab assignment on Canvas.**

d. Was your hypothesis supported or refuted by your data? Why?

LABORATORY 6.

BACTERIAL TRANSFORMATION WITH RECOMBINANT PLASMID DNA

Objectives

Following this lab, students will be able to:

- Compare and contrast plasmid and genomic DNA
- Perform a bacterial transformation
- Explain selection media and reporter genes and their significance in transformation
- Week 7: Describe observations following bacterial growth and assess the efficiency of transformation

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

- Biological Science (8th ed.), Freeman *et al*. 2024, DNA and genes (pp. 334–350), Chapter 15
  - The Molecular Revolution (pp. 422–442), Chapter 20
- Canvas resources

Introduction to DNA and Plasmids

For this lab, you should know the basics about DNA function and structure. DNA encodes the genetic information required to build the proteins that make up the biochemical machinery of every cell. This week we will be investigating DNA using bacteria. Bacteria contain a single large DNA molecule called **genomic DNA** which includes the genes necessary to code for most proteins needed by a cell. Some bacteria also contain smaller circular **plasmid** DNA molecules: accessory DNA that carry relatively few genes. These genes can be passed from one cell to another in conjugation and if released by one cell, through cell death or injury, can sometimes be taken up by another bacterium. The process of uptake of DNA by a bacterial cell from solution is known as transformation that may result in genetic change.

Transformation

Any gene in a plasmid can be transferred during transformation. The plasmid that you will transform carries two genes. The two genes we'll be studying in lab this week — green fluorescent protein and antibiotic resistance. The gene expression of antibiotic resistance in the bacterium indicates a successful transformation. A selection medium is a growth medium that will allow a bacterium with the targeted gene (from the plasmid) to survive, but will not allow bacteria without that gene to grow. The expression of the green fluorescent protein gene on the plasmid provides another way to confirm transformation. Green fluorescent protein (GFP), originally identified in jellyfish, absorbs ultraviolet light and emits green light (longer wavelength, lower energy photons), but doesn't affect the survival of the bacteria on a selection medium. The gene coding for GFP was isolated, and GFP is now widely used as a marker for genetic studies. Normal bacteria do not express GFP, so expression of GFP shows which bacteria were transformed. We call such a marker a **reporter gene**. It cannot, however, be used like an antibiotic resistance gene to select just the bacteria which were transformed.

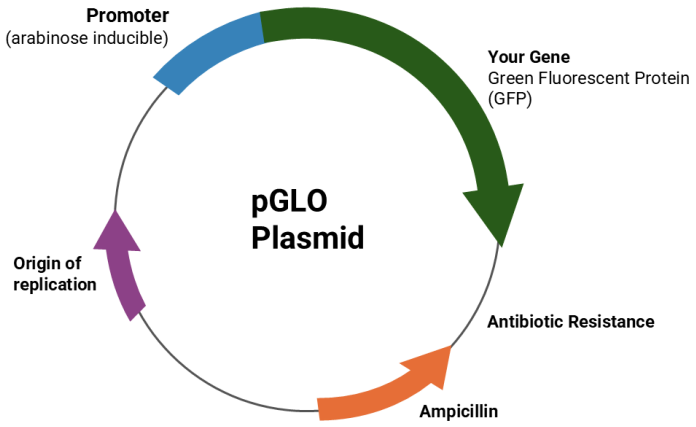


Figure 6.1. pGLO Plasmid.

Adding new genes to a plasmid (= ***recombinant DNA***) and inserting the plasmid into a bacterium is one kind

of genetic engineering. In the lab, we can treat the bacteria with calcium chloride and heat shock to make the bacteria's membranes leaky, making plasmid uptake more likely.

If you insert a new gene into a plasmid, you can use it to change the genetics of the bacterium and get it to make proteins that it does not normally make. The drug industry has utilized this technique to get bacteria to make human growth hormone, insulin, clotting factors, interferons, erythropoietin, and other human proteins. However, plasmid transfer and recombination also occur naturally, which is the basis of rapid evolution of multi-drug resistant bacteria. Any plasmid incorporating an antibiotic-resistance gene spreads rapidly through bacterial populations where antibiotics are frequently used, including those in hospitals and some animal husbandry operations.

In today's lab, you will do a simple transformation using *Escherischia coli* (*E. coli*) bacteria and plasmids that carry 2 genes. One plasmid gene blocks bacteria-killing effects of the antibiotic **ampicillin**. A second plasmid gene codes for synthesis of green fluorescent protein (GFP), a jellyfish protein that fluoresces green light when excited by ultraviolet light. In these plasmids, the GFP DNA has been linked to other DNA that normally functions as a promoter for expression of a gene used to metabolize the sugar arabinose, so GFP expression requires presence of arabinose in the culture medium. The plasmids (from another bacterium) can act as a **vector** for ampicillin resistance and green fluorescent protein expression. In genetic engineering, a vector is a biological tool (plasmid, virus, etc.) used to facilitate gene transfer. Either trait, in connection with an ampicillin selection medium or ultraviolet light, permits fast identification of successful transformations.

Transformation experiments depend on:

1. a **host** in which to transform gene (*E. coli*);
2. ensuring that selected host is **competent** (treatment with calcium chloride and heat shock) to take up new DNA;
3. a **vector** to carry gene (plasmid); and
4. a method (usually selection media) to identify and isolate host cells that took up and now express the new DNA.

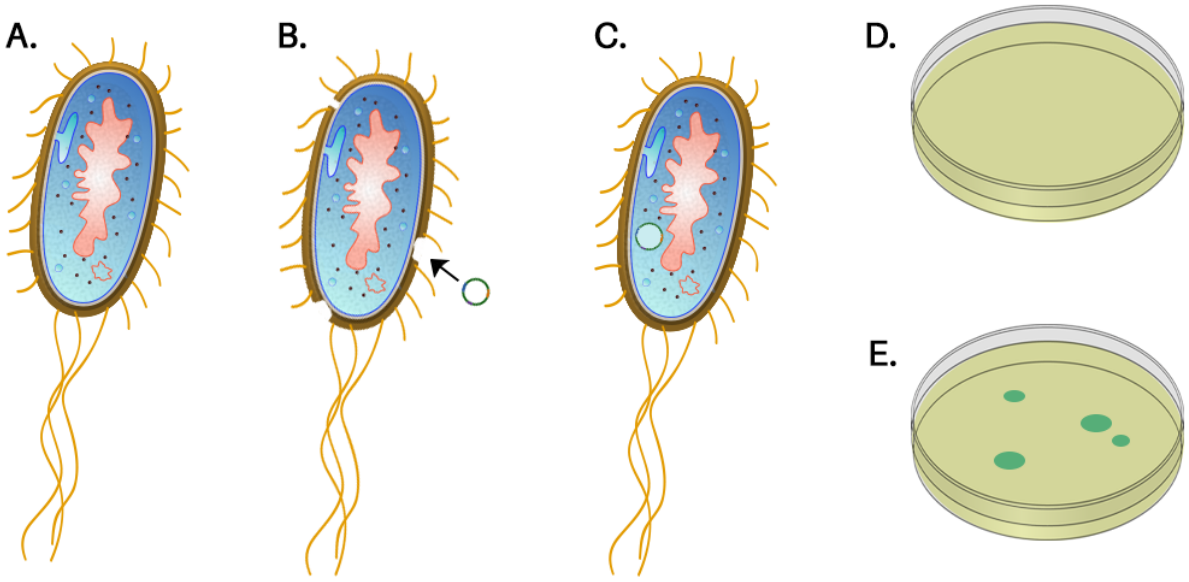


Figure 6.2. Description of bacterial transformation. A. Here is an *E.coli.* bacterium in natural state. Notice how bacterial DNA is circular. B. Extreme cold causes pores (small holes) to appear in the bacterial membrane. Small DNA molecules like our plasmid can move through these holes! C. When the bacteria are heated again, some end up with our plasmid inside them. These are *transformed* bacteria. D–E. We can filter out the untransformed bacteria (the ones that got no plasmid) by growing the bacteria in an antibiotic-containing medium. Untransformed bacteria are killed by the antibiotic in the medium, whereas transformed bacteria grow. Image created by Abbey Elder. E. coli graphics adapted from "Escherichia coli" by the DataBase Center for Life Science, CC BY 4.0.

Part 1: Bacterial Transformation Experiment

NOTE

The techniques needed to do a bacterial transformation require careful attention to details, timing, and sterile technique. Sloppy technique will yield no results and no points!

Note that plasmid concentration is 0.08µg DNA/µl plasmid solution.

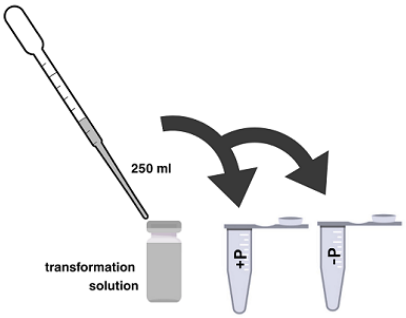
Sterile technique begins with sterile supplies – all materials provided to you in lab have been sterilized for you. It will be important that you begin the lab with a sterile workspace. Ethanol wipes provided at your station should be used to wipe down the bench and inside surfaces of the sterile shield. As you progress through the lab, it will be important that you are mindful of sterile technique. Complete the entire lab activity INSIDE the sterile shield. Wear gloves. Do not re-use pipette tips or sterile loops. Be careful not to touch pipette tips or loops to any surfaces inside your sterile shield.

Properly dispose of all items you use during the lab activity. Carefully following sterile technique will reduce the likelihood of contamination on your experimental plates.

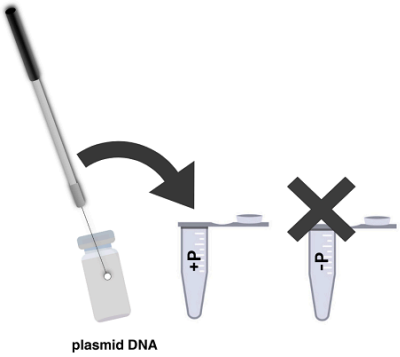
Step 1: Make Competent Cells

1. Work with your lab group to find and surface-sterilize an open sterile shield. Once sterilized, bring two microcentrifuge tubes to your station.
2. Label one closed sterile microcentrifuge tube + plasmid (or +P) and another –plasmid (–P). Label both with your group's name.

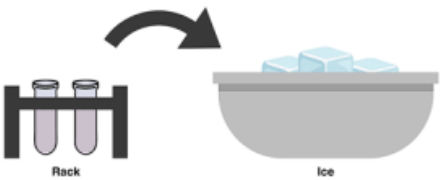
3. Grab a microcentrifuge tube of cold Calcium Chloride ($CaCl_2$) from the appropriate station. Uncap the –P tube and use a micropipette with a sterile tip to add 250 µL of cold 50mM $CaCl_2$. Recap –P tube and repeat with +P tube.
4. Find the petri plate already in your sterile shield containing Luria agar on which *E. coli* lacking genes for ampicillin resistance and GFP production have been growing for about 12-24 hours at 37°C.



5. Use a sterile loop to remove a single colony from the *E. coli* plate. Be careful not to pick up any agar. Select the –P tube and immerse the loop into the $CaCl_2$ at the bottom of the tube. Twirl the loop between your index finger and thumb until the entire colony is dispersed in the solution. Cap the tube, vortex, and place it on the ice. Pack ice around the tube.
6. Using a new sterile loop, add bacteria in the same way to the +P tube. Then ask your TA to use a micropipette to transfer 10 µL of plasmid DNA containing the ampicillin-resistance and GFP genes to the +P cell suspension. Cap the tube, vortex the contents, and place it on the ice. DO NOT add plasmid to the –plasmid tube. Why?

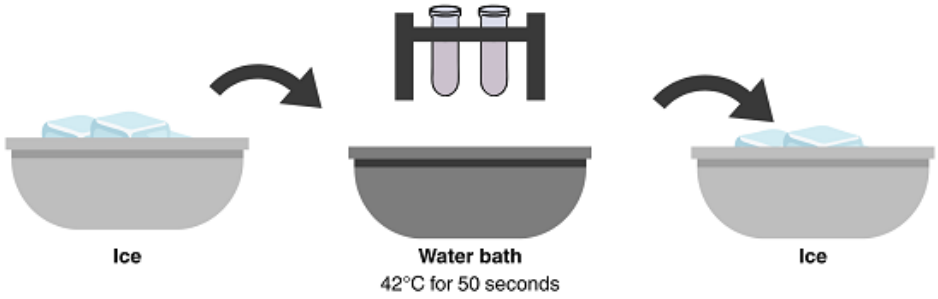


7. Incubate both the tubes in direct contact with ice for at least 10 minutes. You can use this time to label your petri plates. See step #10 for labeling instructions.



Step 2: Perform the Transformation

To be successful in getting *E. coli* to take up the plasmid DNA, follow the temperature and timing in these steps **exactly**. Make certain that the part of the tube containing the bacteria makes good contact with crushed ice or the warm water bath.



Steps 8–9

8. After 10 minutes, remove both tubes from the ice bath, and **immediately** place them in a 42°C water bath (in the Styrofoam floater) for 50 seconds to heat shock the cells. After 50 seconds, return both tubes **immediately** to the ice for at least 2 minutes (longer is better).
9. Remove tubes from the ice and obtain a test tube with Luria broth. Open the +P tube and use a micropipette with a sterile tip to add 250 µL of Luria broth. Recap the tube and gently mix the contents. Repeat the procedure with a new sterile tip for the –P tube. Then incubate both tubes at room temperature for 10 minutes.

Step 3: Plate the Bacteria on Selective Growth Media

To assess the results of your transformation experiment, you will grow both +plasmid and –plasmid bacteria on three different kinds of culture media:

Luria agar + arabinose	(ara)	Blue stripe
Luria agar + ampicillin	(amp)	Orange stripe
Luria agar + ampicillin + arabinose	(amp+ara)	Blue and Orange stripes

Which of these six plates will act as your control(s)? Why is it important to include them in your experiment?

10. While your tubes are incubating, obtain two plates of Luria + arabinose agar, and label the bottom (the agar side) of both plates with "ara," your initials, and date. Label one plate +P and label the other –P. Repeat for the other two kinds of agar, labeling them "amp" or "amp+ara." You will have a total of six labeled petri plates.

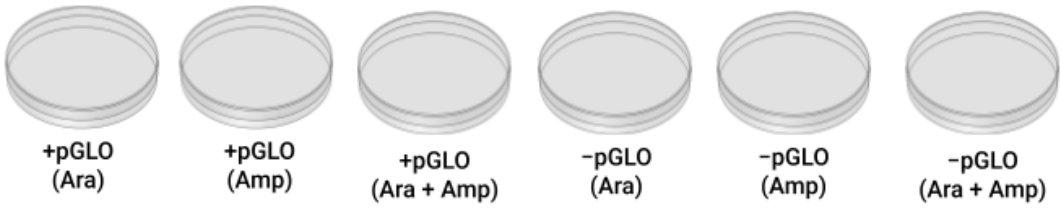


Figure 6.3

11. Follow these directions to inoculate the –plasmid plates with the –plasmid bacteria.

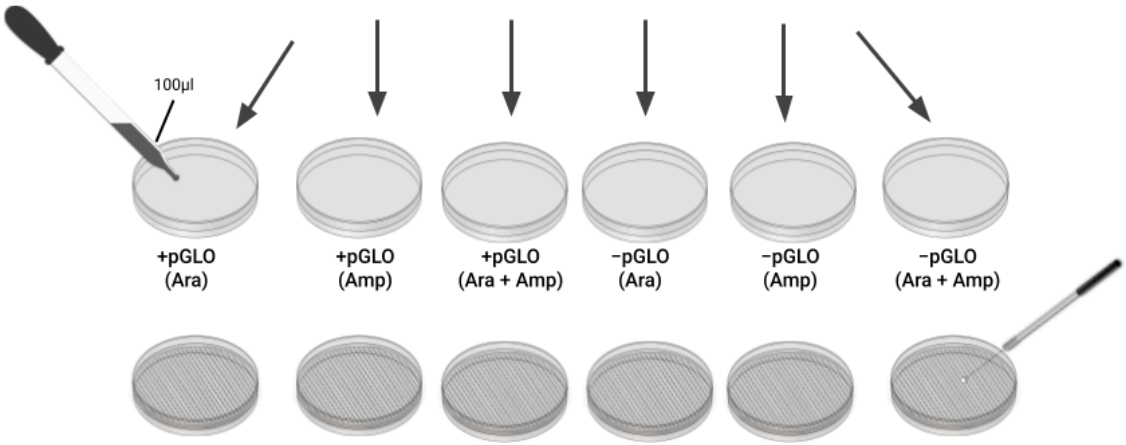


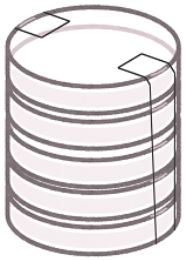
Figure 6.4

a. Gently mix the contents of your –P tube again, then using a micropipette with a sterile tip, pipette 100 µL of the bacterial suspension onto each of your three –plasmid plates. Recap the tube.

b. Use a sterile loop to spread the bacterial suspension evenly across the surface of each plate by skating the flat surface of the loop across the agar. Rub back and forth, gently, across the whole surface of the plate, then repeat at a right angle to your previous direction. Use a new sterile loop for each plate. Be careful not to dig into the agar.

13. Now inoculate the three +plasmid plates with your +plasmid bacterial suspension, following the procedure in step 11 that you used for the –plasmid plates.

Step 4: Plate Incubation and Clean Up



13. Stack your +plasmid plates and tape them together. Likewise, stack and tape the –plasmid plates. Label the tape with your group name and section number. Place the plates lid side down in a bin that has been designated by your TA. The plates will be incubated at 37 °C until the ampicillin/+plasmid plate has visible colonies (about 18–24 hours). Your TA will then move the plates to a refrigerator until next week's lab.
14. Use the signs posted in the lab and instructions from your TA to make sure you dispose of waste in the appropriate locations. Used sterile loops should be placed into the collection bin at the back of the room. Source E. coli plates, used microcentrifuge tubes, gloves, etc. should be placed into the auto-clave bin for disposal.
15. Clean up under your sterile shield to prepare the lab station for the next group of students. Follow the clean-up instructions provided in lab.
16. During week 7 lab you will analyze your data and turn in your post-lab worksheet during class.

Predictions

Based on the information provided in this lab and the set-up you have just completed, predict what you will see on each plate next week. Consider whether or not you should see growth of bacterial colonies, whether those colonies will be isolated or uniformly cover the plate, whether you will see flourescence, and if so, then whether that flourescence will be present in all colonies. Fill out the below table describing what you expect to see for each of the treatments.

Without Plasmid		
Arabinose	Ampicillin	Ampicillin + Arabinose
WithPlasmid		
Arabinose	Ampicillin	Ampicillin + Arabinose

INTER-CHAPTER EXTRA

BACKGROUND SECTION WORKSHEET FOR WEEK 7 PRESENTATIONS

Your group should complete this activity together during the Week 6 lab. Once it is completed, **show it to your TA as a group** and discuss with them **during class**. Your TA will check that your references are appropriate and that you are prepared for the presentation. This worksheet is worth 3 of the 17 points associated with your Week 7 Presentation (see complete rubric on canvas).

This worksheet accompanies the Week 7 student presentations and will give you some practice:

- finding and understanding primary scientific literature
- making connections between concepts explored in lab activities and real-world applications

In your presentations, you are asked to address the "Overall topic":

> "provide some background on the topic and why it is important (big picture: why do people study this topic? What is its importance in everyday life?) You should do some background research and include at least two primary scientific references (see Week 1 info and Citations Inter-chapter in your lab manual on p. 17)"

This worksheet will help you plan what you will say for the background part of the presentation. Find at least two scientific articles that describe a study or experiment related to your topic. Be prepared to talk about these articles in the context of your own experiment and the broader scientific topic you tested.

Requirements for this worksheet and your presentation:

- Answer the following questions as a group.
- Properly cite your sources in question #5 and also on a reference slide at the end of your presentation (see pp. 17–19 of your lab manual).
- Use your own words. When you cite sources, you should paraphrase, not direct quote.

1. **What week (Enzymes, Fermentation, or Photosynthesis) were you assigned to? What variable did you test?**

2. **Briefly summarize your topic.** [E.g., depending on your assigned topic, address: what are enzymes

and how do they work, what is fermentation and when is it used versus cellular respiration, what is photosynthesis and how does it work?, etc. You may use your textbook and lecture notes.]

3. **Importance of topic.** [What is the importance of this topic to humans and our planet?]

4. **What is a primary article?** [How does it differ from secondary or review articles?]

5. **List your two articles in proper citation format.** *Use "et al." after the third author to shorten the citation. [Make sure they are primary articles. Check with your TA if you are not sure.]

6. **Briefly summarize the findings of each article and their importance.** [Think about: Why were these studies done? How do the findings increase understanding of these topics? What fields or industries (e.g., health, food science, ecology, etc.) could have applications for these findings?]

7. **How do the studies in the primary articles connect to what you did in your experiment?**

LABORATORY 7.

DNA TRANSFORMATION RESULTS AND STUDENT PRESENTATIONS

Objectives

Following this lab, students will be able to:

- Plasmid Transformation into Bacteria
    - Identify transformants
    - Calculate transformation efficiency using your results
- Student Presentations
    - Communicate results of an experiment you conducted to classmates
    - Explain how different variables can affect the rates of:
        - an enzyme-mediated reaction
        - fermentation
        - photosynthesis

1. Results from DNA transformation. Describe growth (lawn, isolated colonies and number of colonies, or none). Do any colonies show green fluorescence? If so, how many and what fraction of the total show fluorescence? Are all the cells in a colony fluorescent, or do cells around the periphery look different in any way from cells in the center of the colony? Make sure you describe what you actually see and not what you expected.

a.

Without Plasmid		
Arabinose	Ampicillin	Ampicillin + Arabinose
With Plasmid		
Arabinose	Ampicillin	Ampicillin + Arabinose

b. Your score depends on your results. Did your results match your predictions for what would happen? If not, you can improve your score by identifying any plate which showed unexpected results and explaining why you might have gotten that unexpected result. Be specific about possible causes – an undefined "mistake" is not an adequate explanation. You must explain exactly what sort of mistake or other process might have led to the unexpected results you observed.

2. **Transformation efficiency**: Observe your +plasmid amp plate and +plasmid amp+ara plate. Count the number of colonies you see on each plate, calculate the average, and estimate the transformation efficiency for your experiment by filling out the table and following the instructions below. Remember that each colony includes many thousands of bacteria but resulted from the growth and replication of a single bacterium.

Plate	# Colonies
+ plasmid ampicillin	
+ plasmid ampicillin + arabinose	
Average	

In this experiment, there was 0.157 µg of plasmid DNA per plate. Using this information and your average colony count, you can determine how many bacteria you transformed per microgram (µg) of plasmid DNA (how successful your transformation was). Use the equation below to calculate your transformation efficiency.

Transformation efficiency = Average # colonies on amp plates ÷ µg plasmid DNA on plate

Your Transformation efficiency is _____ units _____

3. What information can you derive from transformation efficiency values? How can knowing the transformation efficiency help you in planning future transformation experiments?

Student Presentations

Student groups will present on the experiment they conducted in either Week 3, 4, or 5. Guidelines for the student presentations are posted on Canvas and more information will be provided by your instructor.

INTER-CHAPTER 4.

MICROSCOPE AND SAMPLE PREPARATION

Objectives

- Understand how to use the lab microscopes and cameras
- Learn how to prepare specimens for viewing under the microscopes

Resources

1. Compound Scope Tutorial (video tutorial, linked on Canvas, and available in student folders in the lab)
2. Stereomicroscope Tutorial (video tutorial, linked on Canvas, and available in student folders in the lab)
3. How to use Preview (video tutorial, linked on Canvas, and available in student folders in the lab)
4. Using Leica Software (video tutorial, linked on Canvas, and available in student folders in the lab)

Special Skills

- Using the lab microscopes (may have several types in your classroom)
- Preparing plant and animal sections (making wet-mount slides)
- Taking digital images with microscope cameras

NOTE

Preparing specimens for examination under a microscope requires a special set of skills that will need to be practiced. Don't expect to get it right on the first try. Be patient and persistent as you work on these skills – you can do it!

Using Lab Microscopes

There are two types of microscopes available for use in the Biology 2120 Lab – the compound microscope and the stereomicroscope (dissecting microscope). Your classroom may have multiple brands or models of scopes, but all will fall into these two categories. For your convenience, there are stereomicroscopes and compound microscopes with digital cameras in each lab classroom, so you can take digital photos of the

specimens you view in lab. There are a limited number of scopes with digital cameras in each room. Please be certain to share these scopes with your classmates so everyone has an opportunity to utilize the cameras.

The **stereomicroscope (dissecting microscope)** assists in your ability to look more carefully at lab specimens that do not require high power viewing (the stereomicroscope will allow you to zoom in to magnify a specimen 8–35×). Magnification of a microscope is the product of the power of the lens in the eyepiece and of the objective. If you want to look at the surface of a plant leaf or look carefully at a dragonfly's wings, this microscope would adequately meet your needs. However, if you wanted to look at the cells inside the plant leaf or the cells that make up the hairs on the dragonfly's wings, you will need a more powerful microscope. In those cases, you would need to prepare a microscope slide of the plant leaf or dragonfly wing and examine it using the more powerful compound microscope. In the case of the plant leaf, you would also need to section it to expose the cells on the interior of the leaf.

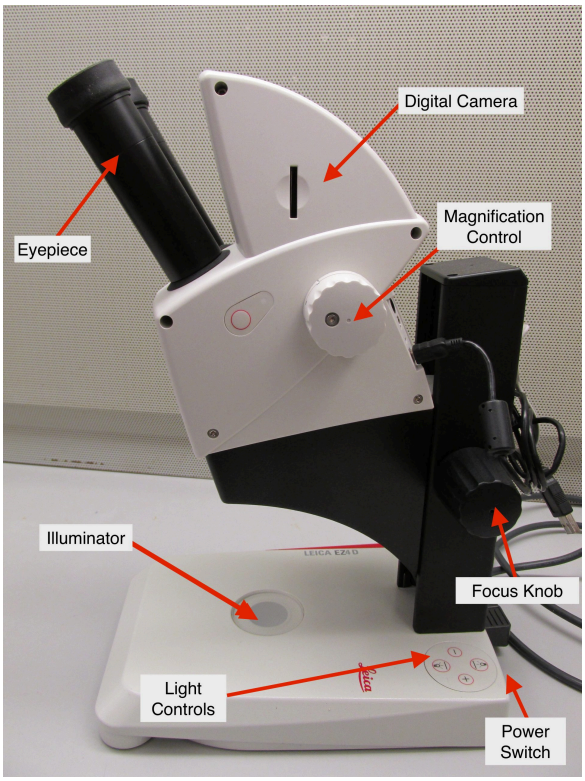


Figure IC4.1. Labeled stereo microscope with digital camera used in Biology 2120L.

The **compound microscope** has 3 objectives that will allow you to magnify the specimen 4×, 10×, or 40× (a few of the compound scopes have a 4th objective that will allow you to magnify by 100×, if using oil immersion). None of the specimens you will view in lab require any magnification greater than 40× (the oil immersion lens will not be utilized in lab). The first week that you will be utilizing the microscopes will be the week in which you study plant cells and tissues (starting week 8). The majority of the specimens you will observe during that week's lab will require you to use the compound microscope to view cell types inside plant structures.

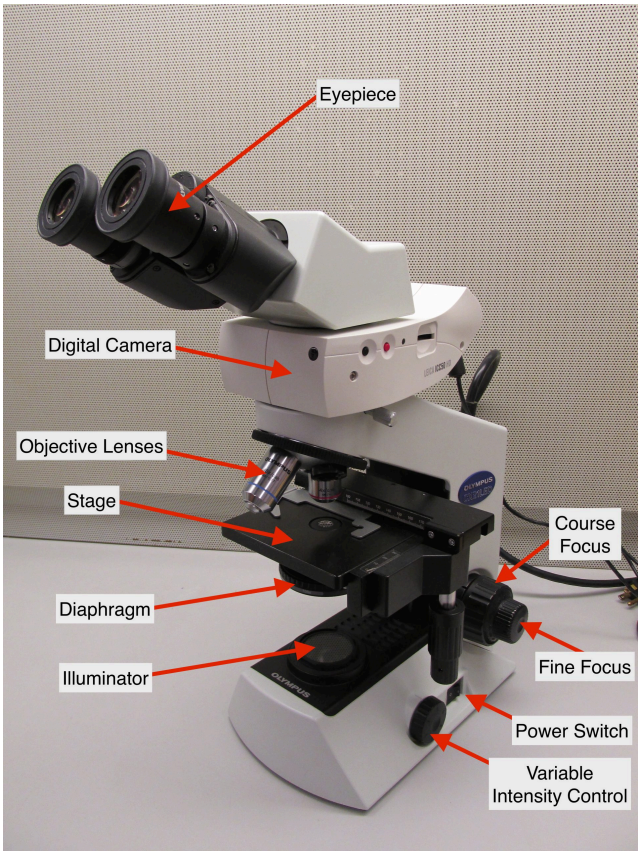


Figure IC4.2.Labeled compound microscope with digital camera used in Biology 2120L. Not all compound microscopes available in the lab are the model pictured, though all have the same parts. You should familiarize yourself with the model you use in class so you are able to get the most out of it.

The microscopes used in lab are valuable pieces of lab equipment. If you use them correctly and with care, they will be of tremendous help as you view specimens for the remainder of the semester. Your TA will spend a few minutes providing you with a brief lesson on how to use the microscopes. In addition, video tutorials describing how to use the lab microscopes can be found both on Canvas and on the laptop computers in lab. Before beginning your lab activities, make sure that you watch the video tutorials and spend some time familiarizing yourself with both types of microscopes. If you have questions, don't hesitate to ask your TA.

Taking Digital Images with Microscope Cameras

Many of the microscopes in lab have a digital camera attached to them, allowing you to take digital images of the specimens you view in lab. This is a great way for you to create your own study guides. You can take digital images of any (or all) of the specimens you see in class. You should then use Cybox or email to save these images for future viewing at home.

You will be required to use the digital cameras periodically during lab for assignments. The assignments associated with the plant anatomy and physiology labs include taking and labeling digital images of various

plant cells and tissues. Please watch the "Using Leica software" and "How to use Preview" video tutorials on the laptop computers in lab before taking your first photo. These video tutorials are also posted on Canvas so you can review them as often as needed.

You should note that all lab laptop computers have a program called "DeepFreeze" installed on them. This program deletes all student files and returns the computer to default settings each time the computer is restarted. You should be sure to save all your files carefully using Cybox or email in order to ensure that you will have them for later use. If you do not carefully save your files, they will be automatically deleted. This also means that you should be mindful of the power source for the lab laptops while they are in use. If the battery runs out on the computer during use, all student files will be lost.

Preparing Specimen Sections (Wet- Mount Slides)

Preparing your specimens for viewing under a microscope is an invaluable skill to learn during lab. To view the specimen properly, you will need to prepare it so you can find the cell or tissue types during your examination. If you do not prepare the specimen correctly, you may not be able to see the structures you are asked to view.

When making a slide of plant (or animal) material to look at under the microscope, it is important to make sure that you section, or slice, the specimen as thinly as possible. When you place the slide onto the microscope, light will pass through it from below, illuminating the specimen for viewing. If your section is too thick, the light will not be able to pass through the specimen and you will not be able to see the desired structures.

Safety Check

Be careful when you cut through specimens. We use very sharp razor blades in the preparation of wet mounts – please hold the specimens carefully and make sure that you do not cut yourself in the process of preparing them for viewing. Make sure you know the location of the first aid kit in your laboratory classroom, in the event that you need it.

When you prepare slides for viewing under a microscope in lab, you will be suspending your sample (plant or animal) in a thin layer of water. This type of preparation is called a **wet mount**. The process for making a wet mount is demonstrated in ***Figure IC4.3*** below.

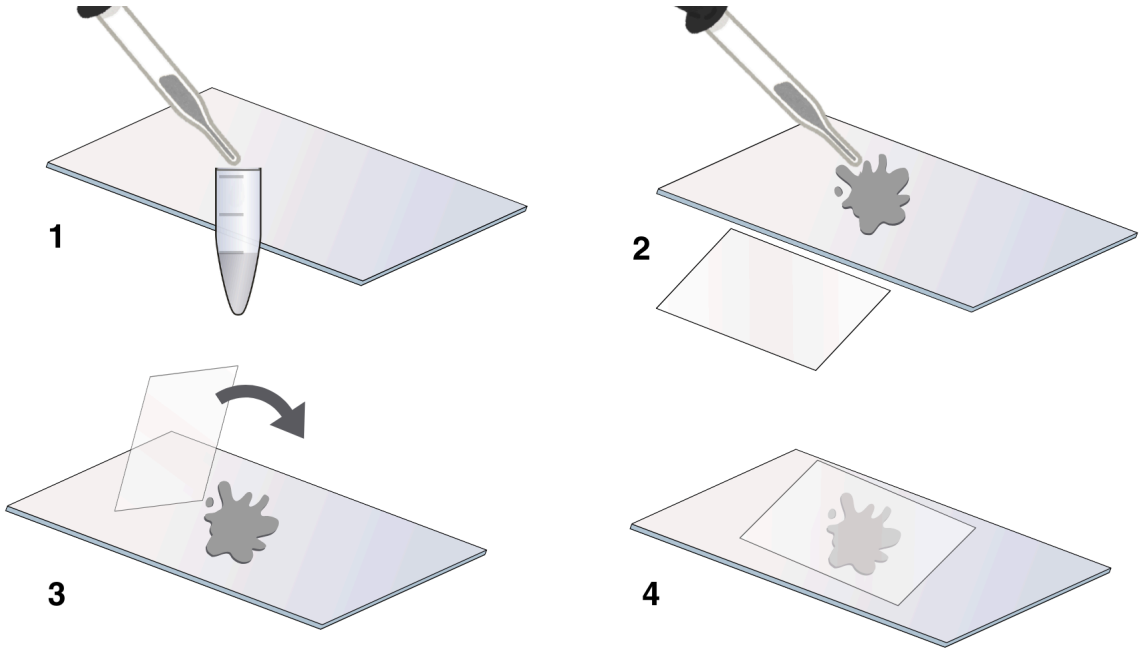


Figure IC4.3. Demonstration of the process of making a wet mount slide for viewing specimens in the lab.

You will need to know the difference between several types of slide preparations. If you are working with very small, very thin specimens you can create a "**whole mount**". In this case, you will carefully place the entire specimen onto your microscope slide.

A "**cross section**" requires you to cut through a specimen at a right angle to the long axis in order to take your sample. A very thin section of the specimen will enable you to view the internal structures more clearly.

A "**longitudinal section**" of a specimen also requires you to cut a thin section of the specimen in order to see internal structures more clearly, but in this case, you cut through the specimen in a plane that is parallel to the long axis to take your sample.

A **tissue peel** requires you to take a very thin sample from the outermost tissue of a plant specimen. In this case, you very carefully peel the outside layer of tissue off of the specimen to get a very thin (preferably one cell thick) sample, , as shown in ***Figure IC4.4***. These preparations can also be called **epidermal peels** because they are often used to peel only the epidermal cells from a plant specimen. One of the specimens which will require this technique is the red onion. To complete the epidermal peel, you will need to carefully peel off only the red (or purple) epidermal layer of cells. When preparing an epidermal peel, you want to make sure that you have a drop of water on your microscope slide before you begin. The sample will be very fragile and has a tendency to roll or stick together if you do not immediately place it into water. Toothpicks are provided at each station in lab and can be of great assistance in rearranging and unrolling a specimen that was not initially lying flat against the glass slide.

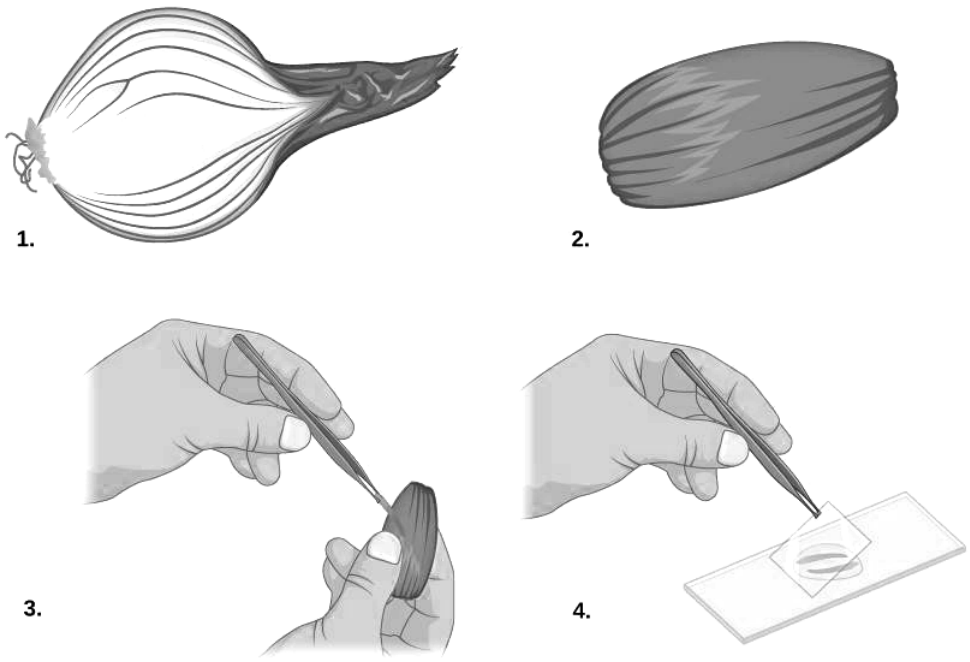


Figure IC4.4. Preparation of an onion tissue peel. Image Source: Lab Manual for Biology, edited by Kelly, Frank, and Payne, CC BY NC SA 4.0.

Important Note

In order to properly view several specimens in lab, it will be necessary to add a stain to your slide so specific structures are more readily visible to you when you view the specimen under the microscope. Specimens that require staining are noted in their description in the lab manual. Each type of stain requires a specific protocol in order to provide reliable results. The instructions for the necessary stains are provided in your lab manual within the topic in which they will be utilized. Make sure that you review the stain protocols carefully before attempting to prepare your specimens.

NOTE

Before you leave the microscope station, make sure that you do not leave any slides on the microscope, that the microscope stage and lens are clean, and the microscope is shut off when you are finished with it.

LABORATORY 8.

PLANT CELLS AND TISSUES

Objectives

Following this lab, students will be able to:

- Properly prepare plant specimens and obtain images of them using lab microscopes and cameras
- Identify key structures in plant cells (e.g., cell wall, vacuoles, plastids) and describe their functions
- Identify different types of plant cells and tissues and describe their functions
- Describe how the morphology of different types of plant cells and tissues connect to their functions

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

- Compound Scope Tutorial (video tutorial, linked on Canvas)
- Stereomicroscope Tutorial (video tutorial, linked on Canvas)
- How to use Preview (video tutorial, linked on Canvas)
- Using Leica Software (video tutorial, linked on Canvas)
- Freeman *et al.* 2024. Biological Science (8th ed.), pp. 751–764. Chapter 34.2
- Botany Photo Atlas (provided for use in lab)
- Canvas Resources

Laboratory 8: Introduction to Plant Cells and Tissues

Plants do not move like animals but require several resources like gases, sunlight, water and minerals for their survival. Plants have evolved diverse forms so that they can harvest these resources successfully and build and maintain their bodies. Plants have an above-ground portion called a shoot system and below-ground portion called a root system. Plants are able to continue to grow these elements throughout their entire life cycle, which is called indeterminate growth.

Plants and animals are both eukaryotes. Therefore, their cells contain many of the same types of structures and organelles. There are some key differences, however, that reflect the different functions these cells perform. Like animal cells, plant cells have cell membranes. In addition, plant cells have an outer cell wall made up of strong cellulose fibers. The cell wall consists of an inner primary and outer secondary cell wall. Plant cells also have chloroplasts and plastids that carry photosynthetic pigments. Plant cells have a large vacuole that is centrally located; it helps the cell maintain osmotic pressure and can act as storage for metabolic enzymes or waste products. Plant cells are connected to each other with the help of cytoplasmic connections known as plasmodesmata. These connections allow for cell-to-cell communication and help transport solutes between cells.

Plant cells are organized into group of cells that can function as a unit, called tissues. Simple tissues contain only one type of cell, while complex tissues contain several types of cells. Plant tissues are further organized into tissue systems that are based upon different structural features, their function and where they are localized within the plant. There are three tissue systems in the plant: **Dermal**, **Ground** and **Vascular**.

The dermal system forms the outermost layer of the plants. It acts as a protective barrier, separating the inside of the plant from the external environment, similar to the skin of animals. It is largely made up of living cells. Epidermal cells are flattened, lack chloroplasts and secrete a waxy substance called a cuticle on the external surface of the plant. Plant surfaces and leaves have specialized structures known as **stomata** (singular: stoma) that help to exchange gases. They consist of an opening, or pore, that is surrounded by two guard cells. The guard cells can change the size of the pore, which allows the plant to regulate the passage of gasses and rate of evaporation.

Some epidermal cells have specialized appendages called trichomes that provide protection. Trichomes come in different forms and therefore perform different protective functions, including reflection of excess sunlight, retention of moisture, protection from feeding or predation.

The ground tissue system makes up much of the interior of plants. It consists mostly of living cells that are involved in metabolism and provide support to the plant body.

The three main types of ground tissue cells are parenchyma, collenchyma and sclerenchyma. Parenchyma cells represent simple living tissue with thin primary cell walls. These are the most abundant living and versatile cells in plants. They may contain chloroplasts and perform photosynthesis or they may have storage functions. They are also found in meristems, where they rapidly divide to form new plant structures. Col-

lenchyma cells are living cells with an unevenly thickened primary cell wall. They are found in simple tissue that functions primarily to provide support during bending and stretching of shoots.

Sclerenchyma cells also provide support, but they are dead at maturity. Sclerenchyma comprises of two types of specialized cells: fibers and sclereids. Fibers are extremely elongated cells, whereas sclereids are relatively short cells and are of variable shapes. They're often found in the tough coats of seeds and in the pits of nuts.

The vascular system is made up of both living and dead cells and functions to transport water and solutes from one part of the plant body to the other. We will look more closely at vascular tissue in next week's lab.

Preparation

You will be utilizing the laboratory microscopes, cameras, and computers in lab today as you observe the specimens provided. Before you begin your observations, make sure you familiarize yourself with the microscopes and cameras so you can use them effectively. Read through the Microscope Interchapter on pages 93–98 and watch the related video tutorials posted on the lab laptops and on Canvas.

The lab microscopes and cameras are helpful tools that you can utilize to create your own study guide. As part of your lab assignment, you will be required to submit labeled photos of two specimens to create study pages. You are welcome to take photos of any of the specimens for review or study guide use, but make sure that you are taking your own photos. Submitting a photo that was not taken **by you** in lab is an example of academic dishonesty and will not be tolerated in lab.

In order to learn about plant cells and tissues, it will be important for you to spend some time looking carefully and critically at the plant material provided to you in lab. The types of plant cells and tissues observed in this week's lab can be found in many different types of plants in nature, but specific specimens have been selected for you to investigate in lab. These specimens should allow you to reliably see specific cell or tissue types.

Examining plant cells and tissues requires a special set of skills that develop *over time* and with *consistent practice*. Don't expect to get it right on the first try. Be patient and persistent as you work on these skills—you can do it!

Special Skills

- Using the Lab Microscopes
- Taking Digital Images with Microscope Cameras
- Preparing Plant Sections (wet-mount slides)
- Staining Plant Specimens with Phloroglucinol
- Staining Plant Specimens with Iodine Potassium Iodide (I_2/KI)

There are several specimens to be viewed in lab this week, many of which need to be carefully prepared before viewing. Where possible, we have chosen to include prepared slides in order to reduce your preparation time. However, it is important to make sure that you know how to prepare specimens for careful viewing. Working with a lab partner to help share the workload is fine, but make sure you know how to section, stain, and prepare the specimens. When you look at the slides under the microscope and take photos, make sure that you do this on your own. Sharing photos of specimens with other students is an example of academic dishonesty and will not be tolerated in lab.

See Interchapter 4 for information on preparing wet-mount slides on pages 93–98. Several of the structures and cell types that you will examine in lab today will be easier to see if they are stained. There are two stains available in lab today – Phloroglucinol and Iodine Potassium Iodide (I_2/KI). Each of these stains has properties that make them a good choice for staining specific structures. The most appropriate stain will be available at the station for you to use. Make sure that you follow the instructions below so that the staining is as effective as possible. Please use the stains with caution. Phloroglucinol is an irritant and Iodine Potassium Iodide is corrosive. Both could stain clothing if spilled. Consult with your TA if you have questions about the stains.

Before lab, your TA will have prepared small pieces of fruits and vegetables for the students in your lab section to use. A 1-inch piece will be more than enough for all of the students in your lab. Remember, the thinner the section, the better you will be able to view the specimen under the scope in order to identify cell types.

Protocol 1: Staining Plant Specimens with Phloroglucinol

Phloroglucinol is a stain that reacts with lignin in the secondary cell walls of plants. To view cells that have lignified cell walls, you will need to prepare the specimen with phloroglucinol stain.

Use the following instructions to stain your specimens with phloroglucinol:

1. Obtain a clean glass slide. Make sure you have a sharp razorblade, a clean coverslip, and a dropper bottle with water.
2. Place the slide on the glass plate in front of you. Add a drop of water onto the center of the glass slide.
3. Carefully cut your section from the desired specimen. Remember thinner is always better.
4. Place your newly-cut section immediately onto the drop of water on your slide. You may want to use a toothpick to adjust the position of your sample.
5. Add a drop or two of phloroglucinol directly on top of your sample.
6. Place the coverslip on top of your sample, being careful not to trap air bubbles over your sample.
7. Let the slide sit for 5–10 minutes for the stain to take effect.
8. View sample under the microscope—you should see that the phloroglucinol has stained some areas red or pink.

Protocol 2: Staining Plant Specimens with Iodine Potassium Iodide (I_2/KI)

In order to see the amyloplasts more easily in the potato cross-section, you will need to stain them with iodine potassium iodide (I_2/KI) solution. This procedure is a bit different than the process of staining with phloroglucinol. To stain with phloroglucinol, you simply added the stain to the slide and let it sit. However, if you leave iodine potassium iodide on the slide with your specimen, it will stain everything purple or black and you won't be able to view the specimen properly. To stain the amyloplasts (and only the amyloplasts), you will need to add the stain to the specimen and then quickly remove it.

Use the following instructions to stain your specimens with I_2/KI:

1. Prepare your workstation with a clean glass slide. Make sure you have a sharp razorblade, a clean coverslip, and a dropper bottle with water available to you.
2. Place the slide on the glass plate in front of you. Place a drop of water onto the center of the glass slide.
3. Carefully cut your section from the desired specimen. Remember that thinner is always better.
4. Immediately place your cut section onto the drop of water on the slide. You may want to use a toothpick to adjust the position of your sample.
5. If needed, add another drop of water to the top of your sample.
6. Place the coverslip on top of your sample, being careful not to trap air bubbles over your sample.
7. Add a drop of I_2/KI to the left side of the coverslip (in contact with the water under the coverslip).
8. Use a clean kimwipe to wick the stain under the coverslip so it comes into contact with your sample. To do this, place an edge of the kimwipe on the right side of the coverslip. As the kimwipe soaks up the water, it will draw the stain from the left side under the coverslip.
9. Immediately place a couple of drops of water onto the left side of the coverslip.
10. Wick the water under the coverslip by placing a corner of a clean kimwipe on the right side of the coverslip, drawing the stain through.
11. It may be necessary to repeat steps 9 and 10 to remove any excess stain.
12. Clean up any messes on your slide and view the sample under the microscope.

The I_2/KI will have reacted with the starch in the amyloplasts, but the water added to the slide after the stain will have rinsed the specimen so it is easier to view. There is no need to wait for this stain to take effect – once you have rinsed the stain from the slide, you can immediately view it under the microscope.

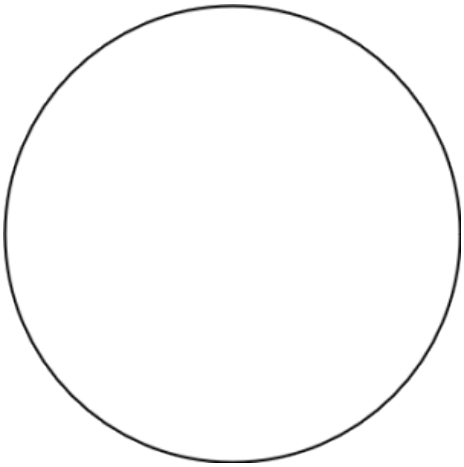
☐ This icon represents a question meant to test your understanding. Answering these questions in the space provided as you go through the lab will help you better understand the topic and study more effectively. Use your text or e-book, botany photo atlases (provided in lab), and appropriate Internet resources to help you.

Plant Cell Structures

1. Plasmodesmata and Middle Lamella

Specimen: Persimmon (*Diospyros virginiana*) endosperm prepared slide

Draw what you see in the space available. Make sure you label the **Plasmodesmata** and **Middle Lamella**.



☐ What are **plasmodesmata**?

☐ What is the functional advantage of having cytoplasmic connections between adjacent cells?

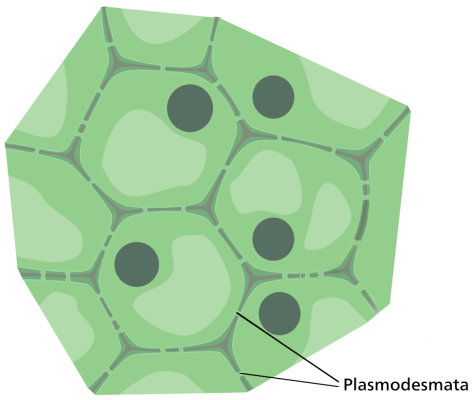


Figure 8.1. Plasmodesmata visualized.

2. Plastids (Chloroplasts)

Specimen: Prepare a fresh wet mount slide of a green bell pepper (*Capsicum annuum*) cross section.

Identify the **Plastids** (**Chloroplasts**) in your specimen and then label them on the illustration below.

☐ What are the functions of chloroplasts?

☐ What gives chloroplasts their green color?

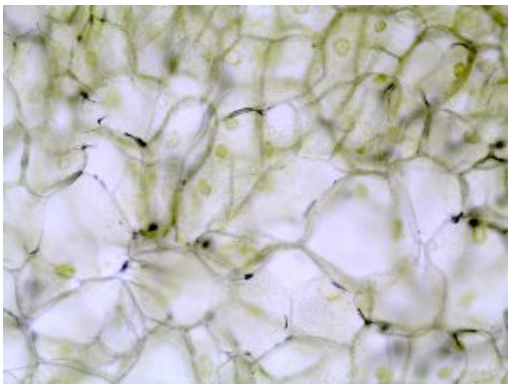


Figure 8.2. Chloroplasts in green pepper cells.

3. Plastids (Chromoplasts)

Specimen: Prepare a fresh wet mount slide of a red bell pepper (*Capsicum annuum*) cross section.

Not all plastids are green. This specimen provides you with an example of plastids of different colors (chromoplasts).

Identify the **Plastids (Chromoplasts)** in your specimen and in the image below.

a. What color are the chromoplasts in this specimen? Can chromoplasts be other colors? If so, what other colors?

b. What are the functions of the chromoplasts?

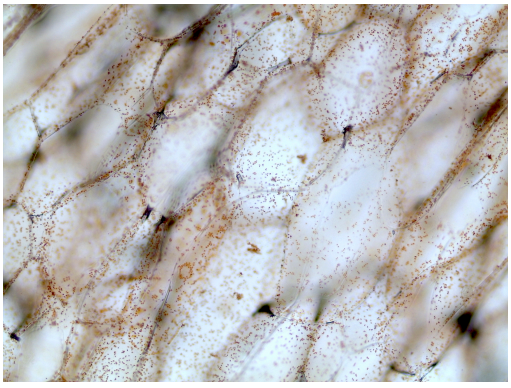


Figure 8.3. Chromoplasts in red pepper cells.

4. Plastids (Amyloplasts)

Specimen: Prepare a fresh wet mount slide of a potato (*Solanum tuberosum*) cross section, stained with iodine potassium iodide (I_2/KI). Refer to the instructions on page 96 for staining.

Identify the **amyloplasts.**

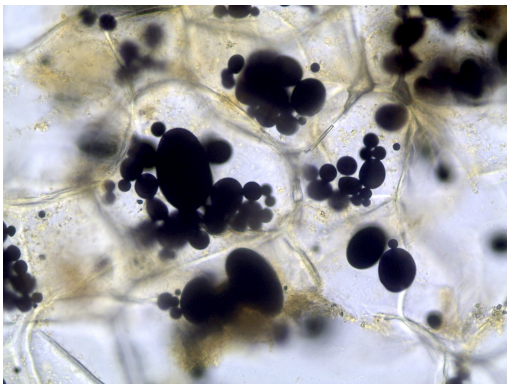


Figure 8.4. Amyloplasts in potato cells.

a. Why are the amyloplasts stained purple with the addition of I_2/KI?

b. What is the function of the amyloplasts?

c. Pages 795–796 (37.4 Gravity: The Gravitropic Response) of the Freeman Biology Textbook describes an interesting function of the amyloplasts. In your own words, describe the role that these plastids play in a plant's ability to sense gravity.

5. Cytoplasm, Central Vacuole, Cell Wall, Nucleus

Specimen: Prepare a fresh wet mount slide of a red onion (*Allium cepa*) epidermal peel.

Identify the **Cytoplasm**, **Central Vacuole**, **Cell Wall**, and **Nucleus.**

a. What functions do the vacuoles serve?

b. In your onion epidermal peel, why do some cells look empty, while others are filled with a purple substance?

To help you understand all of these important cell structures, use the example below to help you label all of the structures in the photo of the onion epidermal peel. Draw arrows from the illustration labels to the corresponding structures in the photograph.

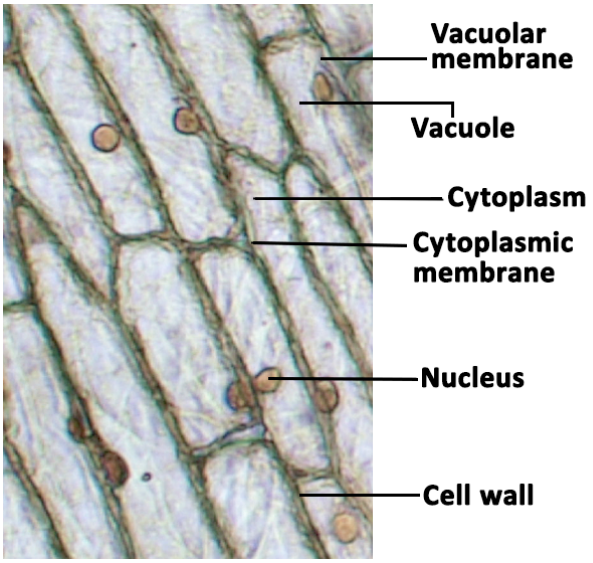


Figure 8.5. Onion cell anatomy

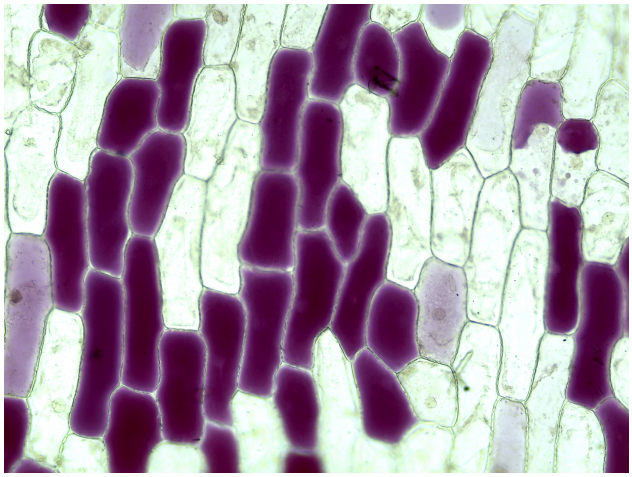


Figure 8.6. Epidermal peel from red onion.

Dermal Tissue

6. Epidermis

Specimen: *Shefflera arboricola* epidermal cells

To view the **epidermis** of *Shefflera*, place a leaf under the dissecting microscope (stereomicroscope) and view it under maximum magnification.

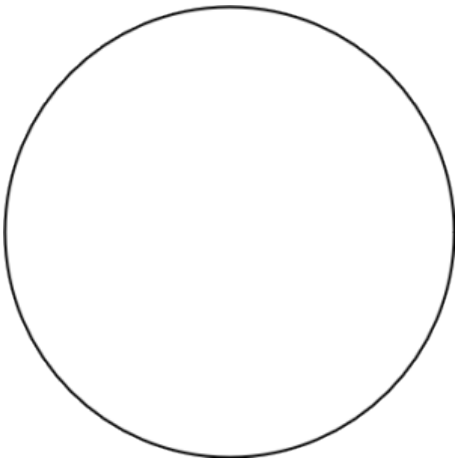
a. How would you describe the surface of a *Shefflera* leaf (hairy, smooth, waxy, dry, shiny, dull, etc.)?

To get a closer look at the **epidermal cells**, you can also make a wet mount of an epidermal peel of the *Shefflera* to view the shape of the epidermal cells using the compound microscope.

7. Epidermal Cells, Stomata, and Guard Cells

Specimen: **Epidermal cells**, **stomata** and **guard cells** (*Tradescantia zebrina*)

Prepare a wet mount slide of a tissue peel from the underside of a *T. zebrina* leaf.



a. Describe the shape of the epidermal cells.

b. Locate stomata (stoma and guard cells). Draw what you see in the space available.

c. What is the function of the stoma and guard cells?

d. The shape of the guard cells determines whether or not the stoma are open. Describe how the shape of the guard cells changes in order to open and close the stoma.

To help you understand all of these important cell structures, use the illustration below to help you label all of the structures in the photo of the *Zebrina* epidermal peel. Draw arrows from the illustration labels to the corresponding structures in the photograph.

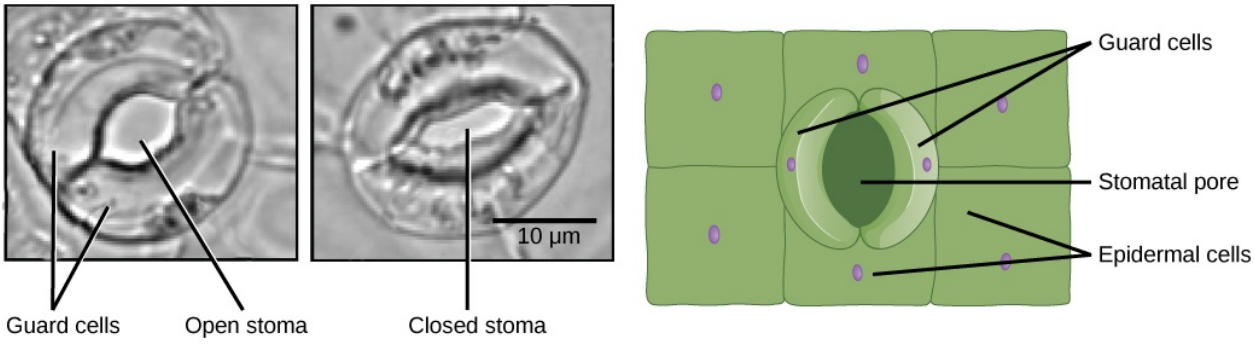


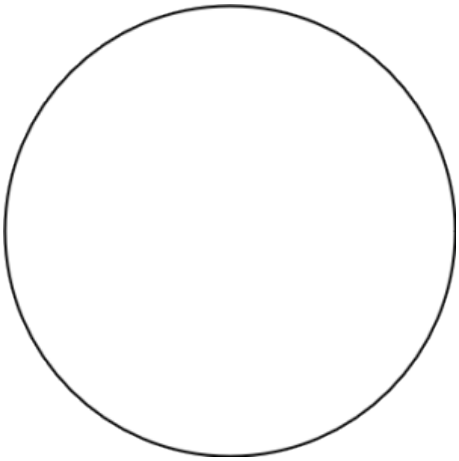
Figure 8.7. Stoma and guard cells. Image adapted from OpenStax Biology, CC BY 4.0.

Trichomes

Specimens: Several different specimens can be used to observe trichomes. We have sunflower (*Helianthis annuus*), African violet (*Saintpaulia ionantha*), and soybean (*Glycine max*) available in the lab. To view the trichomes, place a leaf from one of these plants under the dissecting microscope (stereomicroscope) and view it under maximum magnification.

Note: The species available to view at this station may vary.

a. How would you describe the surface of the leaf (hairy, smooth, waxy, dry, shiny, dull, etc.)?



To get a closer look at the trichomes, you can make a wet mount by carefully shaving some of the trichomes off of the epidermis onto a glass microscope slide, using a scalpel or razor blade. You can then view them under the compound microscope.

b. Draw what you see in the space available.

c. What function(s) do trichomes serve?

Circle the trichromes you see in the photos provided below.

Figure 8.9. Trichomes on African violet petiole.

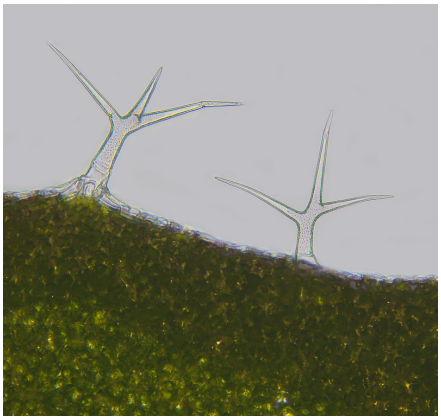
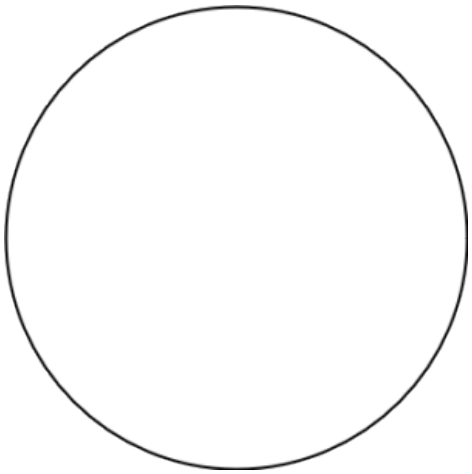


Figure 8.10. Trichomes on arabidopsis, Image Source: Frost Museum via Flickr, CC BY 2.0.

Ground Tissue

9. Parenchyma

Specimen: Prepare a section of sunflower (*Helianthus annuus*) stem in both cross section and longitudinal section for a wet mount slide. The sunflower stem has been placed in dye to help you observe the 3-dimensional structure of the cells.



a. Use the space below to sketch the parenchyma cells you observed in lab. What shape(s) were the parenchyma cells you observed?

b. What function(s) do these cells serve?

c. Do these cells contain cytoplasm? A nucleus?

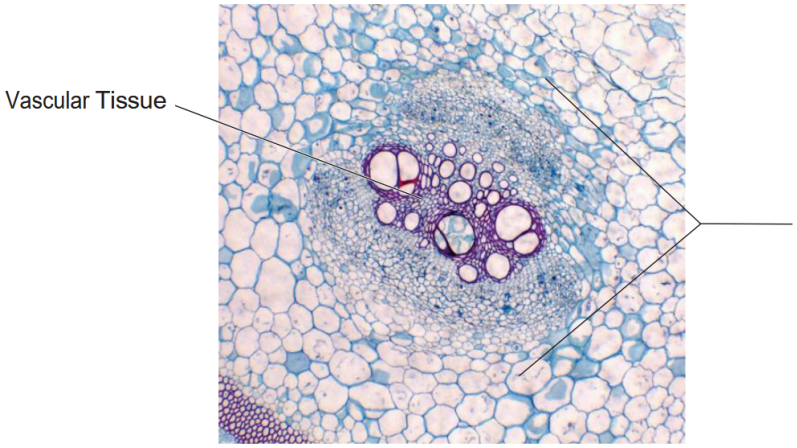


Figure 8.11. Sunflower stem cross section.

10. Collenchyma

Specimen: Prepare a cross section and wet mount slide of celery (*Apium graveolens*). Adding phloroglucinol stain to this section may help you see various cell types within the section. Refer to page 102 for instructions on applying phloroglucinol stain.

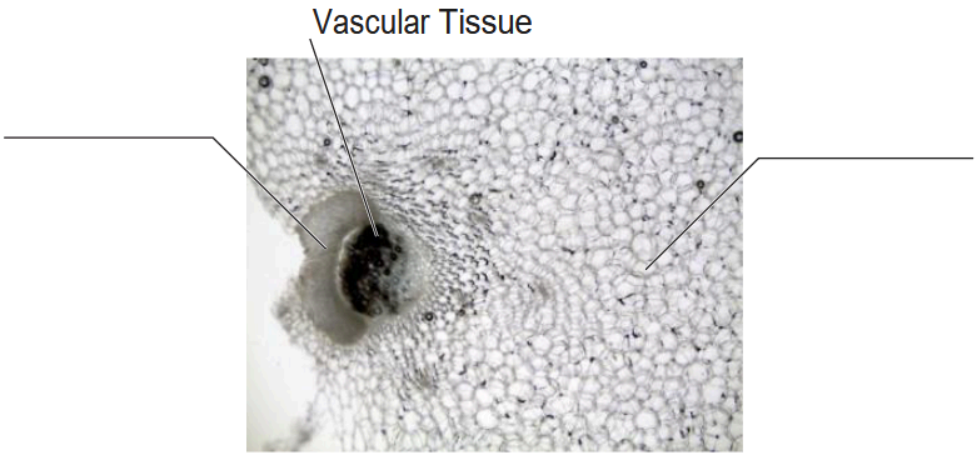


Figure 8.12. Celery petiole cross section.

a. How are collenchyma cells different from parenchyma cells?

b. Do collenchyma cells contain cytoplasm? A nucleus?

c. What is the function of collenchyma?

d. What other tissue types can you see in this specimen?

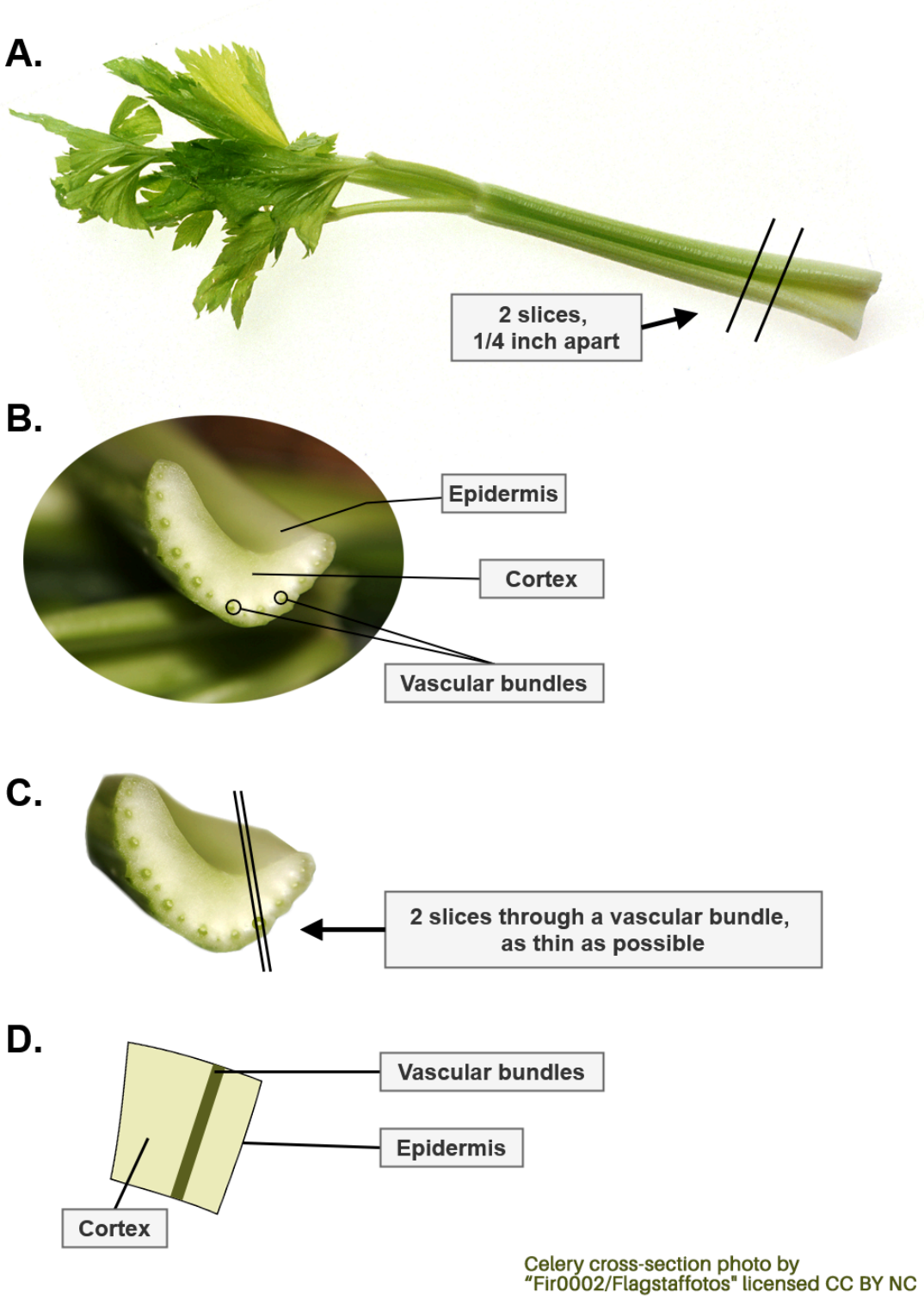


Figure 8.13. Preparation of celery petiole cross section. **A.** Celery stalk. **B.** Celery stalk cross-section. **C.** Cross-section with slice location indicated. **D.** Longitudinal section through vascular bundle. Photo of cross-section originally taken by Fir0002/Flagstaffotos, CC BY NC 3.0.

Sclerenchyma— Rope Fibers

Specimen: Tease a bit of sisal rope or burlap apart. Cut a small sample (2–3mm in length) to place on a wet mount slide. Before adding the coverslip, add a drop or two of phloroglucinol.

Wait 5 minutes before viewing under the microscope.

a. Based on what you view under the microscope, from what cells are these products made?

b. Do these cells contain cytoplasm? A nucleus?

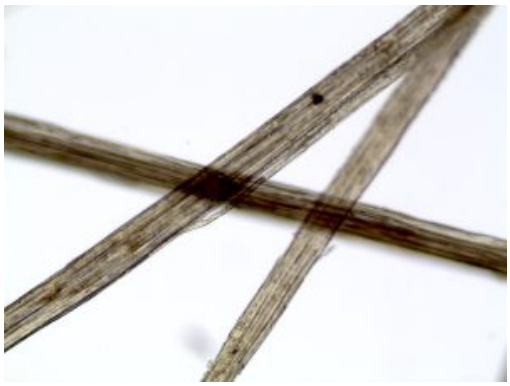


Figure 8.14. Sisal rope fibers.

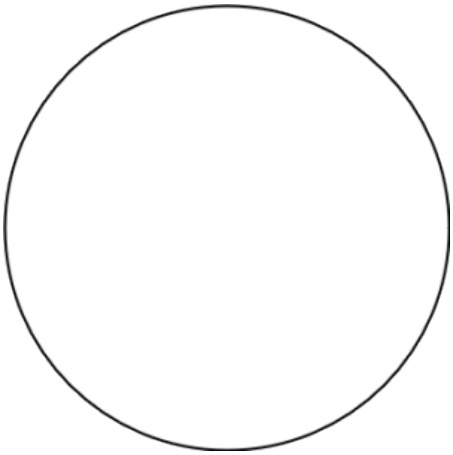
c. What is the function of sclerenchyma?

Sclerenchyma— Sclereids

Specimen: Pear (*Pyrus*) fruit cross section prepared slide. This slide has been prepared with phloroglucinol stain.

Look at the **Cell Walls** in this specimen.

a. Draw what you see in the space available. Make sure you label the cell types visible in the slide. Phloroglucinol is a chemical that will stain _____(a substance found in the secondary cell walls of plants).



b. What is a sclereid?

c. What other cell/tissue types can you identify in this specimen?

13. Sclerenchyma Fibers (Cross Section)

Specimen: View a prepared slide of Linden (*Tilia americana*) cross section.

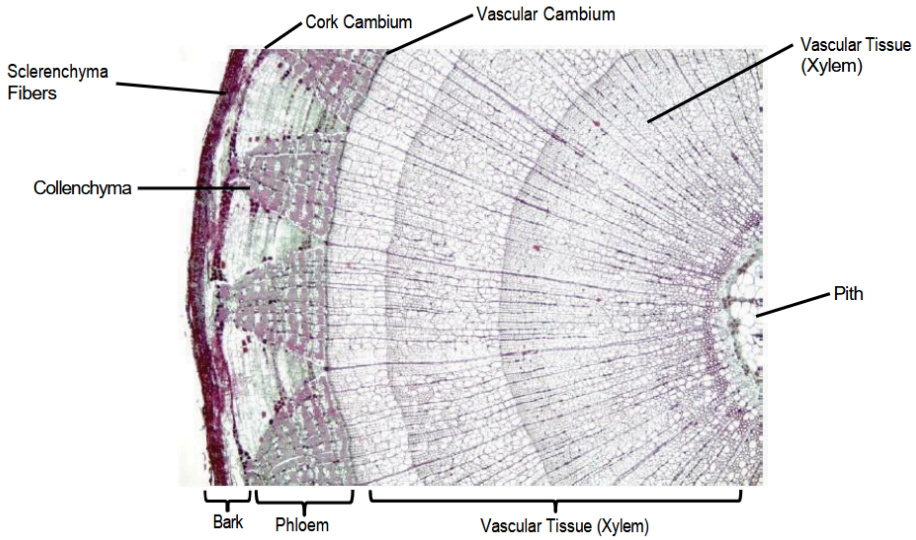


Figure 8.15. Linden stem cross section.

☐ What is the function of the sclerenchyma cells?

☐ How do sclereids differ from fibers?

☐ What is the function of the sclerenchyma fibers?

☐ Do sclerenchyma fibers contain cytoplasm? A nucleus?

☐ What other cell/tissue types can you identify in this specimen?

Describe the differences between these three types of plant cells using the table below. Make sure that you address physical (morphological) differences in the cell types, as well as the differences in their function(s).

Type	Morphology	Function
Parenchyma		
Collenchyma		
Sclerenchyma		

NOTE

All materials submitted as a part of your lab assignment must be your own work. This includes both images and answers to questions. Any items suspected of academic dishonesty will be investigated according to course policy.

LABORATORY 9.

VASCULAR TISSUES & PLANT FUNCTIONAL ANATOMY

Objectives

Following this lab, students will be able to:

- Identify the different cell types of plant vascular tissue and describe how they form the overall tissue structure
- Explain how water and sugar are transported in plants
- Identify the main types of cells and tissues found in plants and describe their distribution in roots, stems, and leaves
- Compare and contrast the arrangement of tissues between different parts of a plant (roots, stems, and leaves) and different phylogenetic groupings of plants
- Describe how the cells and tissues of a plant work together to perform the plant's necessary functions

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

- Compound Scope Tutorial (video tutorial, linked on Canvas)
- Stereomicroscope Tutorial (video tutorial, linked on Canvas)
- How to use Preview (video tutorial, linked on Canvas)
- Using Leica Software (video tutorial, linked on Canvas)

- Biological Science (8th ed.), Freeman *et al.*, 2024. Chapter 34.3–34.4 (pp. 754–763) and Chapter 35 (pp. 766–784).
- Botany Photo Atlas (provided for use in lab)
- Canvas Resources

Background

This week's lab is a continuation from last week's investigation of Plant Cells and Tissues. The focus of this week's lab will be two-fold. First, you will continue studying Plant Cells and Tissues by investigating Plant Vascular Tissues and water transport in plants. You will then take a broader view of plants by considering the plant as a whole and how all of the cells and tissues work together to keep the plant alive.

You'll be using your knowledge of plant cells and tissues to think about how they are utilized within the plant; how the cells and tissue types work together to form the plant and to perform the plant's necessary functions.

In lab you will be looking at plants from the perspective of the three basic plant organs; roots, stems, and leaves. Each of these plant organs performs specific tasks for the plant body as a whole, so each organ will display anatomy that allows the plant to fulfill its function. As you go through this week's material, think about what function each plant organ is responsible for and think about how the anatomy you observe helps to serve that function.

Plant Vascular Tissue

The vascular system enables the transport of fluids and solutes through the plant, similar to the basic function of the cardiovascular system in animals. The vascular system in plants can also provide some support to the plant. It has two main types of tissue: xylem and phloem. Xylem conducts water and dissolved nutrients from roots to shoots through long columns of tissue. Within the tissue there are specialized cells known as tracheids and vessel elements that participate in transport, and parenchyma cells that provide structural support. Tracheids and vessel elements die at maturity and are then able to conduct water through the plant as long columns of hollowed-out dead cells.

Tracheids are long, slender cells with tapered ends. They have gaps in their secondary cell wall that appear as pits. The water moves between tracheids through these pits. Besides water conduction, tracheids can also provide support to the plant. Vessel elements are shorter and wider than tracheids. Along with pits, they also have perforations in their end walls, allowing the vessel elements to be stacked up along each other like a tube. The perforations in vessels are gaps in both the primary and secondary cell walls.

Phloem tissue is made up of two types of specialized cells: sieve-tube elements and companion cells. Sieve tube elements are long, thin cells that can stack together in a tube, similar to xylem cells. They have perfo-

rated ends called sieve plates, which allow sugars and other organic materials to pass between the cells and throughout the plant body. The sieve tube elements are considered living at maturity, but they lack nuclei, chloroplasts and many other organelles. They are connected to companion cells via plasmodesmata. Companion cells contain most organelles and support the sieve tube elements, performing many of their necessary metabolic processes. Phloem tissue also contains more generalized parenchyma cells that provide structural support.

The majority of the specimens you will be observing in lab are provided to you as prepared slides. Not to prepare your own slides (for most specimens) will save you some time this week.

You will be utilizing the microscopes and digital cameras in lab this week. Be mindful when labeling and managing your images to ensure that you do not lose your images or mislabel your photos.

☐ This icon represents a question meant to test your understanding. Answering these questions in the space provided as you go through the lab will help you better understand the topic and study more effectively. Use your text or e-book, botany photo atlases (provided in lab), and appropriate Internet resources to help you.

TA Demonstration

Investigating Water Movement in Bok Choy Petioles

Your TA will play a video tutorial demonstration of water movement in bok choy petioles. In this demonstration, two bok choy leaves (one with the leaves intact, and one where the leaf blades were removed) are cut through the bases of the petioles and placed immediately into a dye solution. The petioles stay in the dye for a short time before they are removed and the movement of the dye through the petioles is examined.

☐ How does the presence of leaf blades affect the rate of water movement through the xylem?

☐ Is this consistent with the evaporation-cohesion-tension model?

1. Xylem – Tracheids

Specimen: Prepared slide of pine stem secondary xylem.

Draw what you see in the space above.

☐ What kind(s) of vascular cells can you identify on this slide?

☐ What are the identifying characteristics of these cells?

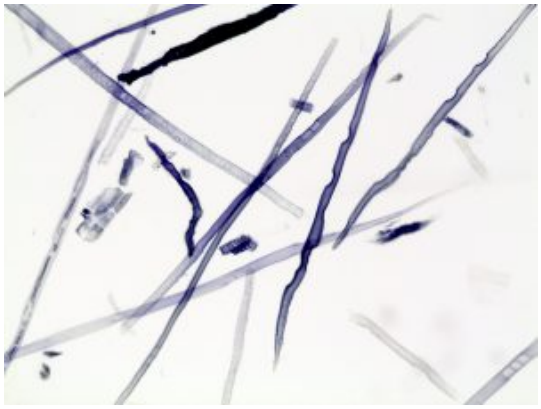


Figure 9.1. Tracheids in pine stem macerate prepared slide.

2. Xylem – Tracheids and Vessel Elements

Specimen: Prepared slide of pumpkin stem macerate.

☐ What kind(s) of vascular cells can you identify on this slide?

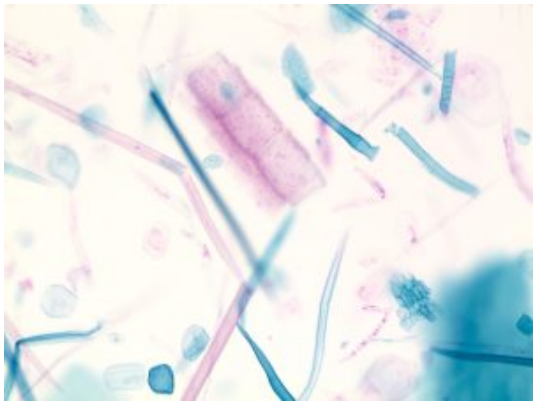
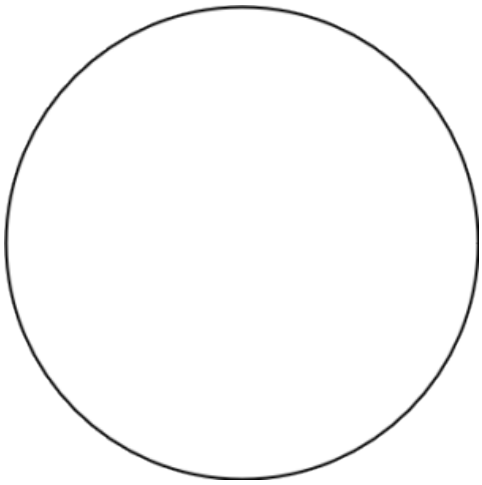


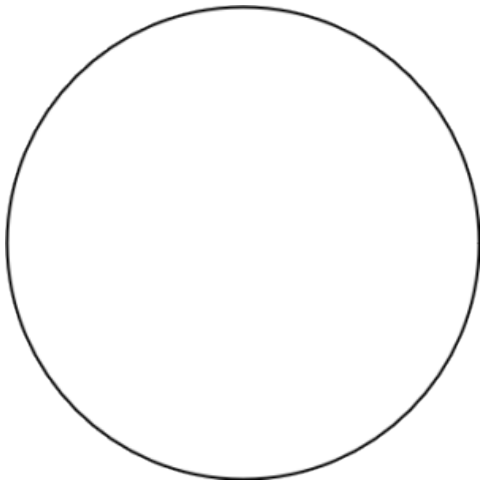
Figure 9.2. Tracheids and vessel elements in pumpkin stem macerate prepared slide.

COMPARISON OF SPECIMEN 1 AND SPECIMEN 2

Draw a tracheid in the circle on the left.

Draw a vessel element in the circle on the right.





☐ Which specimen contains both xylem cell types?

☐ What are the structural and/or functional differences between vessel elements and tracheids?

☐ Do these cells contain cytoplasm and/or a nucleus at functional maturity?

☐ What are the functions of vessel elements and tracheids?

3. Phloem Tissues – Sieve Tube Elements and Companion Cells

Specimen: Prepared slide of pumpkin stem longitudinal section. Label parenchyma, sieve tube member, companion cell, and sieve tube plate on the image below.

☐ What cell types would you find in the phloem tissue?

☐ What are the functions of these cells?

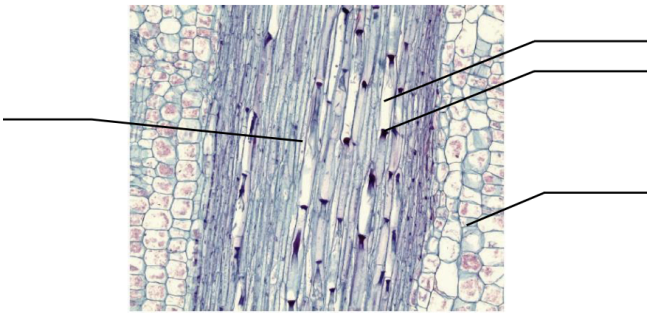


Figure 9.3. Phloem tissues in pumpkin stem longitudinal section.

☐ Do phloem cells contain cytoplasm and/or a nucleus at functional maturity?

4. Xylem and Phloem in Prepared Cross Section

Specimen: Prepared slide of pumpkin stem cross section.

☐ Locate and label the xylem and phloem tissues in images of this cross section below.

☐ Locate and label a sieve plate in images of this cross section below.

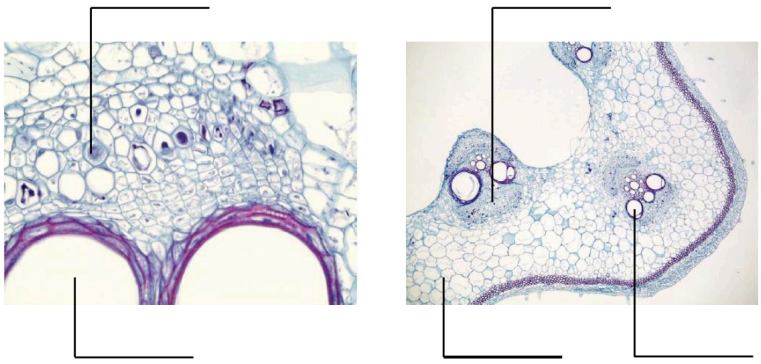


Figure 9.4. Xylem and Phloem tissues in pumpkin stem cross section prepared slide.

COMPARISON OF SPECIMEN 3 AND SPECIMEN 4

These two specimens give you different views of the same plant. Compare what you see in these two specimens to give you a better idea of how the vascular tissue cells are shaped.

☐ Describe the shapes and functions of the following cell types:

Vessel Element

Sieve Tube Elements

Companion Cell

Xylem and Phloem in Fresh Cross Section

Specimen: Prepare a wet mount slide of a sunflower (*Helianthus*) or tomato (*Solanum*) in both cross section and longitudinal section.

Locate the xylem and phloem in your cross section.

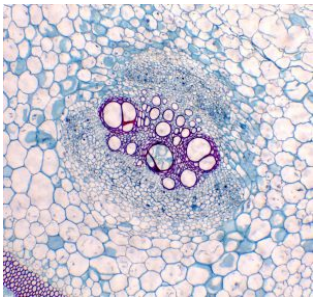


Figure 9.5. Xylem and Phloem tissue in sunflower stem cross section.

☐ What is the function of xylem?

☐ What is the function of phloem?

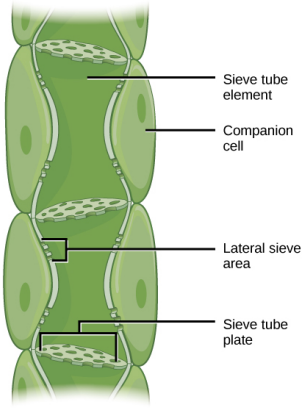


Figure 9.6. Phloem cell structure.

ROOTS

6. Root Morphology

The figure below illustrates the three major types of roots found in plants; tap roots, fibrous roots, and adventitious roots.

☐ In your own words, describe the differences between the three types of roots below.

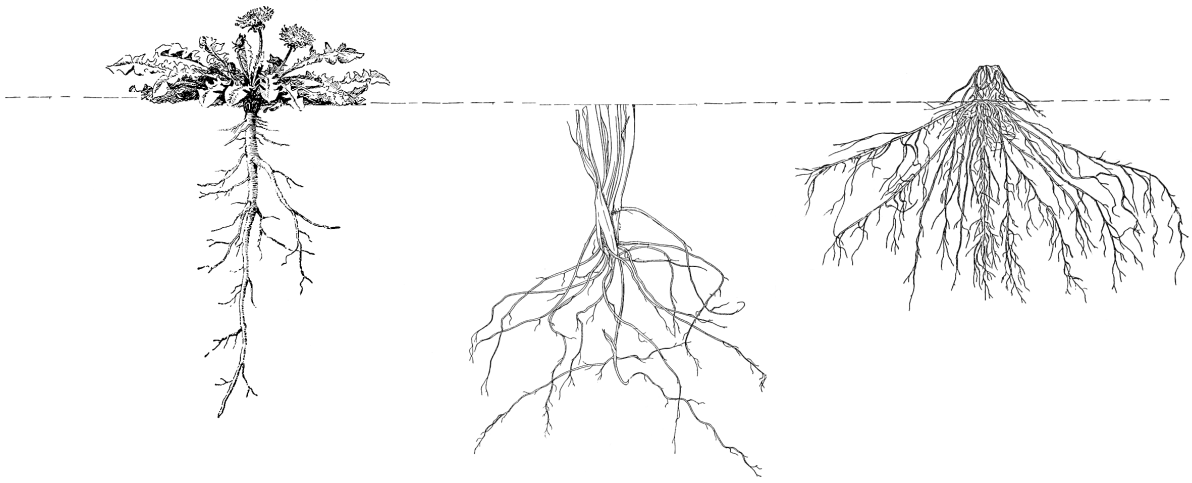


Figure 9.7. Plant Root Types. Left: dandelion with fleshy tap root; middle: wheat plant with fibrous roots; left: corn crop with adventitious (prop) roots.

7. Radish Seedling Observations (shoot, root, root hairs, root cap)

Specimen: Make observations of the radish seedlings from the plate provided.

☐ Locate the shoot, root, and root hairs on the radish seedlings. What is the function of the root hairs?

8. Root Anatomy (root cap, region of cell division, region of cell elongation)

Specimen: Prepared slide of longitudinal section of young dicot root (radish, *Raphanus*).

☐ Locate the root cap, region of cell division, and region of elongation in the images below.

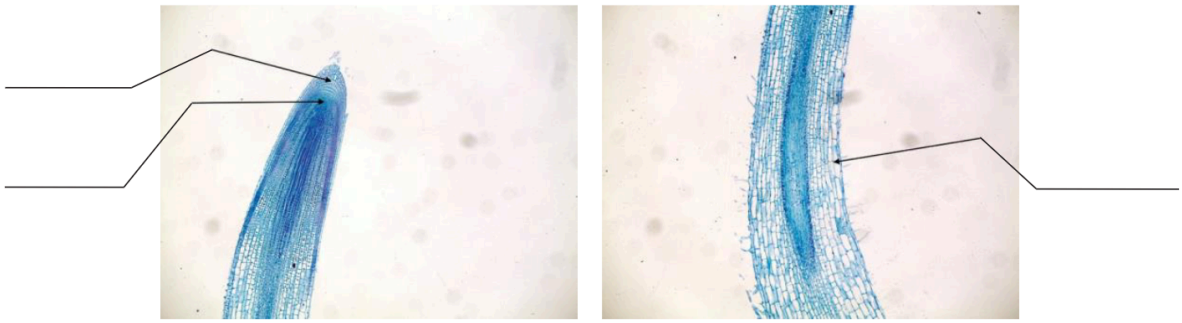


Figure 9.8. **Radish root longitudinal section.**

9. Eudicot Root Anatomy

Specimen: Eudicot Roots—Prepared slide of buttercup (*Ranunculus*) cross section. Examine the slide carefully under the compound microscope.

☐ Label the following structures on the image below: epidermis, cortex, xylem, phloem, pericycle, and endodermis.

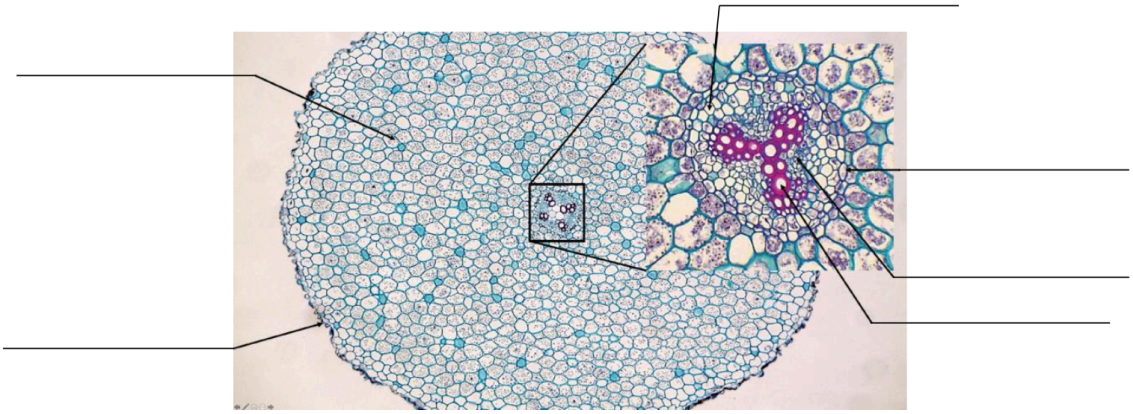


Figure 9.9. **Ranunculus root cross section.**

☐ What is the purpose of the Casparian Strip?

☐ Can you see the vascular cambium, secondary xylem, secondary phloem, and cork cambium in this slide? Why or why not?

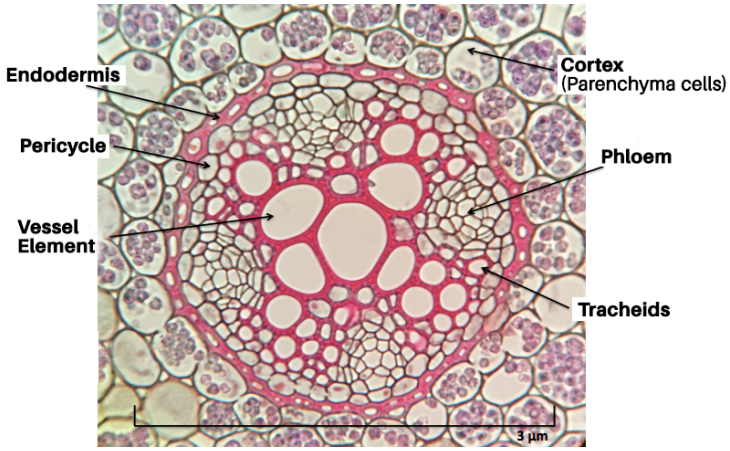


Figure 9.10. Eudicot root anatomy. The epidermis (not pictured here) is external to the cortex cells. Image Source: Sadierath via Wikimedia Commons, CC BY SA 4.0.

10. Monocot Root Anatomy

Specimen: Monocot Roots— Prepared slide of cross section of corn (*Zea mays*) root (typical monocot root).

☐ Examine the organization of the vascular tissue of the corn root. How does it differ from a eudicot root?

☐ Label the following structures on the photo below: pith, endodermis, cortex, xylem, and phloem.

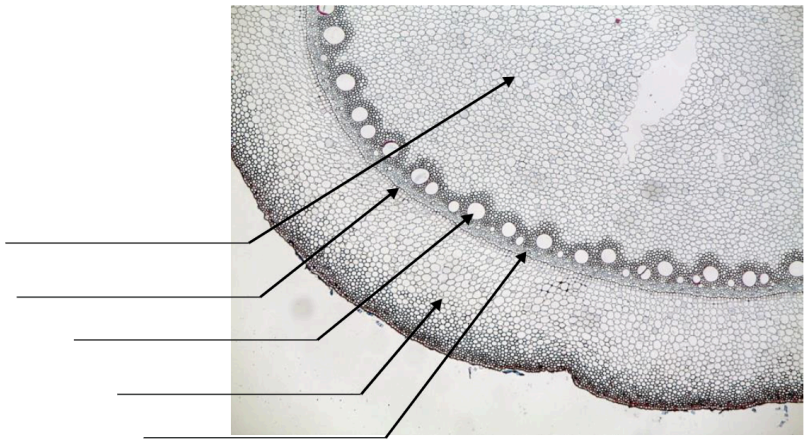


Figure 9.11. Monocot root cross section.

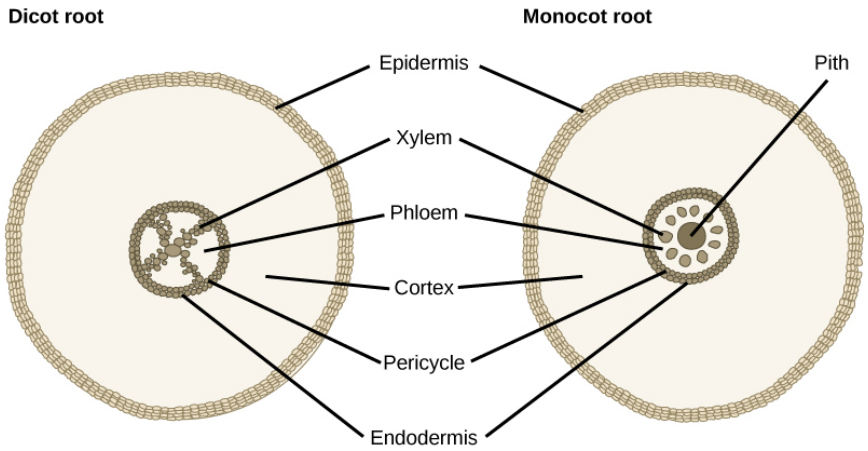


Figure 9.12. Monocot root anatomy (left side of figure), Eudicot root anatomy (right side of figure). Source: OpenStax Biology, CC BY 4.0.

Stems

11. External Eudicot Stem Structure

Specimen: Woody twig displays (various local tree species)

Locate the apical buds, lateral buds, leaf scars, and lenticels on the specimens provided in lab.

☐ Why is bark important to woody stems?

☐ What function do the lenticels perform?

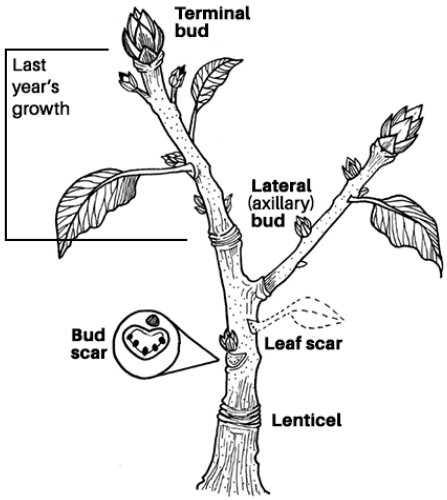


Figure 9.13. External eudicot stem anatomy (twig structure). Shoot morphology by Maria Morrow, CC BY NC 4.0.

12. Herbaceous (Non- Woody) Eudicot Stem Histology

Specimen: Prepared slide of longitudinal section of *Coleus* terminal bud.

☐ Locate the leaf primordium, vascular bundle, apical meristem, and lateral bud on the photo below.

☐ What is the difference between the terminal bud and the lateral bud? What is similar?

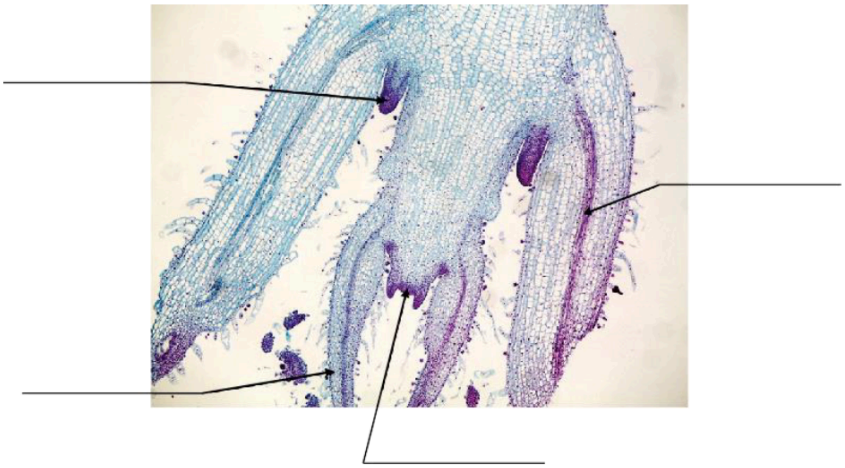


Figure 9.14. ***Coleus*** **apical meristem cross section.**

13. Eudicot Stem Fresh Cross Section

Specimen: Prepare your own cross section of sunflower (*Helianthus*) or tomato (*Solanum*) stem.

☐ Carefully examine the slide and locate the pith, epidermis, and vascular bundles on the photo below.

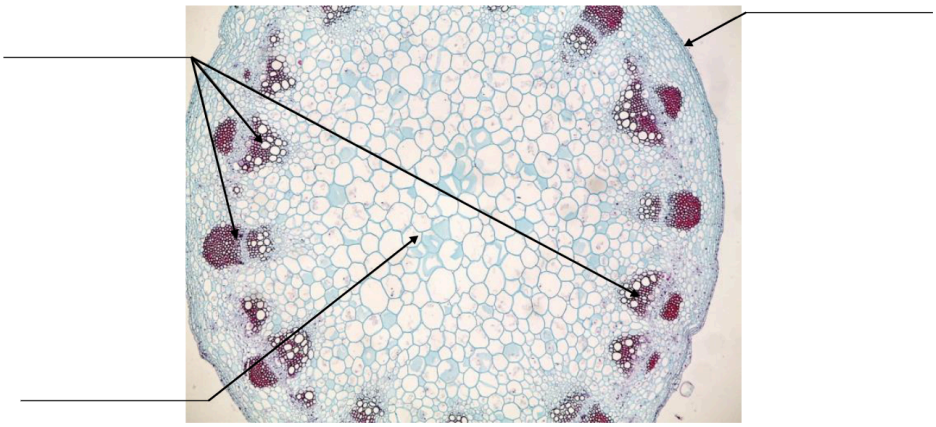


Figure 9.15. Eudicot stem cross section

14. Eudicot Stem Cross Section Prepared Slide

Specimen: Prepared slide of alfalfa (*Medicago*) stem cross section.

☐ Carefully examine the prepared slide and locate the pith, epidermis, and vascular bundles on the image below.

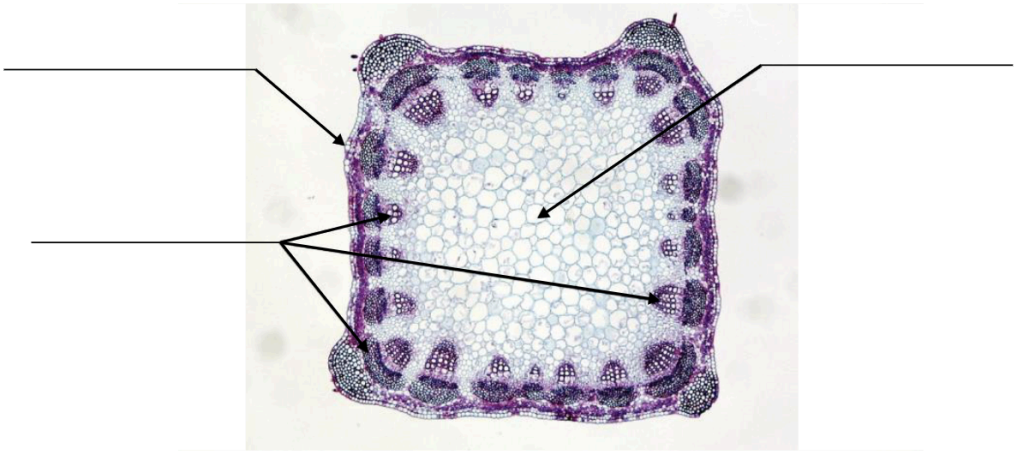


Figure 9.16. Eudicot stem (alfalfa) cross section.

15. Monocot Stem Histology

Specimen: Prepared slide of corn (*Zea mays*) stem cross section.

☐ Carefully examine the prepared slide and locate the ground tissue (parenchyma), epidermis, and vascular bundles on the image on the next page.

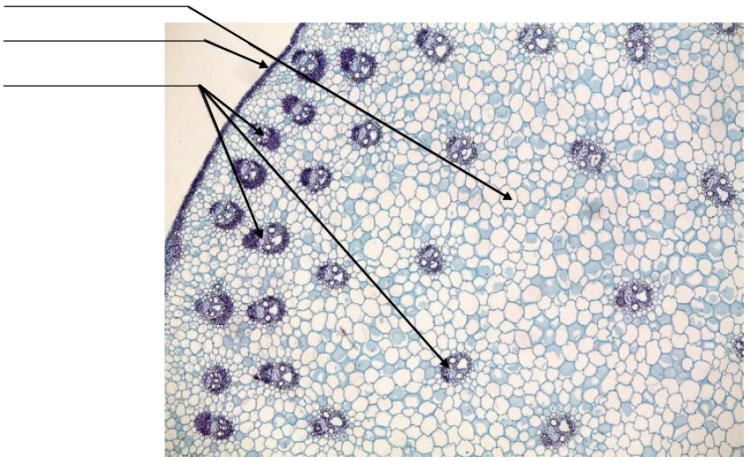


Figure 9.17. Monocot stem (corn) cross section.

☐ Referring to ***Figure9.18***, how is the organization of the vascular tissue in monocot stems different from that in eudicot stems?

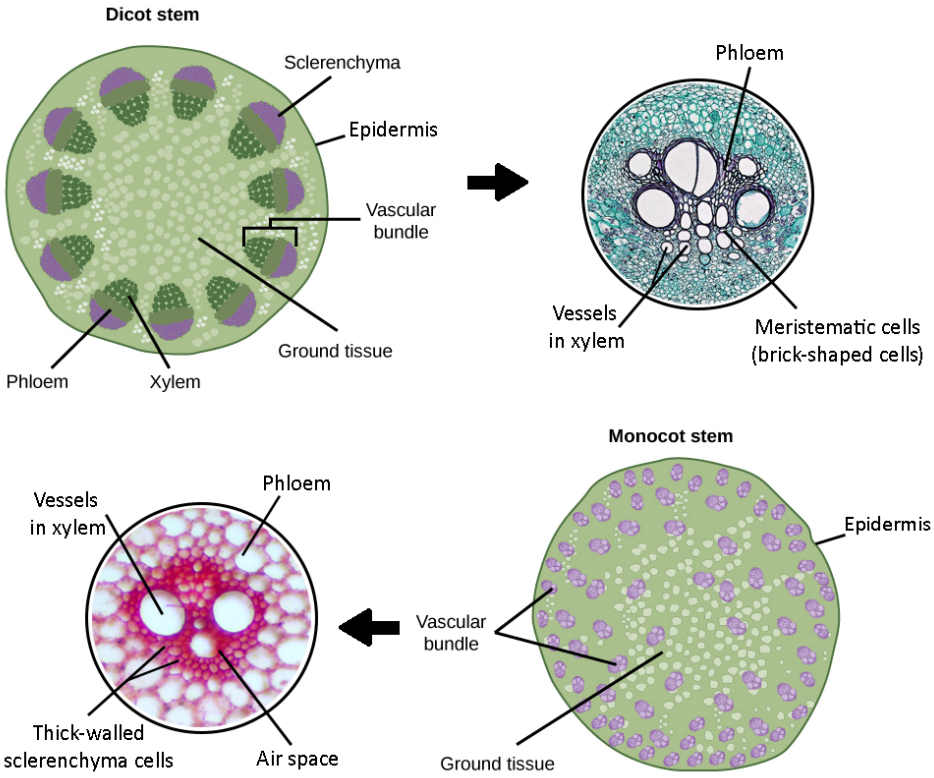


Figure 9.18. Monocot stem anatomy compared with Eudicot stem anatomy. Image Source: OpenStax Biology,CC BY 4.0. Adapted by Abbey Elder to add inset photos with labels.

Review of vascular tissue placement in monocot and eudicot stems and roots. Draw a line to match the options on the right to the statements on the left to complete them:

Vascular tissue in monocot roots is:	found in the center.
Vascular tissue in monocot stems is:	arranged in a ring.
Vascular tissue in eudicot roots is:	arranged in a ring.
Vascular tissue in eudicot stems is:	scattered throughout.

Leaves

16. Internal Eudicot Leaf Anatomy

Specimen: Structure of eudicot leaf. Prepared slide of cross section of eudicot leaf AND fresh cross section of eudicot leaf.

☐ Review from Laboratory 8: What is a stoma/stomate? What is the function of the guard cells?

☐ Carefully look at both your own cross section of fresh material and the prepared slide of the eudicot leaf cross section. Describe what you see. Do you notice any differences between the prepared slide and the fresh specimen?

☐ Locate and label the following structures on the photo below: cuticle, epidermis, palisade mesophyll, spongy mesophyll, bundle sheath, vascular bundle, and stoma.

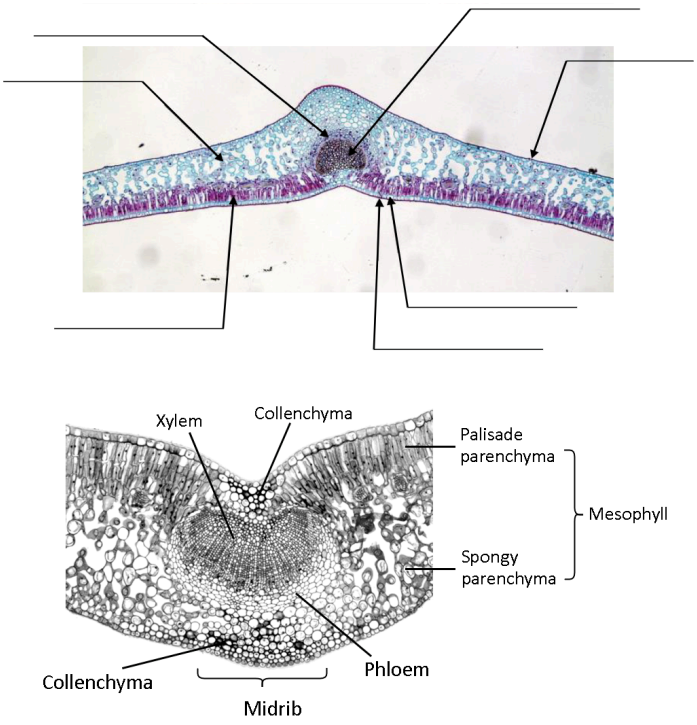


Figure 9.19. Internal eudicot leaf anatomy. Photomicrograph source: Jon Houseman via Wikimedia Commons, CC BY SA 4.0. Adapted by Abbey Elder to add labels.

17. Internal Monocot Leaf Anatomy

Specimen: Structure of monocot leaf. Prepared slide of corn (*Zea mays*) leaf cross section AND fresh cross section of corn leaf (*Zea mays*).

☐ Carefully look at both your own cross section of fresh material and the prepared slide of the monocot leaf cross section.

☐ Describe what you see. Do you notice any differences between the prepared slide and the fresh specimen?

☐ Locate and label the following structures on the image below: cuticle, epidermis, bundle sheath, vascular tissue, mesophyll, and stoma.

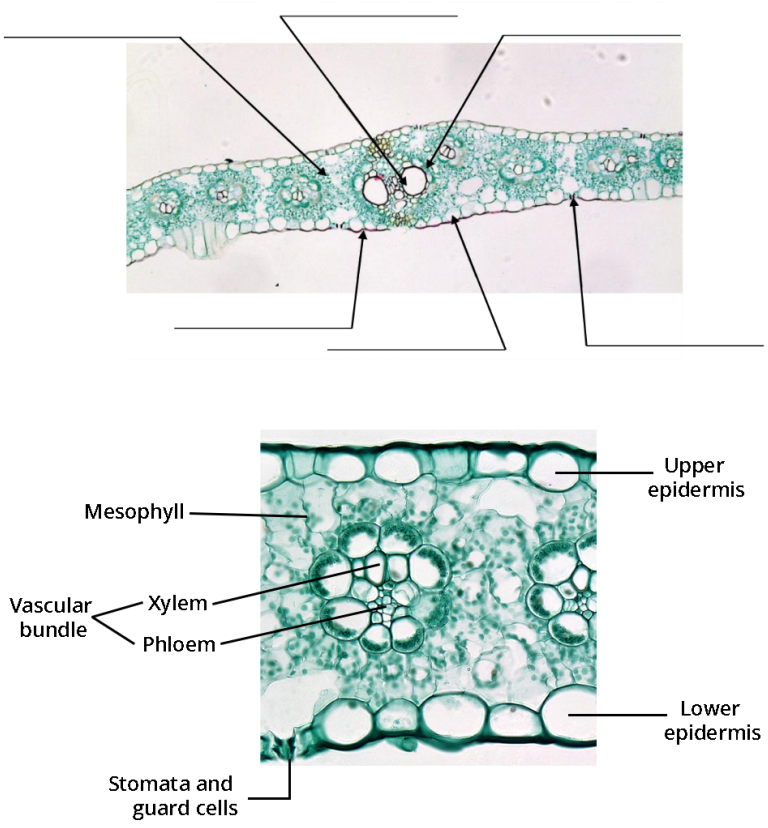


Figure 9.20. Internal monocot leaf anatomy.

18. Internal Anatomy of Gymnosperm Leaf

Specimen: Structure of gymnosperm needle. Prepared slide of cross section of Austrian pine (*Pinus nigra*) needle.

☐ Carefully examine the slide and describe what you see. What is the general tissue and cell organization of a gymnosperm needle?

☐ What structure(s) do you see in the gymnosperm leaf cross section that you did not see in the monocot or eudicot leaf cross sections?

☐ Do you notice anything special about the stomata in the pine leaf compared to other leaves you observed in lab?

☐ Locate and label the following structures on the image below: epidermis, mesophyll, vascular bundle, stoma, resin duct, bundle sheath.

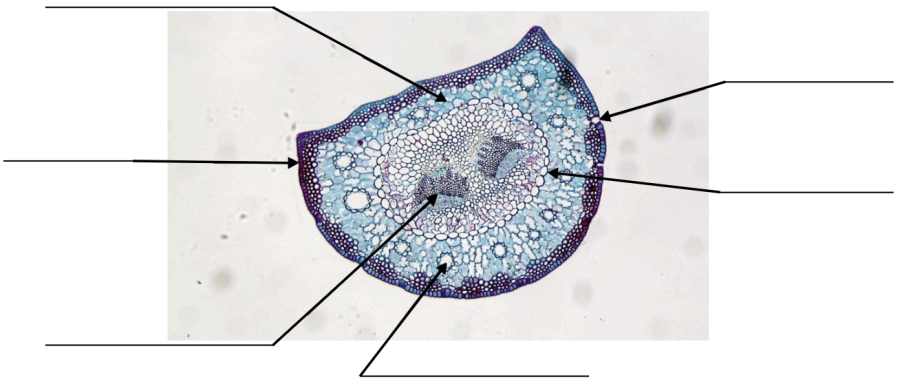


Figure 9.21. Pine needle cross section.

☐ Compare the pine needle (gymnosperm leaf) to the leaves of monocots and eudicots. Specifically look at the epidermal thickness, cross-sectional shape, position of vascular tissue, and presence of resin ducts. Describe what you see in the gymnosperm leaf vs. the monocot/eudicot leaves for each of these characteristics.

NOTE

All materials submitted as a part of your lab assignment must be your own work. This includes both images and answers to questions. **Any items suspected of academic dishonesty will be investigated according to course policy.**

INTER-CHAPTER 5.

DISSECTION TECHNIQUES AND TERMINOLOGY

Why Do Dissections?

Dissections are an important part of any Biology class. It is one thing to read about anatomy and physiology or look at pictures, but another entirely to explore an organism yourself. For the third part of Biology 2120L, we will use fetal pig dissections to explore mammalian anatomy and physiology. Though we will not study human anatomy directly through dissection, the fetal pig is a relatively good substitute so you can learn a great deal about your own anatomy and physiology through the upcoming labs.

Care of Your Specimen

Fetal pigs are unborn fetuses from the mother pig and are a byproduct of meat production. The veins and arteries of our fetal pigs have been injected with colored latex to help you more easily identify them as you explore. The veins will be blue and the arteries red. The fetal pig will also be preserved with chemicals that prevent the breakdown of tissue while you are embarking on your multi-week dissection. Make sure that you follow the directions in the "fetal pig dissection video" that you will watch in class to keep your pig in top condition and to keep yourself safe.

You will be working with your lab partner over four weeks to complete all of the necessary dissections on your fetal pig, so taking proper care of your specimen will be extremely important. To prevent mold and bacteria growth on your pig, thoroughly spray your specimen with the provided preservative, Wardsafe, at the end of each lab period. Once sprayed, wrap your pig in paper towels, and spray those with Wardsafe as well. When done, place your wrapped pig in a Ziploc bag and return it to the class storage bin for cold storage.

It is important to note that dissection specimens and parts require special disposal and must stay in the lab classroom. You are welcome to take photos of your dissections to help you review the information at home, but do not remove your fetal pig (or any part of it) from the lab classroom.

Dissection Safety

Although dissections can be engaging and fun, there are many safety hazards that you need to keep in mind. Refer back to the safety scavenger hunt you did during week 1 to remind you where the safety equipment in your classroom is located. Feel free to ask your TA any safety related questions you may have.

Gloves: Whenever you are dissecting any specimen, always protect your hands by wearing gloves. Inspect them frequently and replace them if you notice any holes or cuts. The gloves we use in lab are latex-free nitrile gloves.

Safety goggles: Always wear safety goggles to prevent preservative or other liquid animal products from getting in your eyes. In the event that you do get preservative splashed in your eyes, make sure you know where the eye wash station is and how to use it.

Proper attire: As is the case for all labs, it is extremely important to wear closed-toe shoes during dissections. If a scalpel or razor falls on your foot, the fabric of your shoe will offer you a layer of protection. Dress appropriately for dissections to provide your skin with the most protection.

Food and drink: As always, it is important to make sure you do not bring in any food or drink into the lab. All food or drink should be securely stored inside your book bag. There are tables outside the doors of your classroom where you can keep any food or drink that you need to access during lab.

Sharps: In the next few labs we will be using sharp scalpels, scissors, and razor blades. Make sure you know how to properly use these tools. The blade should never be pointed towards you while walking or while dissecting. For razor blades, make sure you are opening them correctly to prevent accidentally pressing into the blade. Consult with your TA for instructions if you need to change the blade on your scalpel.

Fainting: During dissections it is important to watch out for fainting. The sights and smell of dissections may bother some students (especially if they have not slept well or eaten recently). Make sure you are always sitting down when doing dissections (in the case that you faint, there will be a shorter distance to fall). If you or your lab partner is feeling ill, let your TA know and feel free to take a break for some fresh air.

Anatomical Terms

Like all aspects of Biology, each new topic includes its own set of terminology. In anatomy, there are a variety of terms to describe the location of structures and their orientation to other structures and parts of the specimen (tail, head, center of body). The main terms you need to know are described and are shown in the figure of a cat below. When you receive your fetal pig, take a few minutes to make sure you understand these terms in relation to your specimen.

- **Anterior**: towards the front (head) of the organism
- **Posterior**: towards the rear of the organism
- **Dorsal**: towards the upper side (back) of the organism
- **Ventral**: towards the belly (lower area) of the organism
- **Distal**: away from the center of the organism's body
- **Proximal**: close to the center of the organism's body

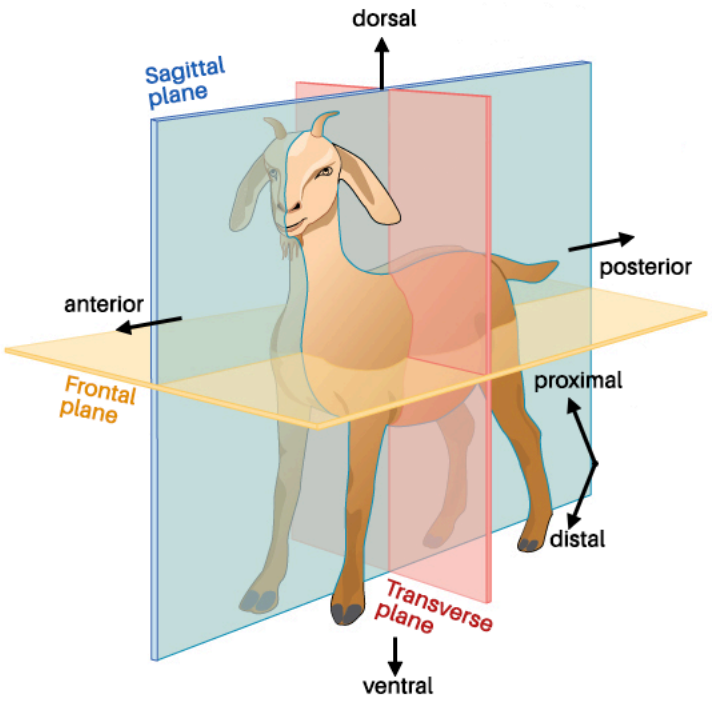


Figure IC5.1. Common anatomical terms used during dissections. Image adapted by Abbey Elder from OpenStax Biology figure, CC BY 4.0.

Tools of the Trade

There are many dissection tools available to you in class. Each has its own use and is best for a certain type of motion/dissection. To do the best dissection possible, make sure to use the right tool for the job. The selection of provided tools are described below. The dissection tools we use in lab are not disposable tools. When you finish your dissection each week, make sure that you carefully wash, dry, and put away the tools you used in lab. If you discover any damaged tools, inform your TA so they can be replaced.

Scissors (large and small): Best for cutting away skin, organs, and connective tissue. Use the appropriate size for your needs.

Scalpel: Best for precise cuts. This tool is extremely sharp and it is easy to cut your specimen too deep if you're not careful. Consult with your TA if the blade on your scalpel needs to be replaced.

Blunt probe: Best for exploring the inside of your pig, gently pushing organs and structures aside to find underlying structures. Once you make the initial cuts with your scalpel, you will find that the blunt probe is a very effective tool during dissections.

Dissection needle: Best for teasing apart connective tissue or other small structures. Be careful not to puncture anything when using this tool.

Tweezers: Best for holding or gently picking up structures.

Razor blades: Best for making thin slices for cross sections. These will only be available at specific stations in which you need to make wet mounts.

LABORATORY 10.

DIGESTIVE, RENAL, AND REPRODUCTIVE SYSTEMS

Objectives

Following this lab, students will be able to:

- Demonstrate proper dissection technique and respect for animal specimens and dissection tools
- Identify the structures and describe the functions of the mammalian digestive, renal, and reproductive systems
- Compare and contrast fetal pig anatomy and physiology with that of humans and other mammals
- Predict the consequences of a defect in the anatomy and/or physiology of mammalian systems
- Name the substances that are created by and/or pass through the digestive, renal, and reproductive organ systems and describe the pathway they take through these systems

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Your TA will check that you have attempted to complete the dissections associated with this week's lab activity.

Resources

- Dissection Interchapter 6 "Dissection Techniques and Terminology" (pp.137–140)
- Fetal pig dissection tutorial video (tutorial video on Canvas)
- Fetal pig dissection guide, Smith and Schenk 2011 (provided for use in lab)
- Biological Science, Freeman *et al*. 2024 (8th ed.) Digestive System (41.3), Renal System (40.4), Reproductive System (47.2–47.3)
- Internet and Canvas resources

☐ This icon represents a question meant to test your understanding. Answering these questions in the space provided as you go through the lab will help you better understand the topic and study more effectively. Use your text or e-book, pig dissection guide, and the internet to help you.

1. All About Your Pig

This is your first day of fetal pig dissection. Working with a partner, obtain a pig and prepare your specimen and workstation as shown in the "Fetal pig dissection video tutorial". Before you begin your dissection, do a thorough investigation of its external features.

☐ Based on your observations of your fetal pig's external anatomy (specifically the location of its urogenital opening), is your pig a male or female? How can you tell?

After you have determined the sex of your pig, you can begin to make the incisions shown in the next section to expose its internal organs.

2. Making the First Cuts

Use ***Figure 10.1*** below to make your first incisions. Follow the numbers to make the incisions in order. Make sure you don't slice too deep and puncture the organs. It is always better to cut slowly and shallowly to start, then cut deeper later if you need to. After you make the cuts, identify the organs shown in ***Figure 10.2***. **BE CAREFUL** not to remove the thymus or thyroid gland when you take off the skin on the neck. Leave the esophagus in place.

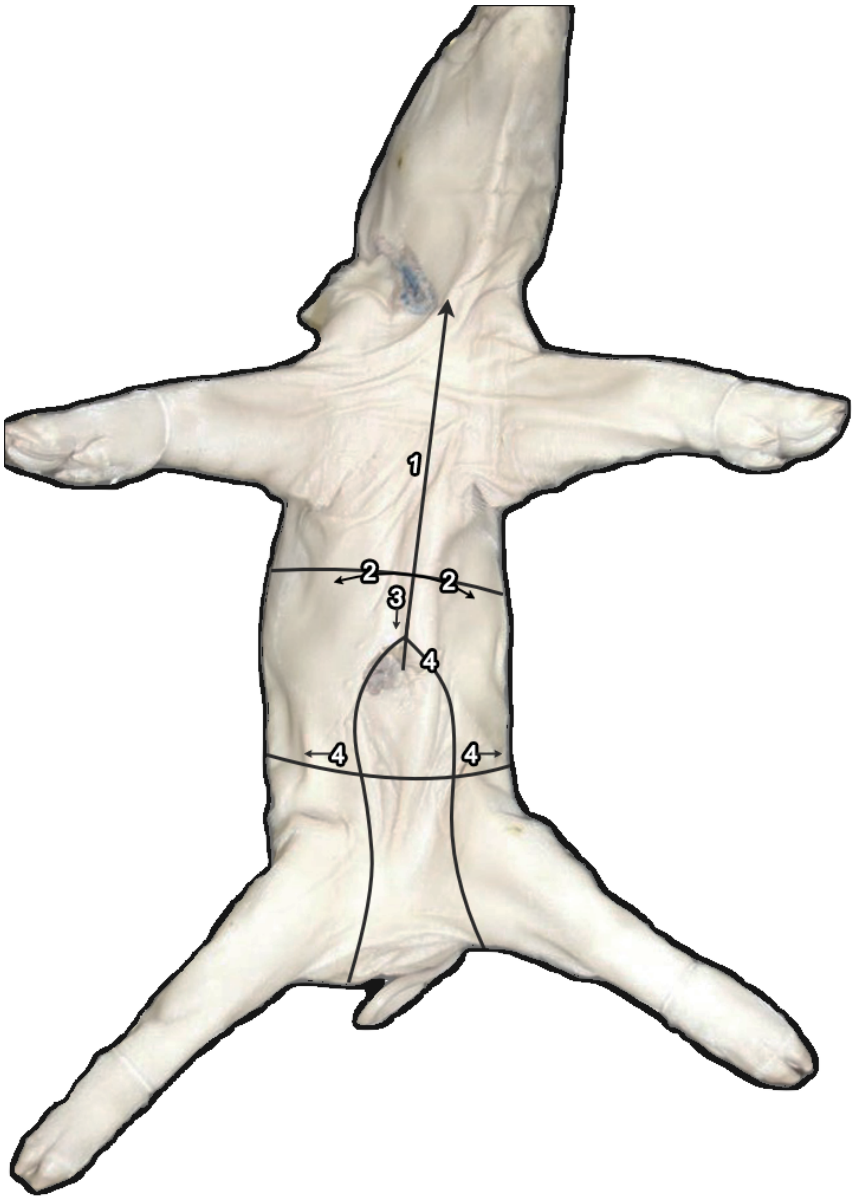


Figure 10.1. The order and direction of the initial incisions to open your fetal pig. Image Source: Biology II Laboratory Manual, CC BY NC SA 4.0.

3. Body Cavity and Digestive Tract Anatomy

Locate each organ in your pig shown in ***Figures 10.2*** and ***10.3***. Have your partner test you for recognition.

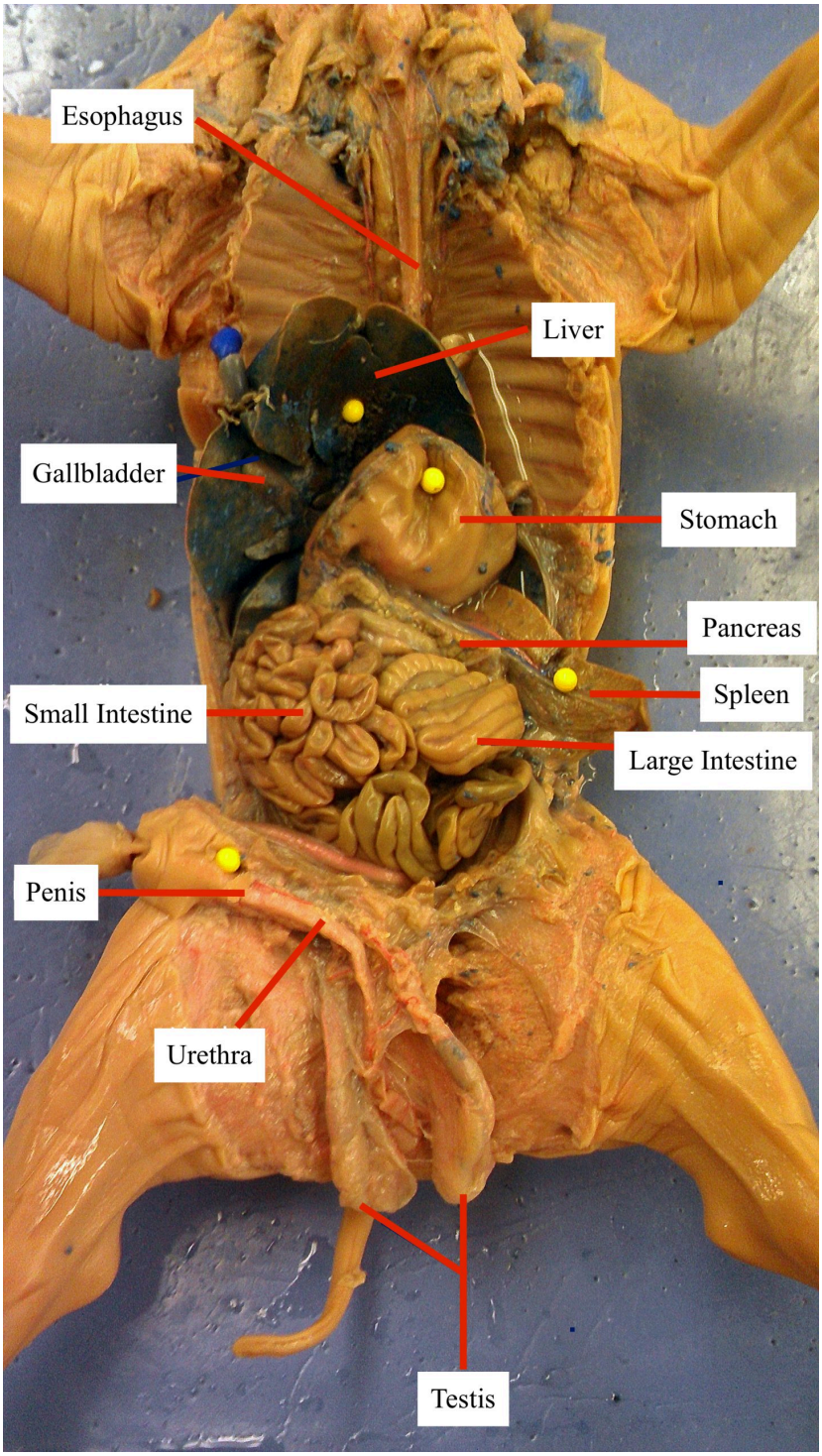


Figure 10.2. Dissected male fetal pig with a sampling of digestive, renal, and reproductive structures labeled. In this image the heart, lungs, and trachea have been removed. **DO NOT** remove these organs from your dissections yet. **Note:** The reproductive structures of the female fetal pig will look different.

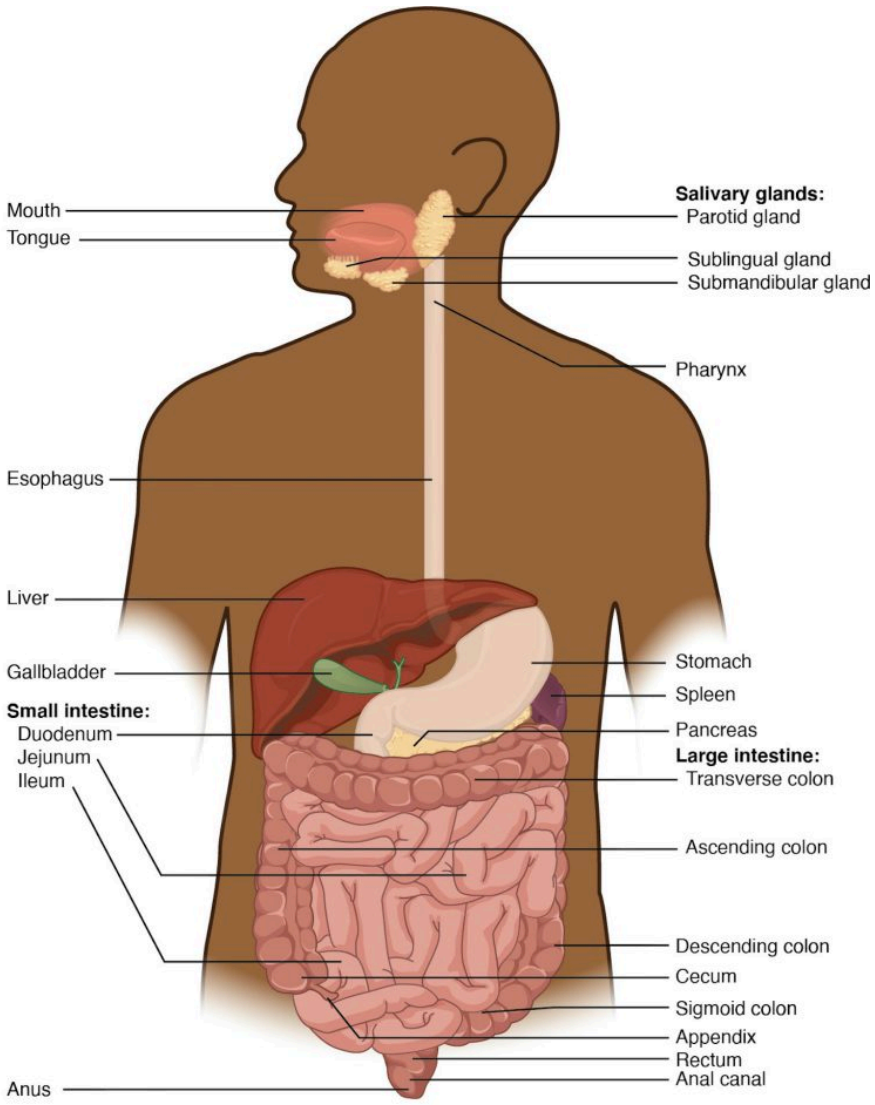


Figure 10.3. Body cavity of a human. Image Source: OpenStax Anatomy & Physiology, licensed CC BY 4.0.

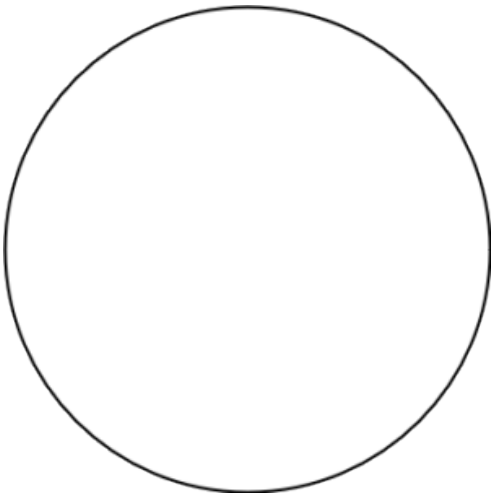
☐ What are the largest organs visible in your fetal pig? What are the functions of these organs and why do you think they are so large?

One group from each class should remove the intestines from their fetal pig for measuring. Estimate or measure the length of the removed small and large intestines.

Intestine Length =

☐ Did the length of your fetal pig's intestines surprise you? Why do you think the intestines are so long?

After you have thoroughly investigated your fetal pig's intestines, go to the **Prepared Slide of Jejunum (intestines) Station** and observe the prepared microscopic slides of the small intestine. Make sure you locate the **villus, microvilli, lumen,** and **smooth muscle**. Sketch what you see in the adjacent circle and/or take a photo using the Leica software.



☐ How do the villi and microvilli relate to the intestine's function?

☐ Celiac disease occurs in some individuals when ingested gluten causes an autoimmune response. This response often causes damage to the small intestine, including shortening of the villi. Symptoms of celiac disease include diarrhea, weight loss, anemia, and other mineral and nutrient deficiencies. Why would damage to the villi cause these symptoms?

4. Esophagus, Trachea, and Mouth Anatomy

When you are working to uncover the esophagus, trachea, and larynx, BE CAREFUL not to cut any blood vessels. We will need these intact for next week's lab. Use ***Figure 10.2*** and ***Figure 10.3*** to help you locate the esophagus in your pig. For help in locating the trachea and larynx, look ahead to ***Figure 12.2*** in the Gas Exchange chapter for a detailed image.

☐ What are the functions of these structures? Fill in the table below.

Table 10.1. Student worktable: function of esophagus, trachea, salivary glands.

Structure	Function
Esophagus	
Trachea	
Salivary Glands	

The mouth anatomy of mammals is quite complex and dissection of this area can be complicated. Demonstration dissections have been provided for you, but you may choose to do your own dissection of the mouth and salivary glands if you would like a challenge and have time. Using the demo dissections or your own, locate the mouth anatomy structures shown in ***Figure 10.4*** of the fetal pig.

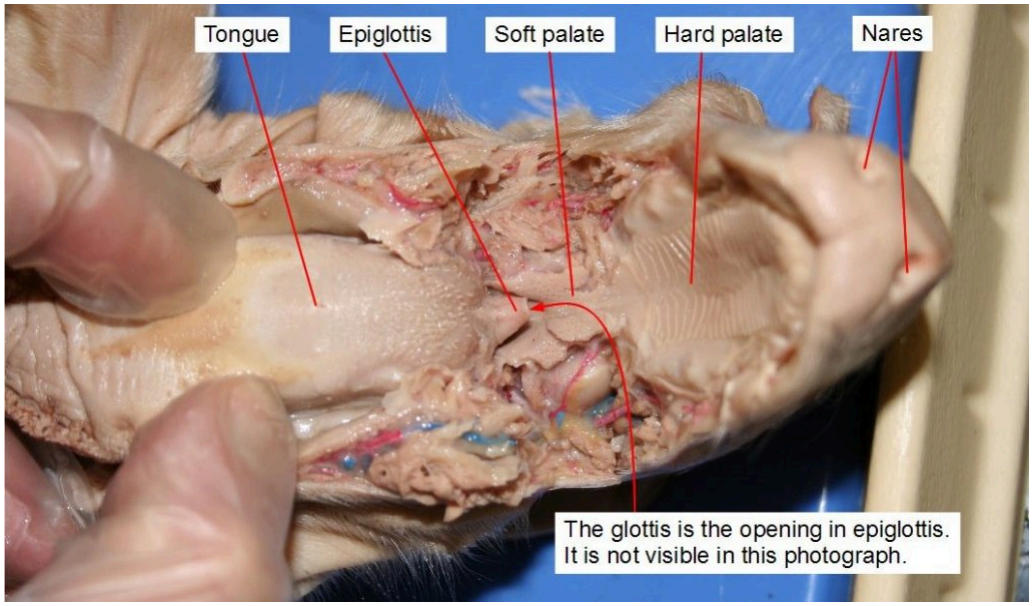


Figure 10.4. Mouth anatomy of a pig. Image Source: "Biology II Laboratory Manual," by Michael J. Gregory, Ph.D., CC BY NC SA 4.0 License.

5. Accessory Organs

Be sure to locate the **liver, gallbladder, pancreas, spleen, thyroid,** and **thymus**. Refer to ***Figures 10.2***, ***10.3***, and ***10.5*** to help you. Note that the thyroid is an endocrine gland and the thymus is part of the lymphatic system, but because we don't study those systems separately, we will examine them here. What are their functions?

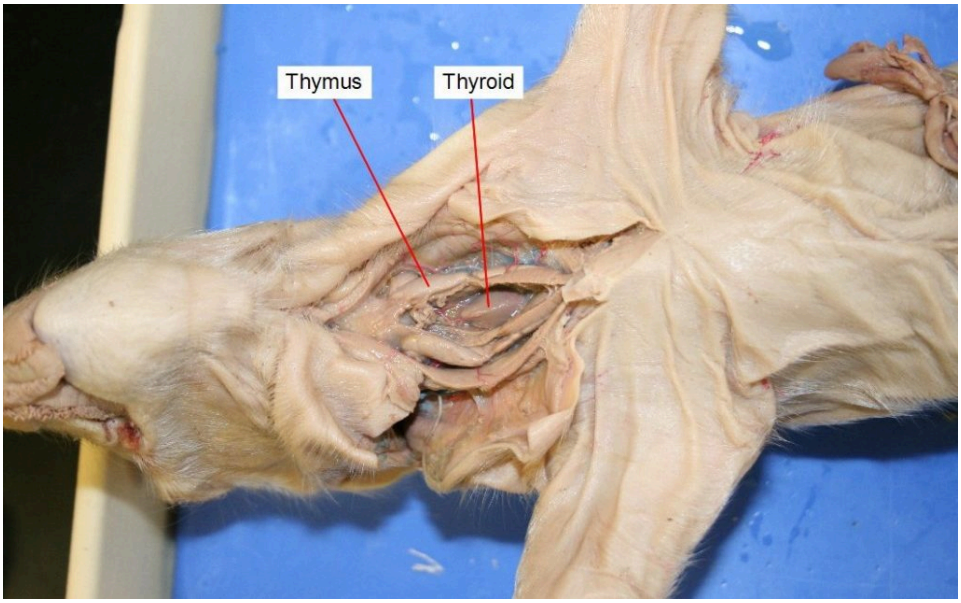


Figure 10.5. Thymus and thyroid.Image Source: "Biology II Laboratory Manual," by Michael J. Gregory, Ph.D., CC BY NC SA 4.0 License.

Table 10.2. Students worktable: function of accessory organs.

Structure	Function
Salivary Glands	
Liver	
Gallbladder	
Pancreas	
Spleen	
Thyroid	
Thymus	

6. Mammalian Excretory System

The excretory system is extremely important in mammals to help eliminate waste, filter materials, and regulate water and salt levels. Blood is continually filtered through the kidney; every time the heart beats, about 20% of the blood is routed through the kidneys. Plasma and solutes leak out of capillaries in the kidneys and is then modified into urine. Once created, urine travels through the ureters, the bladder, the urethra, and then exits the body. Identify the components of the excretory system as shown in***Figure 10.6*** in your dissected fetal pig and then we will explore these in more detail.

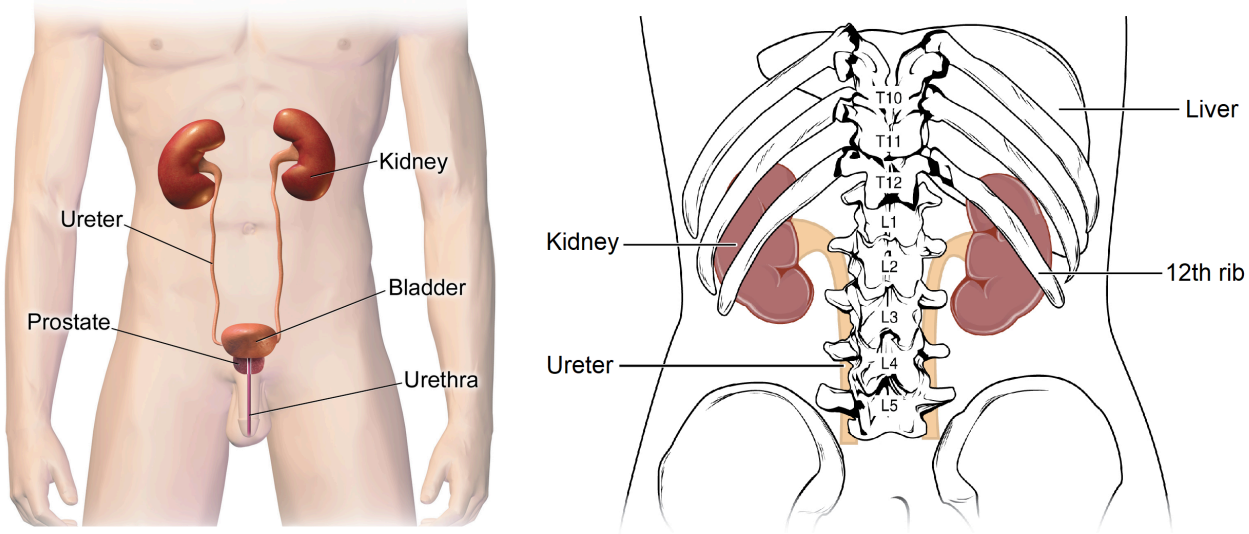


Figure 10.6. Mammalian excretory system. Image Sources: Urinary system by Bruce Blaus, CC BY SA 4.0, OpenStax Anatomy & Physiology, CC BY 4.0.

After you have located all the structures above, remove one kidney from your fetal pig. Take it to **the Kidney Station** and cut a thin section from it using a razor blade. Find the **cortical** and **medullary** regions. Compare your section to the prepared kidney cross section slide available to you at the station.

The kidney is very complex; use the kidney model and poster in the lab to explore the internal structure of the kidneys that you cannot see with your naked eye. Utilizing the model while viewing the prepared slides of the kidney cross section may help you interpret what you see under the scope.

☐ Based on your observation of the kidney model, what do you think is the functional unit of the kidney? Why?

Locate the following kidney and nephron structures in ***Figure 10.6*** using the model and provided slides.

☐ Why do you think the arteries leading to the kidneys are so large?

☐ What do the kidneys filter from the blood?

☐ Trace the flow of fluid through a nephron from the glomerulus to the collecting duct in ***Figure 10.7*** using your pencil. What is the order of structures through which the fluid passes?

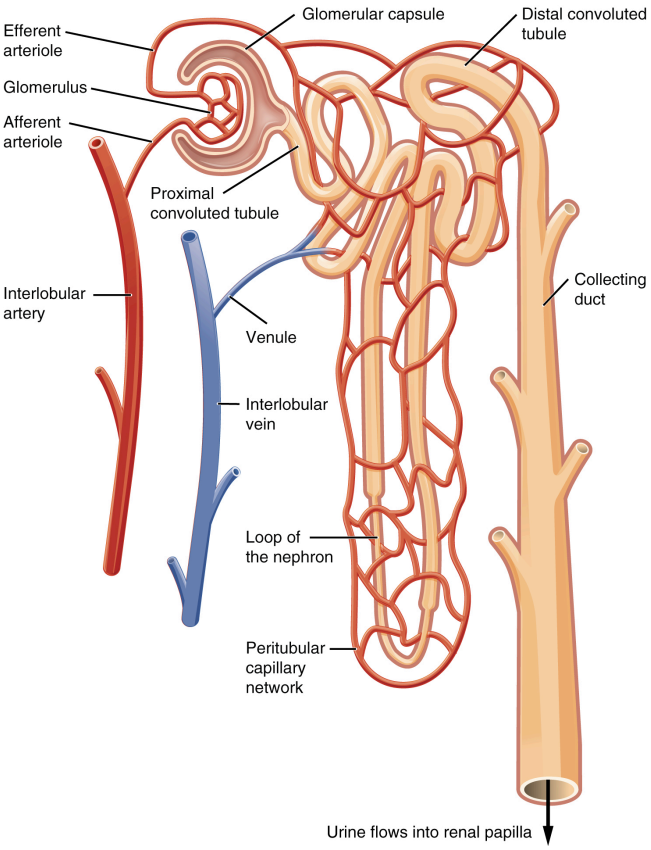


Figure 10.7. Blood flow in the nephron. Image Source: OpenStax Anatomy & Physiology, CC BY 4.0.

7. Mammalian Reproductive System

Identify the internal reproductive organs of your pig. This may take some careful dissection to prevent accidentally cutting through structures. Locate a group of your classmates or carefully observe the demonstration dissection of a pig of the opposite sex and make sure you can identify both male and female reproductive organs, as shown in ***Figures 10.8*** and ***10.9***.

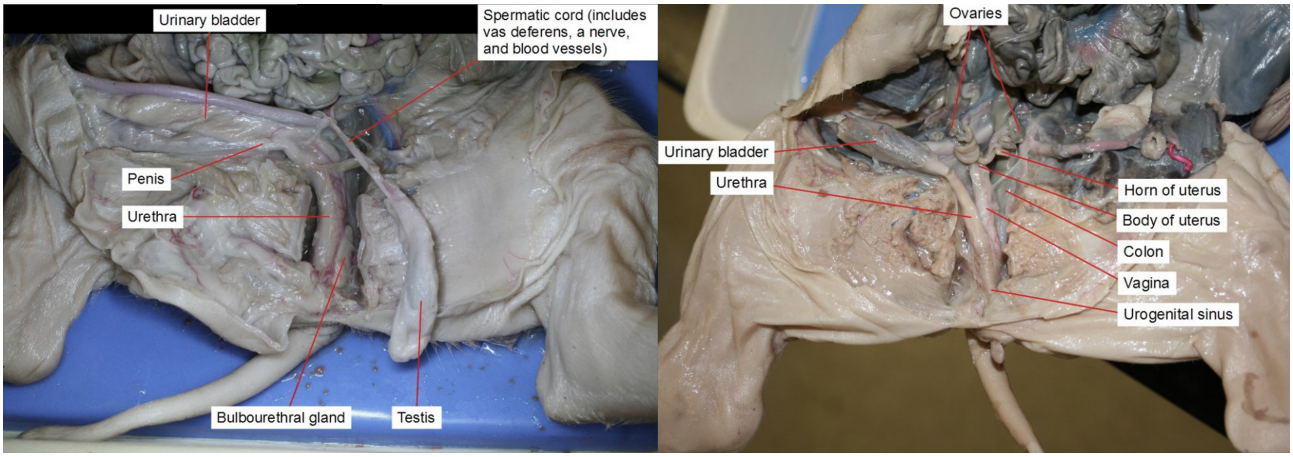


Figure 10.8. (left) Male fetal pig reproductive system. Figure 10.9. (right) Female fetal pig reproductive system.Image Source: "Biology II Laboratory Manual," by Michael J. Gregory, Ph.D., CC BY NC SA 4.0 License.

Describe the pathway that eggs and sperm take to leave the body.

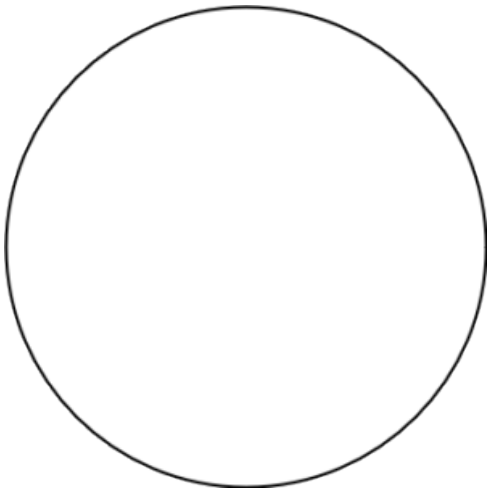
Eggs:

Sperm:

Make sure you check out the pregnant sow uterus. Can you find the embryos?

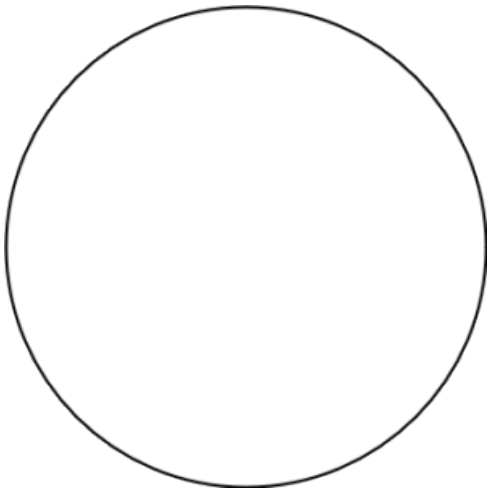
☐ You know that the reproductive anatomy of males and females differ on the macroscopic scale, but they also differ on the microscopic scale. Look at the slides for male and female gonads (testes and ovaries) and sketch what you see under the microscope below.

MALE



Magnification:

FEMALE



Magnification:

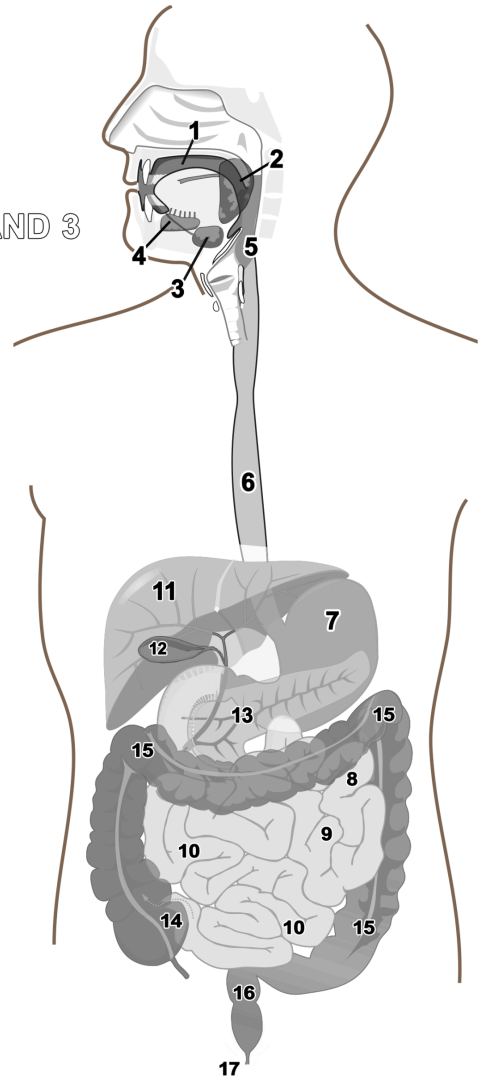
Study Material

The following material will not be collected or graded but is provided to further test your understanding of the material in the lab. These will be valuable assets for quiz and practical studying.

Review Activity 10 .1

Help yourself visualize the different body organs in respect to other structures in the system. Using crayons, colored pencils, and/or markers, fill in the name of each organ with a specific color and using that same color fill in the corresponding organ. Use different colors for different organs/structures. For an extra challenge, test yourself on their function.

ORAL CAVITY 1
PAROTID GLAND 2
SUBMANDIBULAR GLAND 3
SUBLINGUAL GLAND 4
PHARYNX 5
ESOPHAGUS 6
STOMACH 7
DUODENUM 8
JEJUNUM 9
ILEUM 10
LIVER 11
GALLBLADDER 12
PANCREAS 13
CECUM 14
LARGE INTESTINE 15
RECTUM 16
ANUS 17

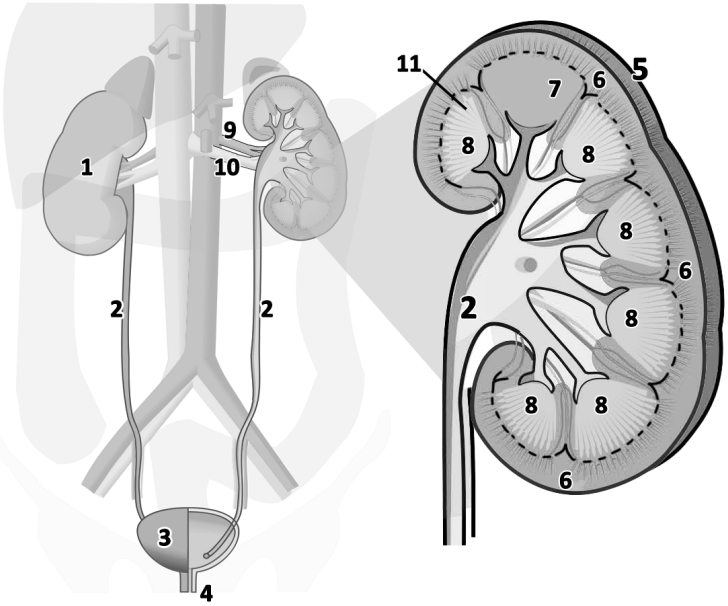


Review Figure 10.1.

Review Activity 10 .2

Help yourself visualize the different body organs in respect to other structures in the system. Using crayons, colored pencils, and/or markers, fill in the name of each organ with a specific color and using that same color fill in the corresponding organ. Use different colors for different organs/structures.

KIDNEY **1**
URETER **2**
URINARY BLADDER **3**
URETHRA **4**
RENAL CAPSULE **5**
RENAL CORTEX **6**
RENAL MEDULLA **7**
RENAL PYRAMIDS **8**
RENAL ARTERY **9**
RENAL VEIN **10**
NEPHRON **11**

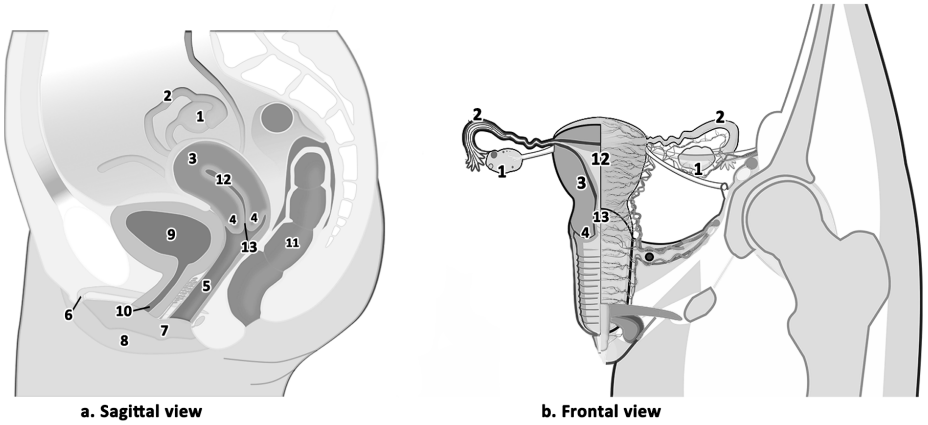


Review Figure 10.2. Image Source: adapted by Abbey Elder from "Urinary system large unlabeled" by Andrew Meyerson (adapted from Jordi March i Nogué), CC BY SA 3.0.

Review Activity 10 .3

Help yourself visualize the different body organs in respect to other structures in the system. Using crayons, colored pencils, and/or markers, fill in the name of each organ with a specific color and using that same color fill in the corresponding organ. Use different colors for different organs/structures.

OVARY **1**
UTERINE TUBE **2**
BODY OF UTERUS **3**
CERVIX OF UTERUS **4**
VAGINA **5**
CLITORIS **6**
LABIUM MINUS **7**
LABIUM MAJUS **8**
URINARY BLADDER **9**
URETHRA **10**
RECTUM **11**
UTERINE CAVITY **12**
CERVICAL CANAL **13**



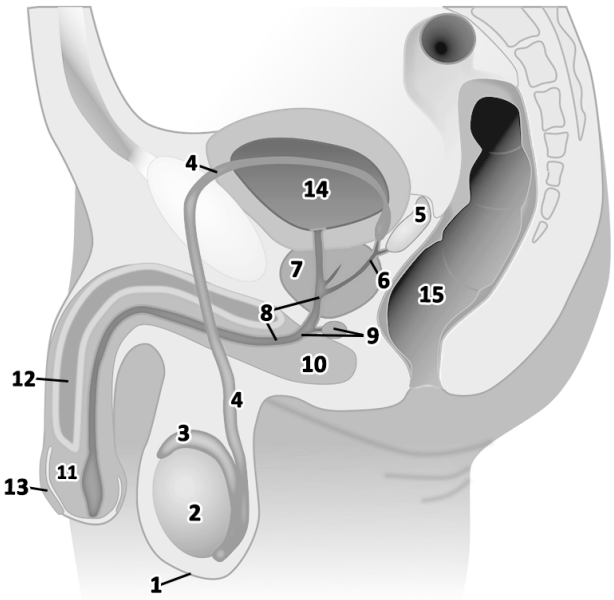
Review Figure 10.3. Adapted from "Male and female anatomy" (sagittal view) by Tsaitgaist and "Scheme female reproductive non-labels" (frontal view) by Jmarchn, both CC BY SA 3.0.

Note: You are **not** expected to know every term listed on this review page. Review ***Figure 10.9*** to study for the lab practical exam.

Review Activity 10 .4

Help yourself visualize the different body organs in respect to other structures in the system. Using crayons, colored pencils, and/or markers, fill in the name of each organ with a specific color and using that same color fill in the corresponding organ. Use different colors for different organs/structures.

SCROTUM **1**
TESTIS **2**
EPIDIDYMIS **3**
DUCTUS DEFERENS **4**
SEMINAL VESICLE **5**
EJACULATORY DUCT **6**
PROSTATE GLAND **7**
URETHRA **8**
BULBOURETHRAL
GLAND AND DUCT **9**
CORPUS SPONGIOSUM **10**
GLANS PENIS **11**
CORPORA CAVERNOSA **12**
PREPUCE **13**
URINARY BLADDER **14**
RECTUM **15**



Review Figure 10.4.Adapted from "Male and female anatomy" (sagittal view) by Tsaitgaist, CC BY SA 3.0.

Note: You are **not** expected to know every term listed on this review page. Review ***Figure 10.8*** to study for the lab practical exam.

LABORATORY 11.

CIRCULATORY SYSTEM

Objectives

Following this week's lab, students will be able to:

- Identify structures and describe the function of the mammalian circulatory system
- Recount the pathway that blood flows through the heart and the major vessels in the mammalian circulatory system and state at which points blood is oxygenated and deoxygenated
- Identify blood cell types and describe their functions
- Measure human heart rates and blood pressures and explain how these values relate to human health
- Demonstrate proper dissection technique and respect for animal specimens and dissection tools

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Your TA will check that you have attempted to complete the dissections associated with this week's lab activity.

Resources

- Dissection Interchapter 6 "Dissection Techniques and Terminology" (pp. 137–140)
- Fetal pig dissection tutorial video (tutorial video on Canvas)
- Fetal pig dissection guide, Smith and Schenk 2011 (provided in binders for use in lab)
- Biological Science, Freeman *et al.* 2024 (8th edition)
    - Circulatory System (42.5)
- Internet and Canvas resources

☐ This icon represents a question meant to test your understanding. Answering these questions in the space provided as you go through the lab will help you better understand the topic and study more effectively. Use your text or e-book, pig dissection guide, and the internet to help you.

NOTE

Printed instructions for the Blood Pressure PowerLab activity are provided at the PowerLab stations in the classroom. The instructions are also provided on Canvas for students to review from home. Please read through the instructions carefully while working through the PowerLab activity. Record the data on the data sheets printed on the following pages of the lab manual.

Part 1: Blood Pressure and Heart Rate Lab Activity

WARNING

This lab activity is not a replacement for medical advice. If you are concerned with your blood pressure, please see a doctor. This procedure involves stopping blood flow to the arm. This is potentially dangerous. Do not leave the cuff inflated for a prolonged period of time (no more than around 90 seconds).

During contraction and relaxation of the heart, the pressure of blood in the arteries changes depending on the stage of the cycle. The ventricles of the heart contract to push blood into the arteries and then relaxes to fill with blood before pumping once more. When the heart pushes blood into the arteries there is a sudden increase in pressure. Then, when the heart muscle relaxes, blood pressure declines slowly until the heart contracts again. Blood pressure is at its highest (**systolic pressure**) shortly after contraction begins and at its lowest (**diastolic pressure**) at the end of relaxation just before the ventricle contracts again. When you see a blood pressure reading, the first number is the **systolic pressure** and the second number is the **diastolic pressure**. Both pressures are measured in units of millimeters of mercury (mm Hg). The table below shows National Institutes of Health values for normal, prehypertension, and high blood pressure categories.

Table11.1. Blood pressure values from the National Institutes of Health of different categories of health.

Category	SystolicPressure (mmHg)	DiastolicPressure (mmHg)
Normal	<120	<80
Prehypertension	120–139	80–89
High blood pressure	>140	>90
Hypotension	<90	<60

In this lab, you will get to measure your blood pressure by using an inflatable cuff to apply external pressure around the upper arm to collapse the brachial artery and block blood flow to the forearm. You will record the applied pressure and record blood flow to a finger or thumb using the PowerLab and the Lumiscope.

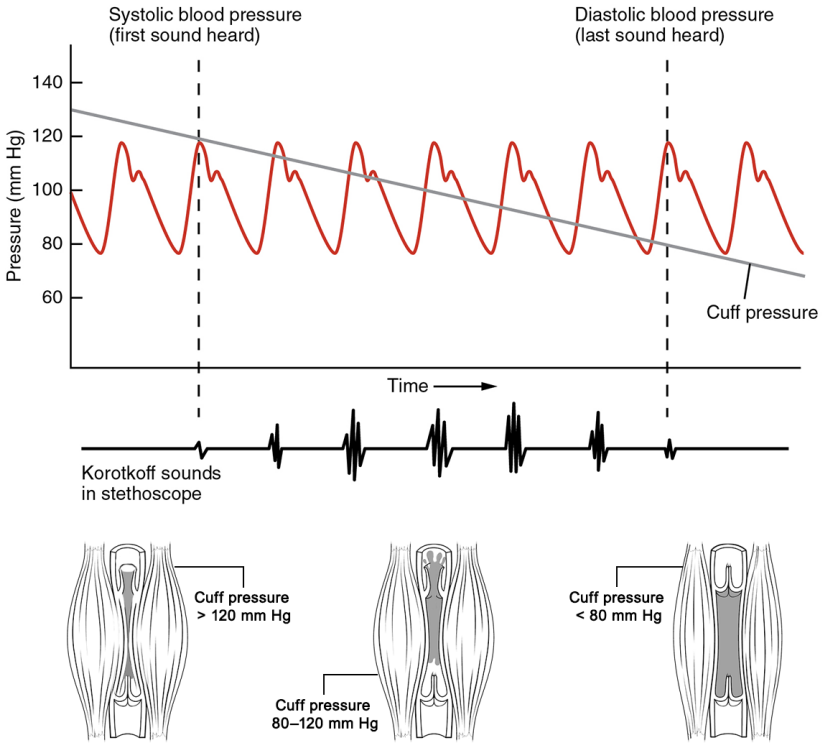


Figure 11.1. Diagram depicting what happens in your arm during a blood pressure measurement. Image Source: Figures 20.12 & 20.15 in OpenStax Anatomy & Physiology, CC BY 4.0. Adapted by Abbey Elder.

PowerLab Activity Data

Blood Pressure and Heart Rate Activity

1. Enter your data from the **Lumiscope blood pressure monitor**. Include units of measurement.
    - Systolic Pressure:
    - Diastolic Pressure:
    - Heart Rate:
2. Enter the data you collected to measure your heart rate (pulse) from your **Chart recording** described on page 160. Include units of measurement.
    - Time of first detectable heartbeat:
    - Time of final detectable heartbeat:
    - Calculated heart rate:

3. Are these pressures above, within, or below the "normal" blood pressure range for young adults?

4. Based on what you have learned about the cardiovascular system, why is high blood pressure bad for your health?

Part 2: What Factors Affect Blood Pressure and Heart Rate?

CREATE YOUR OWN EXPERIMENT

Use the guide below to create your experiment.

For this activity, you only need to take readings from **one** of your group members. After you have completed the in-class activity and before you move on to dissections, create your own blood pressure experiment and carry it out in your group using the information provided below. Record your hypotheses, protocol, and data in the following document. Once completed, you can move on to the Circulatory system dissections and stations.

TEST A HYPOTHESIS

In your group, brainstorm some potential variables that may affect blood pressure and heart rate. Be prepared to share your idea with the class. Your TA will compile some options on the board and your group can choose one that is most interesting to you. Once you have decided on a variable, answer the questions below before you test your experiment.

1. Choose one of these variables to test in lab. How will your variable affect blood pressure and/or heart rate? Below, identify the independent and dependent variables, and write a short hypothesis and protocol. Compare your experimental values to the values you already collected in lab.

   Independent Variable(s):

   Dependent Variable(s):

Hypothesis:

Brief Protocol:

Table 11.2. Student worktable for results from the blood pressure and heart rate experiment and create your own experiment.

Variable	Predicted effect (increase ordecrease)	Resting value	Experimental value	Was your prediction correct?
Heart rate				
Systolic pressure				
Diastolic pressure				

2. Carry out your experiment. Use the ***Table 11.2***, above, to enter your predictions and results. Fill in the resting value column with the data you collected in the beginning of the lab. ***Include units of measurement with your data!***

3. Compare your results to the hypothesis you wrote out in #2. Did your experimental results support your predicted effects on heart rate and/or blood pressure? Why or why not? What could be done to improve or expand your experiment in the future?

When complete, continue on to Part 3: Circulatory System Dissections and Stations

Part 3: Circulatory Dissections and Stations

1. Circulatory System Dissection

Carefully remove the following organs from the abdomen of your pig: intestines, stomach, liver, gall bladder, pancreas, spleen, bladder, thymus, and thyroid. Do not remove the lungs from your fetal pig, since you will study the respiratory system next week. Using your dissecting tools, remove connective tissue surrounding the remaining organs as needed to show the veins and arteries of your pig. If you haven't already, remove the tissue from your pig's neck and chest to expose the carotid and jugular veins. Identify the veins and arteries in your pig using the diagram below. You can also check out the demo dissection provided in lab to test yourself. Put a check mark next to each vein and artery as you find it.

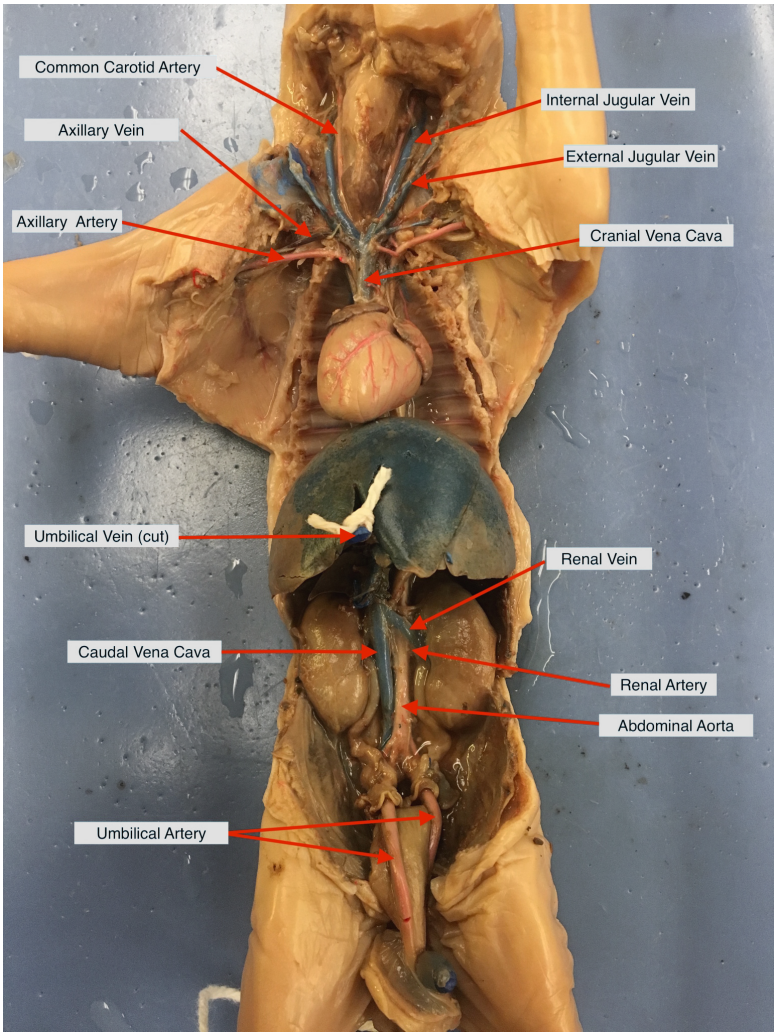


Figure 11.2. Circulatory system of a fetal pig. This image shows the lungs and diaphragm removed so circulatory system structures can be viewed more easily. Do not remove them from your own dissections.

☐ What color are the veins and arteries in your pig?

Vein color:

Artery color:

Note: The arteries and veins have been injected with colored latex so that you can easily differentiate them in the fetal pigs. In life, blood is always red due to the hemoglobin found in red blood cells. When hemoglobin is bound to oxygen, it is bright red and when it is not bound to oxygen, it is darker red.

☐ The definition of an artery is any vessel taking blood away from the heart. The definition of a vein is any vessel carrying blood towards the heart. What does the color of the veins and arteries in the fetal pig indicate about oxygenation levels in most arteries vs. most veins? Why does this make sense in terms of the function of the heart and circulatory system?

☐ Should umbilical and pulmonary veins and arteries match the color-coding scheme of the other arteries and veins in the body? Why or why not?

☐ Where does gas exchange occur for fetal pigs? Which blood vessel (the umbilical artery or vein) carries oxygenated blood?

2. Heart Structure and Function

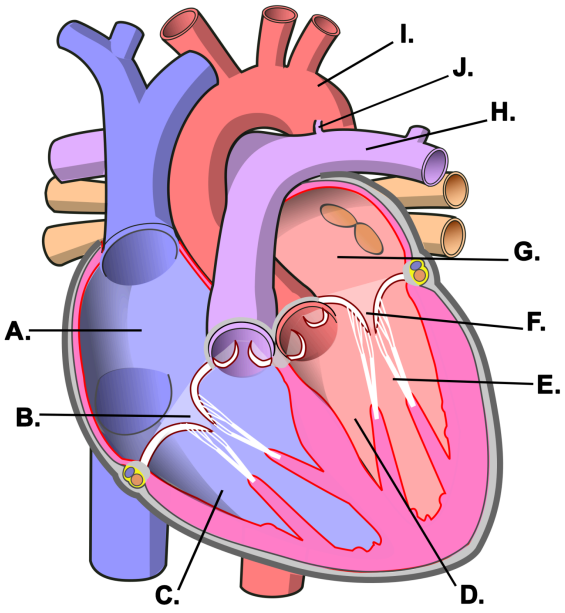
Use the stations provided in lab to observe the heart models and the demonstration dissection of an adult pig heart. Using your dissection guide and the information provided at the station, identify the parts of the heart in the diagram below. Make sure you can identify structures in both the model and the actual heart.

☐ Use red and blue markers or highlighters to trace the path of blood through the heart. Use blue to represent deoxygenated blood and red to represent oxygenated blood.

☐ Carefully remove the heart from your fetal pig and cut it in half longitudinally. Look at the chambers of the heart and trace blood flow.

Use the following list of structures to label the figure on the right:

1. Bicuspid valve
2. Chordae tendinae
3. Right atrium
4. Left atrium
5. Right ventricle
6. Left ventricle
7. Tricuspid valve
8. Pulmonary trunk
9. Ligamentum arteriosum
   (Ductus Arteriosus in the fetus)
10. Aorta



A. _____ **F.** _____

B. _____ **G.** _____

C. _____ **H.** _____

D. _____ **I.** _____

E. _____ **J.** _____

Figure 11.3. Mammalian heart structure. Image Source: "Diagram of the human heart" by Yaddah and MesserWoland, CC BY SA 3.0.

Be sure to know the path of blood starting at different parts of the heart and traveling to various parts of the body. Work with your partner to describe these potential pathways. Use ***Figures 11.2*** and ***11.3*** to help you.

☐ List the structures that blood travels through starting from the **lung** and going to the **kidney**. Is the blood in this pathway oxygenated or deoxygenated? Do oxygenation levels change at any point in this pathway?

☐ List the structures that blood travels through starting from the **right atrium** and going to the **head**. Is the blood in this pathway oxygenated or deoxygenated? Do oxygenation levels change at any point in this pathway?

☐ List the structures that blood travels through starting from the **liver** and going to the **left ventricle**. Is the blood in this pathway oxygenated or deoxygenated? Do oxygenation levels change at any point in this pathway?

3. Vein and Artery Structure and Function

Observe the prepared slide of a vein and artery at the station provided in lab.

☐ Using the photo below, label the vein with an **"A"** and the artery with **"B."**

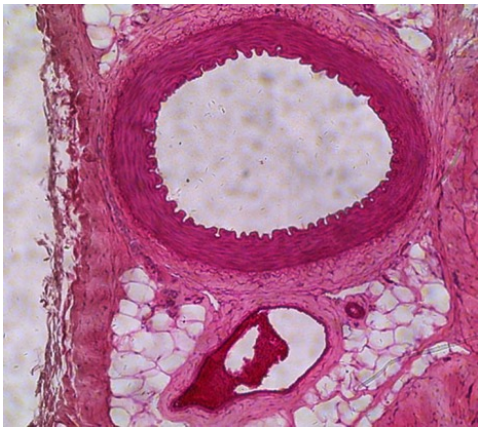


Figure 11.4. Mammalian vein and artery.

☐ How did you tell them apart? Why are these differences important to the function of the vein and artery?

4. Blood Smear

Look at the prepared slide of a blood smear at the station in lab. Observe it under the compound microscope at high enough magnification that allows you to see the individual blood cells. You can also use ***Figure 11.5***, below, to help visualize the different cell types.

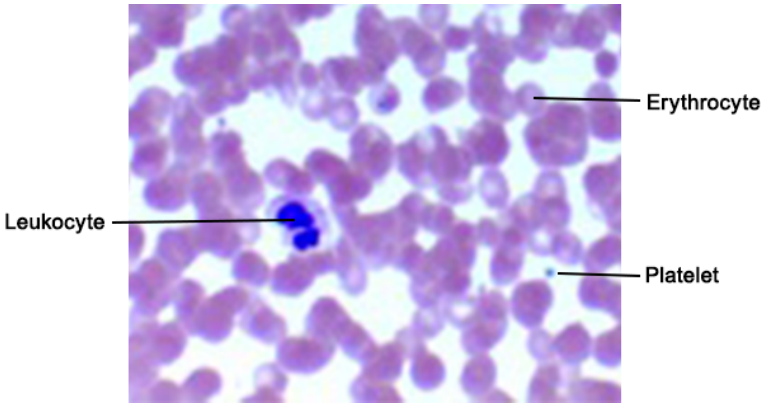


Figure 11.5. Magnified view of a blood smear at 40X magnification.

☐ There are many different types of cells in blood. Find each under your microscope, sketch what they look like, and describe their function(s) in ***Table 11.3***.

Table11.3. Student worktable to describe differences between blood cell types.

Cell Type	Sketch or take descriptive notes	Function
Erythrocytes		
Leukocytes		
Platelets		

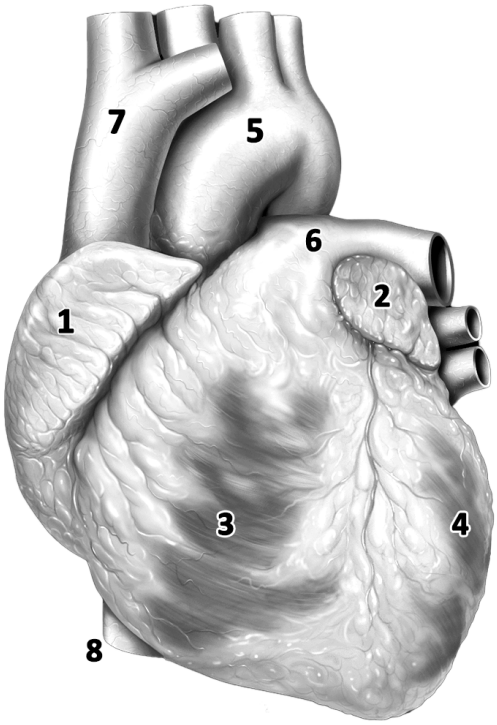
Study Material

The following material will not be collected or graded but is provided to further test your understanding of the material in the lab. These will be valuable assets for quiz and practical studying.

Review Activity 11.1

Help yourself visualize the external parts of the heart. Using crayons, colored pencils, and/or markers, fill in the name of each structure with a specific color and using that same color fill in the corresponding structure. Use different colors for different structures.

RIGHT ATRIUM **1**
LEFT ATRIUM **2**
RIGHT VENTRICLE **3**
LEFT VENTRICLE **4**
AORTIC ARCH **5**
PULMONARY TRUNK **6**
SUPERIOR VENA CAVA **7**
INFERIOR VENA CAVA **8**

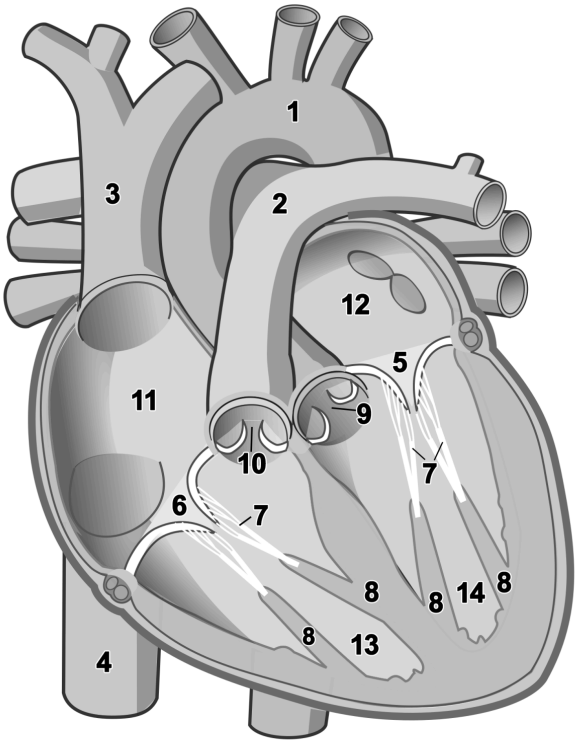


Activity Figure 11.1 Exterior heart structure. Image Source: Patrick Lynch via Wikimedia Commons, CC BY 2.5. New version adapted by Abbey Elder, CC BY 4.0.

Review Activity 11.2

Help yourself visualize the internal parts of the heart. Using crayons, colored pencils, and/or markers, fill in the name of each structure with a specific color and using that same color fill in the corresponding structure. Use different colors for different structures.

AORTA 1
PULMONARY TRUNK 2
SUPERIOR VENA CAVA 3
INFERIOR VENA CAVA 4
BICUSPID VALVE 5
TRICUSPID VALVE 6
CHORDAE TENDINAE 7
PAPILLARY MUSCLE 8
AORTIC SEMILUNAR VALVE 9
PULMONARY SEMILUNAR VALVE 10
RIGHT ATRIUM 11
LEFT ATRIUM 12
RIGHT VENTRICLE 13
LEFT VENTRICLE 14



Activity Figure 11.2 Human heart interior structure. Image Source: Yaddah and MesserWoland via Wikimedia Commons, CC BY SA 3.0. Adapted by Abbey Elder to add labels.

Bonus:

Using a blue and red marker, trace the pathway of blood through the vessels and heart structures. Use red for areas where the blood is oxygenated and blue for where it is deoxygenated. Draw in lungs and color those purple. (Why would they be purple in this conceptual figure?)

LABORATORY 12.

GAS EXCHANGE AND RESPIRATORY SYSTEM

Objectives

Following this week's lab, students will be able to:

- Identify structures and describe the function of the mammalian respiratory system
- List at least two similarities and two differences each between the respiratory systems of
- fish, insects, and mammals
- Describe the pathway air or water takes through the above animal respiratory systems and where gas exchange occurs
- Define at least four different human lung volumes and explain their significance in a health context
- Demonstrate proper dissection technique and respect for animal specimens and dissection tools

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Your TA will check that you have attempted to complete the dissections associated with this week's lab activity.

Resources

- Dissection Interchapter 6 "Dissection Techniques and Terminology" (pp. 137–140)
- Fetal pig dissection tutorial video (tutorial video on Canvas)
- Fetal pig dissection guide, Smith and Schenk 2011 (provided in binders for use in lab)
- Biological Science, Freeman *et al.* 2024 (8th edition)
    - Respiratory System (42.1–42.4)
- Internet and Canvas resources

☐ This icon represents a question meant to test your understanding. Check the box once you answer it. Answering these questions in the space provided as you go through the lab will help you better understand the topic and study more effectively. Use your text or e-book, pig dissection guide, and the internet to help you.

Part 1: Human Lung Volumes

Ever wonder how much air your lungs can hold? When you inhale and exhale, you use muscles to expand your rib cage to let air in and compress your rib cage to push air out. This is a form of ventilation, which is the movement of air or water past a respiratory surface so that gas exchange can occur between the external environment and body tissues. In this case, the respiratory surface is in the lungs, but in other animals it can be a part of gills or tracheae. The diagram below exhibits what happens to your lungs when you inhale (inspiration) and exhale (exhalation). In lab today we will explore what is happening in your lungs during these actions and how efficient your lungs are during normal breathing to better understand how lungs function.

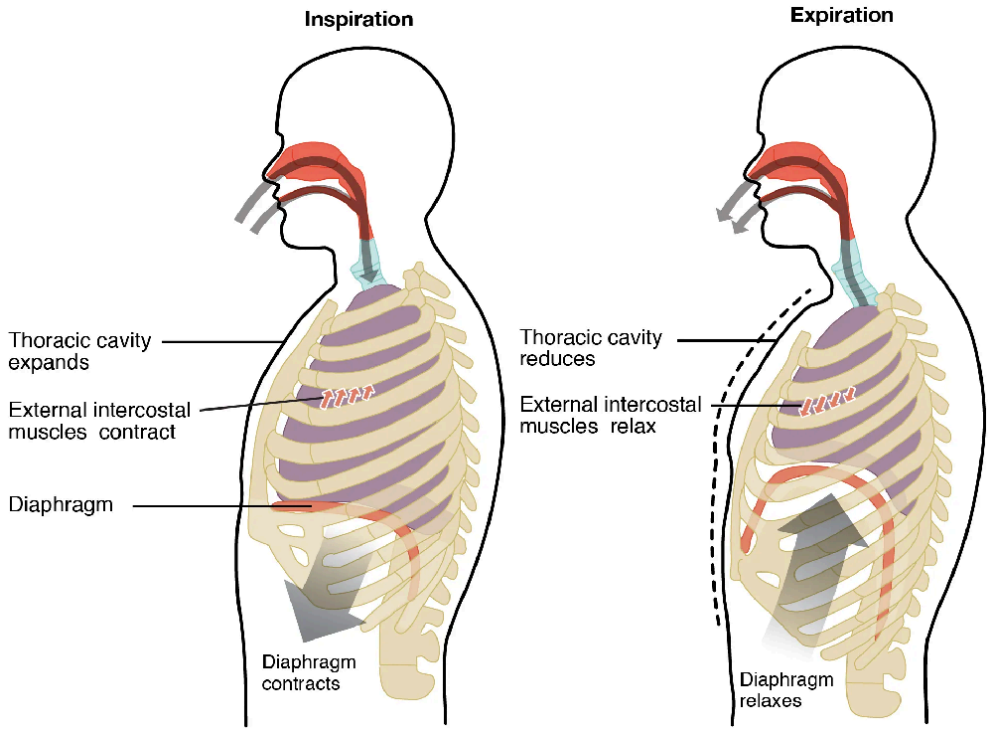


Figure 12.1. Inspiration and expiration of human lungs. Image Source: OpenStax Anatomy & Physiology 2E, CC BY 4.0.

There are several lung volume measurements that are important for assessing an individual's health and understanding how human lungs function. ***Table 12.1*** shows values for human respiratory volumes for the typical adult. Using this table and information from the video shown in class, work with your group mates to define the following:

Tidal Volume:

Inspiratory reserve volume:

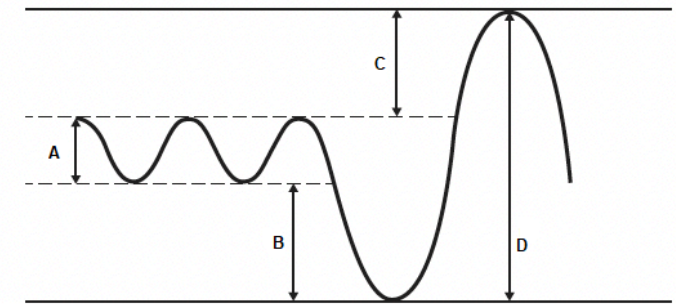
Expiratory reserve volume:

Vital Capacity:

Table 12.1. **Averages of typical adult respiratory volumes (in liters). Adapted from Ganong, William. "Fig. 35-7". Review of Medical Physiology (21st ed.)**

Volume	Value (liters) Men	Value (liters) Women
Inspiratory reserve volume	3.1	1.9
Tidal volume	0.5	0.5
Expiratory reserve volume	1.2	0.7
Vital capacity	5.8	4.2

1. The diagram below is an unlabeled drawing depicting the above ventilation volumes. Fill out the blanks below with the name of each segment.



A:

C:

B:

D:

Part 2: Respiratory System Dissections and Stations

During this portion of the lab, fill in ***Table 12.2*** to help organize your knowledge about the respiratory structures of mammals, fish, and insects. You will need the table to complete the post-lab worksheet.

Table 12.2. Characteristics of the respiratory system of mammals, fish, and insects.

Characteristics	Mammals	Fish	Insects
Structures and actions involved in ventilation			
Respiratory medium (air or water)			
Structures and actions involved with the respiratory surface			
Delivery to cell			

Fetal Pig Respiratory System Dissection

If you haven't done so already, open your pig's rib cage to expose its lungs and diaphragm. Remove any extra tissue around the neck, including the thyroid and thymus if you have not removed them already, to expose the trachea and larynx. Identify the organs in the figure below in your pig.

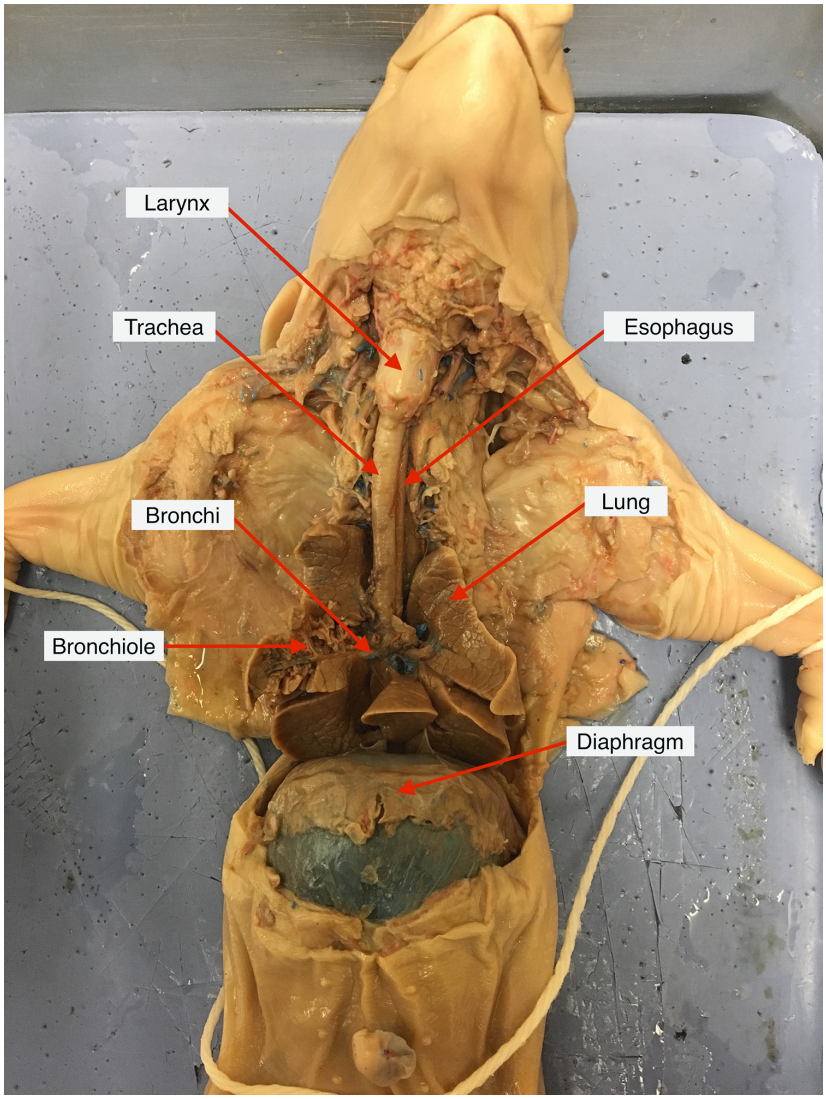


Figure 12.2. Labeled dissection of the fetal pig respiratory system.

As mammals, we breathe through our nose and mouth and ingest food and water through our mouth. Both the nose and mouth empty into a shared passageway, called the pharynx. The pharynx then leads to both the esophagus and the larynx. We learned about the route of food and water through the esophagus in Laboratory 10. Air goes through the larynx to the trachea and eventually reaches the lungs. It is vitally important that the food we eat does not end up in our lungs! ***Figure 12.3*** (below) shows where the epiglottis is located within the human pharynx.

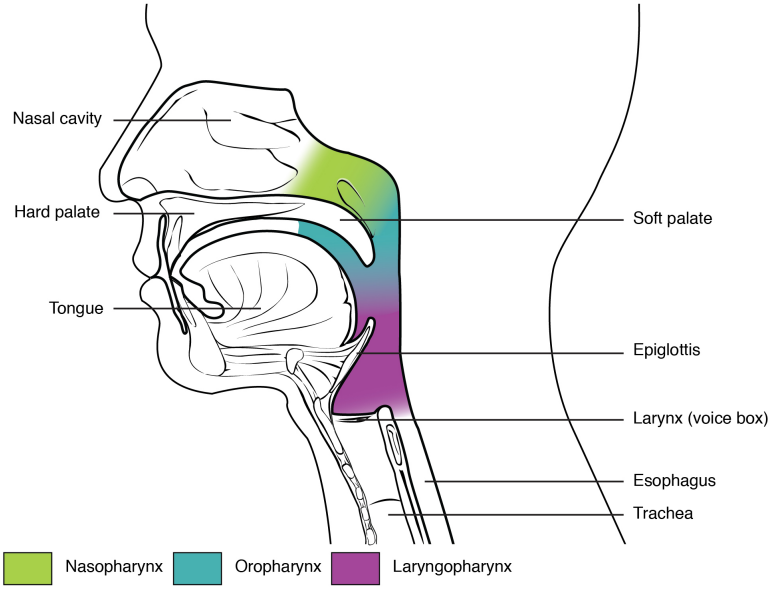


Figure 12.3. Epiglottis and other sections of the pharynx. The epiglottis covers the larynx when we swallow and is open when we breathe. Image Source: OpenStax Anatomy & Physiology, CC BY 4.0.

☐ Trace the passage of food through the pharynx into the esophagus on the illustration above.

☐ Trace the passage of air through the larynx into the trachea on the illustration above.

☐ Why is the epiglottis important?

Once air enters the body through the mouth and trachea, it moves from the respiratory tree to the alveoli as described in **Figure 12.4** below.

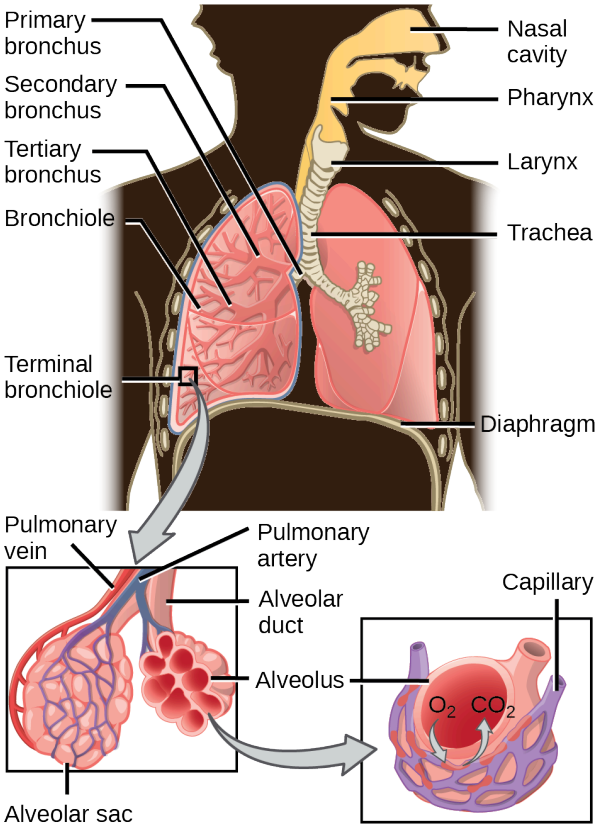
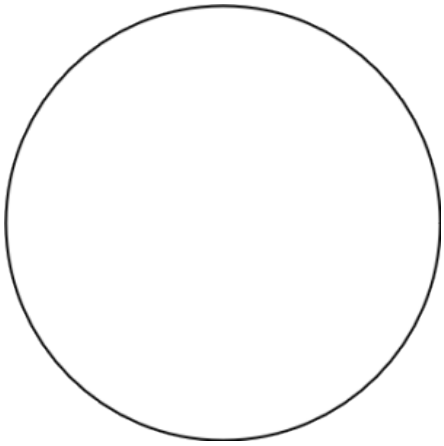


Figure 12.4. Human lungs, bronchiole, and alveoli. Image Source: OpenStax Biology 2E, CC BY 4.0.

You can't see some of these structures with the naked eye, so we have provided cross sectional slides of mammal lungs to show you the microscopic structures. Observe this slide at the lab station and sketch what you see below.



Magnification: _____

Insect Tracheal Respiratory System

Now that you have explored the mammalian respiratory system, it is time to observe the respiratory systems of different organisms. They are all quite different! Go to the insect station in the lab and check out the live hornworms. Look on the side of the hornworm to find the **spiracles**. They are found on the side of the body, one pair in each abdominal segment. At this station there is also a laptop computer with a video of a giant katydid breathing! You can also find the video linked on Canvas.

☐ What is the function of the spiracles in insects?

☐ Do insects have lungs like mammals? If not, what do they have instead?

Next, look at the demonstration dissection of a cockroach under the dissecting scope. The shiny mass of tubes you see in the abdomen are called **tracheoles** (use the photo posted at the station to help you distinguish the tracheoles from the insect digestive system). You may notice **air sacs** attached to the tracheoles. These are expansions of the tracheoles that are especially important in larger insects to help push air throughout the body. Use ***Figure 12.5*** to identify the structures in a bee.

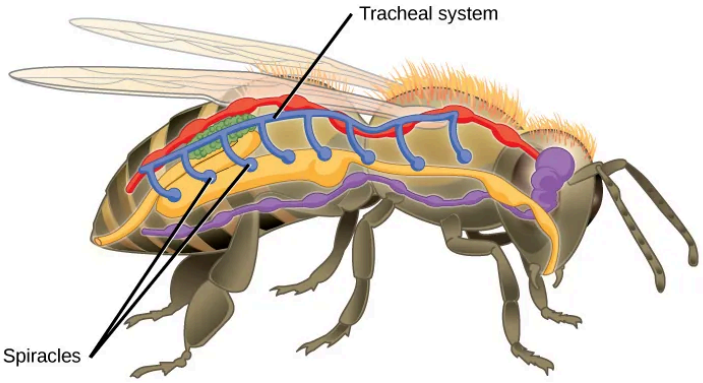


Figure 12.5. Respiratory system of a honeybee. Image Source: OpenStax Biology 2E, CC BY 4.0.

Fish Respiratory System

Go to the fish station to observe the photos, illustrations, and videos available. **Make sure you can identify the operculum, gill arches, and gills.**

☐ What is the function of the operculum in fish? What might happen if the operculum was damaged?

Since fish live underwater, their respiratory system is quite different than ours. Check out ***Figure 12.6*** which depicts the path of water across a fish's gills.

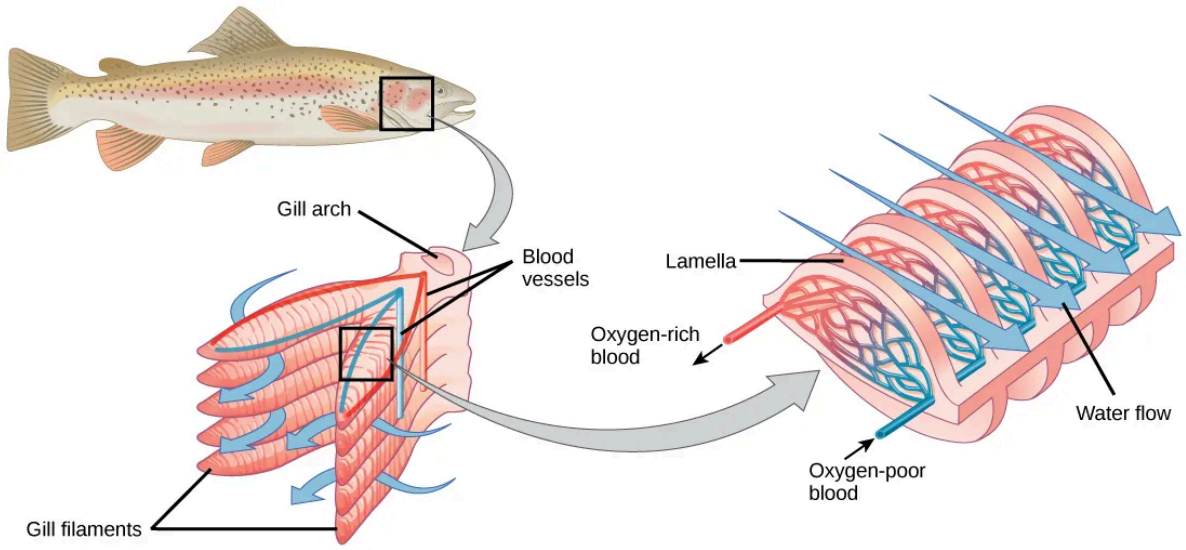
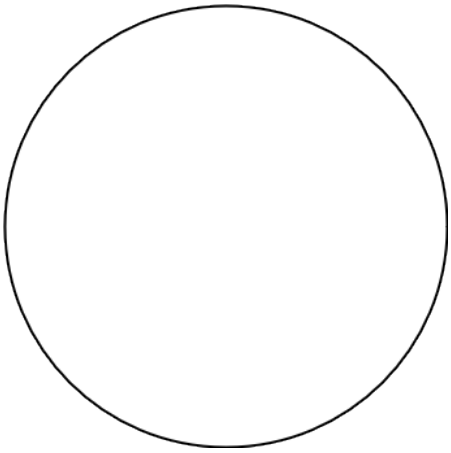


Figure 12.6. Respiration in a fish. Image Source: OpenStax Biology 2E, CC BY 4.0.

☐ Refer to the figure above to trace the pathway of water flow over the gills of the fish. How do gills work? What happens to the water as it enters the gills? Where does the water go after it leaves the gills?

☐ Look at the gill arch slide under the compound microscope. Sketch what you see below.



Magnification: _____

☐ Based on your observations, what structural features in the gills increase surface area for gas exchange?

☐ Why do you think gills stop functioning when a fish is taken out of the water?

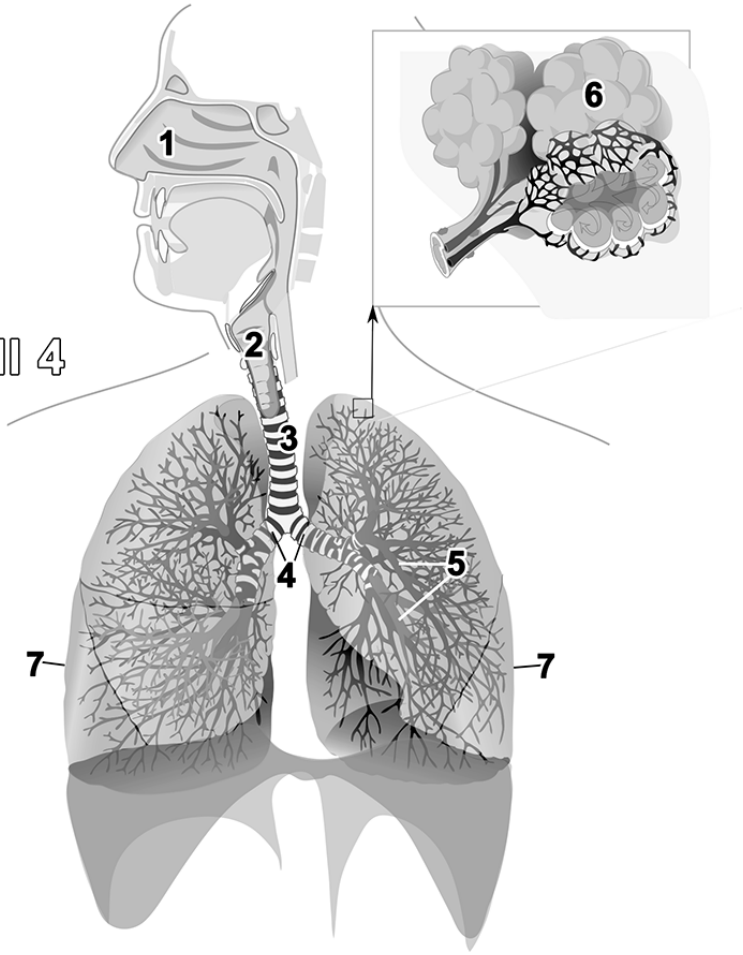
Study Material

The following material will not be collected or graded but is provided to further test your understanding of the material in the lab. These will be valuable assets for quiz and practical studying.

Review Activity 12 .1

Help yourself visualize the external parts of the mammalian respiratory system. Using crayons, colored pencils, and/or markers, fill in the name of each structure with a specific color and using that same color fill in the corresponding structure. Use different colors for different parts.

NASAL CAVITY 1
LARYNX 2
TRACHEA 3
PRIMARY BRONCHI 4
BRONCHIOLE 5
ALVEOLUS 6
LUNGS 7



Review Figure 12.1. Adapted from Respiratory System figure by Bibi Saint-Pol and Jmarchn, CC BY SA 3.0.

LABORATORY 13.

NERVOUS AND SENSORY SYSTEMS

Objectives

Following this week's lab, students will be able to:

- Identify the structures and describe the function of the mammalian nervous system
- Identify the structures and describe the function of the mammalian visual system
- For various phenomena in the eye (e.g., color blindness, bleaching, the blind spot) name the structures involved and describe how those structures contribute to each phenomenon
- Demonstrate proper dissection technique and respect for animal specimens and dissection tools

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Your TA will check that you have attempted to complete the dissections associated with this week's lab activity.

Resources

- Dissection Inter-Chapter 5 "Dissection Techniques and Terminology" (pp. 137–140)
- Fetal pig dissection tutorial video (tutorial video on Canvas)
- Fetal pig dissection guide, Smith and Schenk 2011 (provided in binders for use in lab)
- Biological Science, Freeman *et al.* 2024. 8th Edition. Nervous System (Chapter 43) Visual System (Chapter 44, specifically 44.3)
- Canvas resources

☐ This icon represents a question meant to test your understanding. Answering these questions in the space provided as you go through the lab will help you better understand the topic and study more effectively. Use your text or e-book, pig dissection guide, and the internet to help you.

LAB ORGANIZATION

Lab today will be organized a bit differently from previous labs. Work within your dissection group (2 people per group) to go through activities A-F in any order you'd like. Spend time looking at the appropriate slides, doing the dissections, completing the activities, and answering the questions. Be sure to clean up all of your supplies and dispose of your fetal pig (as directed by your TA) at the end of lab.

Station A: Nervous System Microanatomy and Models

Locate the prepared slide showing a smear of cells from a spinal cord and observe it under a compound microscope. Look through the smear to identify **neurons** and **glial cells**, and observe the structural differences between these cell types. Label these structures on **Figure 13.1** below. You are encouraged to take your own photos to help you study.

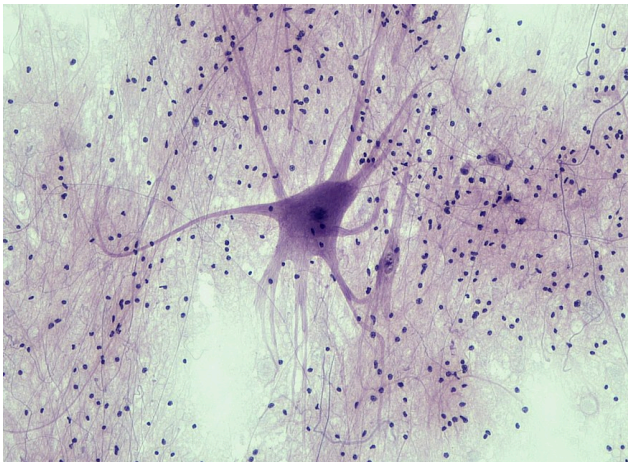


Figure 13.1. Microscopic view of a mammalian spinal cord smear.

☐ What is a glial cell?

☐ What is a neuron?

Look at a spinal cord cross-section prepared slide under the compound scope. Find the structures in the slide that are shown in **Figure 13.2** below.

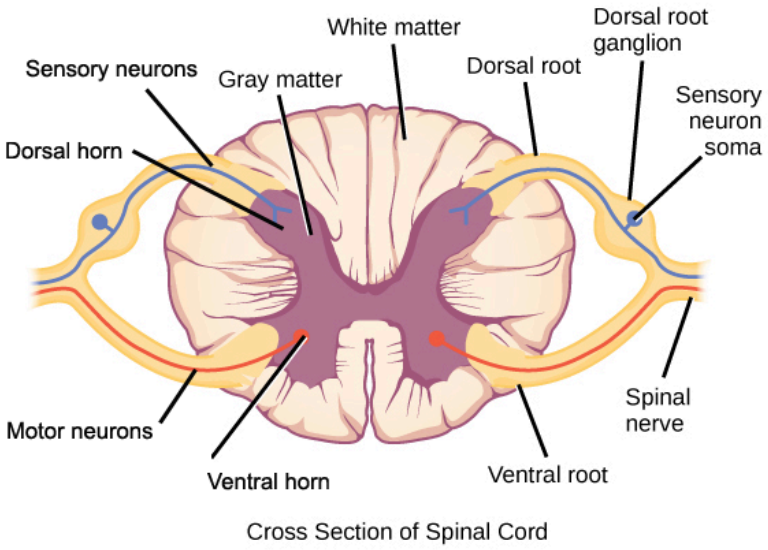


Figure 13.2. Model depicting the cross-section of a human spinal cord. Image Source: OpenStax Biology 2E, CC BY 4.0.

☐ What parts of the cells making up nervous tissue contribute to gray matter? What parts of the cells making up nervous tissue contribute to white matter?

☐ What is the difference between the appearance of gray matter and white matter? Why do you think those differences exist in terms of their function?

Now take a look at the model of a nerve cell provided in lab. Compare it to the **Figure 13.3** below.

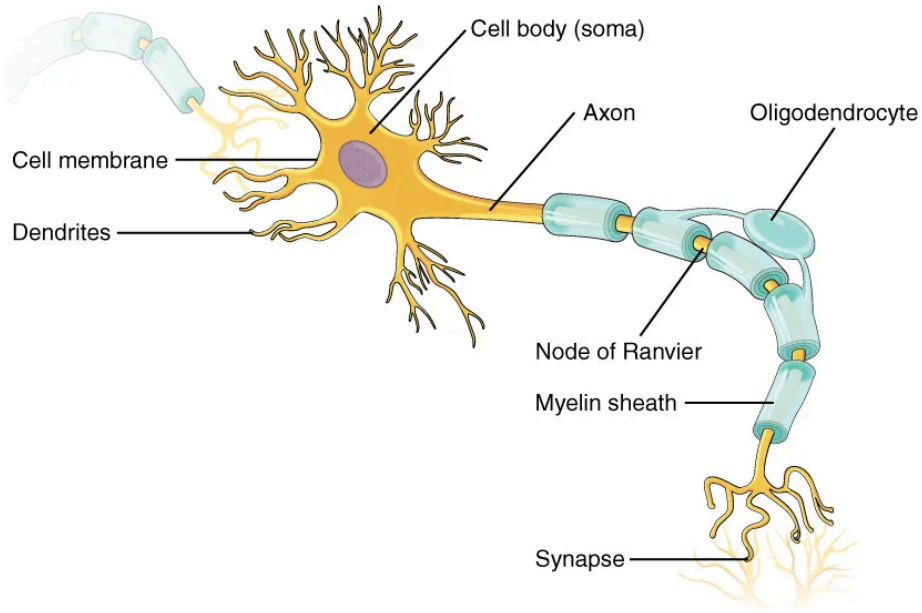


Figure 13.3. Illustration of a nerve cell. Image Source: OpenStax Anatomy & Physiology 2E, CC BY 4.0.

☐ What structures can you identify in the model?

☐ How does a signal pass through these structures?

Station B: Stretch Reflex

Watch the video provided at this station to learn about the patellar stretch reflex and answer the questions below. There are reflex hammers at this station if any volunteers would like to test their reflexes. If you would like to volunteer, follow these instructions:

Have the volunteer sit in a chair with their legs bent and their body relaxed. The tester should look at **Figure 13.4** below to determine the area they will need to tap with the reflex hammer. Then, the tester should gently hit the volunteer's patellar tendon, right below the kneecap, with the broad side of the reflex hammer. Observe what happens to the volunteer's leg. You may need to try a few times to get the right location.

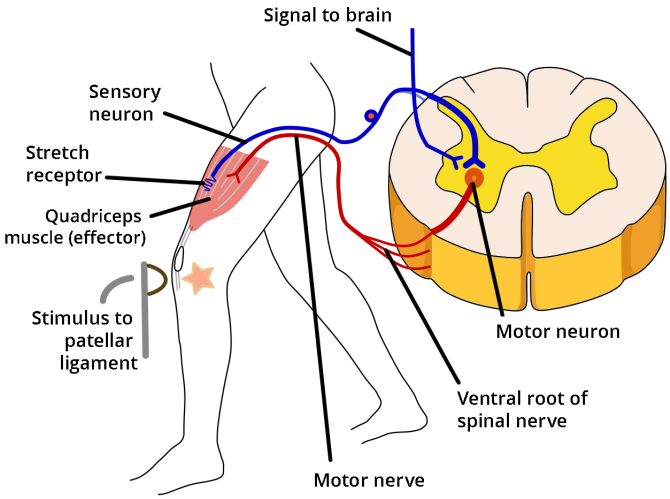


Figure 13.4. Patellar stretch reflex. Image Source: Andreas Raether via Wikimedia Commons, CC BY SA 4.0.

☐ For the patellar stretch reflex to occur, signals need to be sent between the peripheral (PNS) and central (CNS) nervous systems. a) In the sensory neuron, which direction is the signal traveling (towards the PNS or CNS) and at which structure does the neuron end?

b) In the motor neuron, which direction is the signal traveling (towards the PNS or CNS) and at which structure does the neuron end?

☐ What can a slow patellar reflex tell your doctor about how your nervous system is functioning?

☐ What part of the nervous system controls reflexes?

☐ Can you stop or control your reflexes?

Station C: Pig Nervous System Dissection

NOTE: This is the last time you will use your fetal pig. When you are finished with your dissection, place the pig back in your class storage bin for disposal. Do not remove any part of your pig from the lab.

In this activity, retrieve your pig and dissection supplies and return to your student bench. You and your partner will expose and observe the spinal cord of your fetal pig. You are going to look at both the ventral and dorsal views of the spinal cord. Begin by removing all the large organs of the body cavity. After removal, you should be able to see the **sympathetic trunks** and **spinal nerves** along the back of the body cavity of your pig. You can look at the demonstration dissection provided in class to make sure you are visualizing the right structures.

☐ Which structures are in the central nervous system? Which structures are in the peripheral nervous system?

☐ How do sympathetic nerves and parasympathetic nerves differ in function?

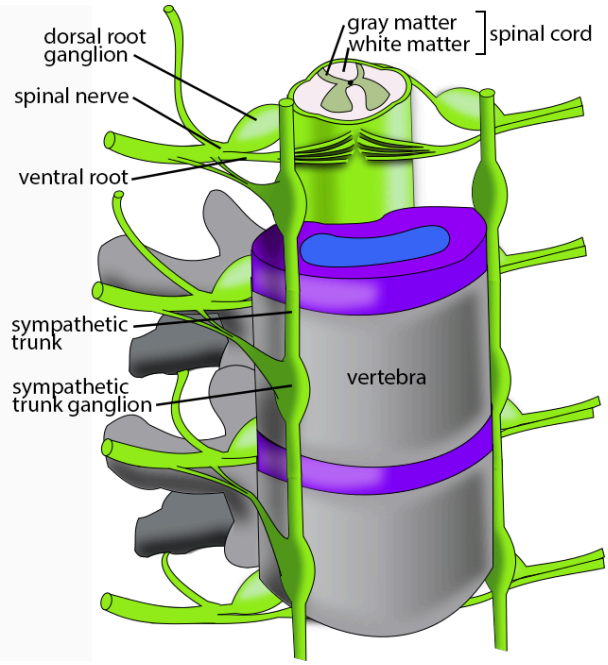


Figure 13.5. Spinal cord and spinal nerve illustration. Image Source: Sheldahl via Wikimedia Commons, CC BY SA 4.0.

Next, turn the fetal pig over and begin the dorsal spinal cord dissection. Start by removing the skin around the spine and up to the base of the skull. Work slowly to reveal the meninges, dorsal and ventral roots, and spinal nerves. Feel free to look at the demonstration dissection to help lead your dissection. Use the fetal pig dissection guides provided in lab to help find the structures.

☐ What are the functions of meninges in the spinal cord?

☐ How do dorsal and ventral roots differ in function?

☐ Why do nerves, but not ganglia, appear white?

Station D: Sheep Brain Dissection

SAFETY NOTE

The dissections being completed in lab this week can cause some students to feel queasy and/ or uncomfortable. Please be alert and mindful this week. Look out for your lab partner and make sure that you practice safe dissection protocols.

Retrieve a sheep brain from the station in the lab, place it on your dissection tray, and bring it back to your workstation for dissection and observation. You should identify major regions on the outside of the brain first (**Figure 13.6**) and then cut the brain longitudinally (cut between the lobes) if it has not been done already. Use the fetal pig dissection guide and laminated guides provided at the station to identify all of the areas of the brain shown in **Figure 13.6**. Test each other on the structures.

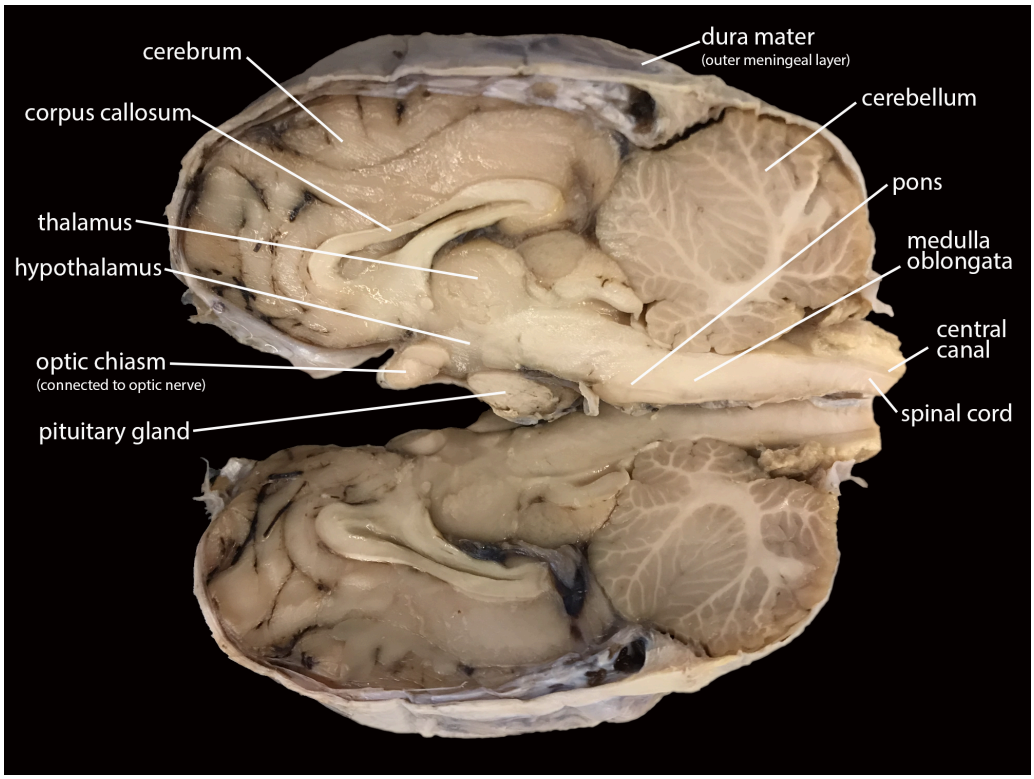


Figure 13.6. Ventral view of the mammalian (sheep) brain.

After thoroughly observing the sheep brain, fill out **Table 13.1** to help you learn about the functions associated with various areas of the brain.

Table 13.1. **Student worktable to describe the functions that occur in various areas of the brain.**

Structure	Function
Cerebrum	
Cerebellum	
Pons	
Hypothalamus	
Pituitary Gland	
Medulla Oblongata	
Optic Chiasm	
Olfactory Bulb	
Meninges	

Station E: Sheep Eye Dissection and Retinal Microanatomy

SAFETY NOTE

The dissections being completed in lab this week can cause some students to feel queasy and/ or uncomfortable. Please be alert and mindful this week. Look out for your lab partner and make sure that you practice safe dissection protocols. Students are required to wear safety goggles while completing dissections.

For this activity, each pair of students should retrieve a sheep eye from station E and then return to their student bench. Before cutting into it, observe the external structures of the eye. After you have observed the outer structures, use a scalpel to slice the eye **longitudinally**.

Wear goggles to prevent any liquid from the specimen from getting in your eyes. Once you have cut the eye, identify the structures shown in **Figure 13.7** below. Check out the eye model in the lab to test each other on structure identification.

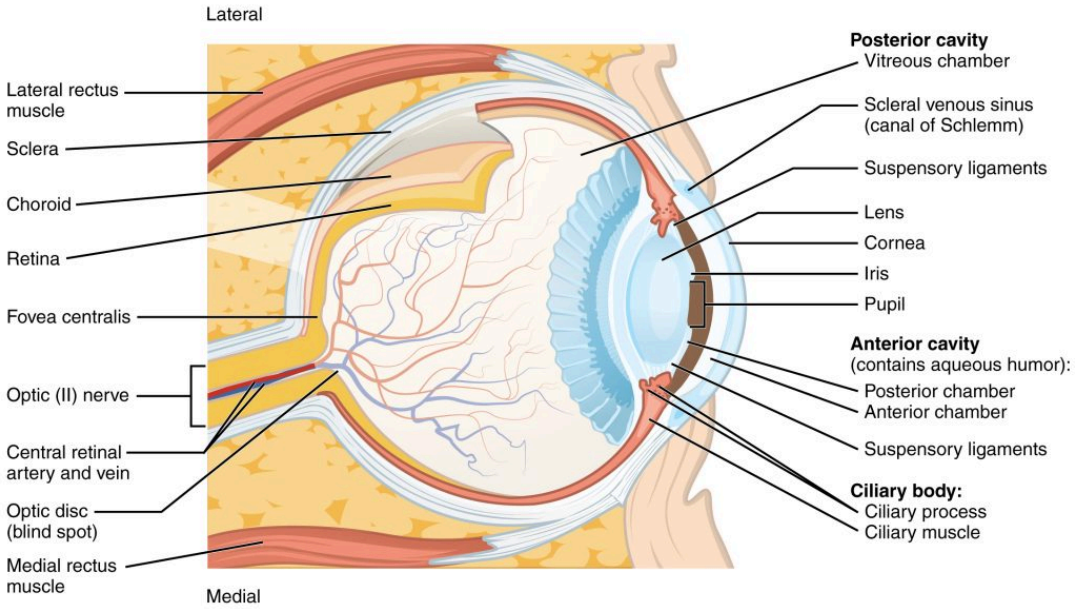


Figure 13.7. Illustration depicting structure of the mammalian eye. Image Source: OpenStax Anatomy & Physiology 2E, CC BY 4.0.

After you have finished your dissection, work with your partner to fill out **Table 13.2** to describe the functions of a few eye structures.

Table 13.2. **Student worktable to describe the functions that occur in various areas of the eye.**

Structure	Function
Pupil	
Cornea	
Retina	
Lens	
Optic Nerve	
Optic Disc	

Examine a cross section of the retina of a mammalian eye (prepared slide, compound microscope). Use **Figure 13.8** as a guide. Try to identify the layers of structures—the sclera and blood vessels in the choroid externally, then the photoreceptor layer deep to the choroid, and then the bipolar and ganglion layers of neurons internally.

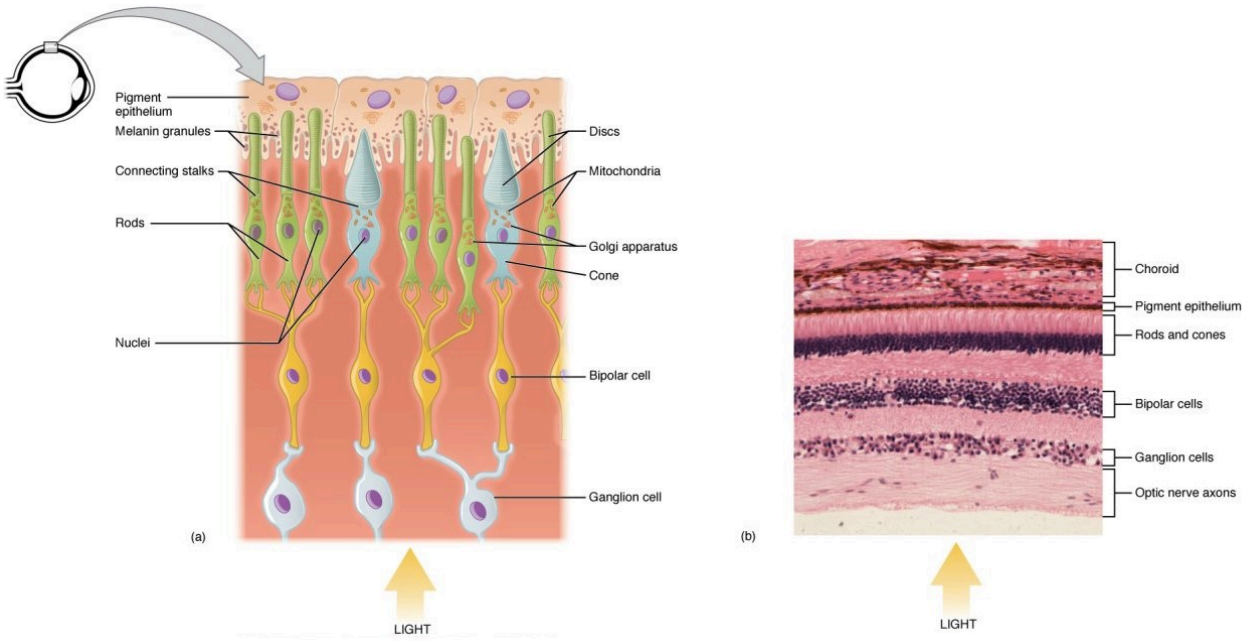


Figure 13.8. Microscopic image depicting the mammalian retina. Image Source: OpenStax Anatomy & Physiology 2E, CC BY 4.0.

☐ What is the function of the retina?

In the retina, there are photoreceptor cells that respond to light and help you "see" clear and colorful images. There are two types of these cells: **rods** and **cones**. Both are extremely important to the proper function of your eye and create images in different ways. Check out pages 952–956 in the Freeman textbook for more information about rods and cones. Use the information to help you fill in **Table 13.3**.

Table 13.3. Student worktable to compare and contrast rod and cone structures of the eye.

	Rods	Cones
Function		
Optimal Conditions (i.e. bright light, dim light)		
How many types?		

Station F: Blind Spot, After-images, and Color Blindness

At this station, you will learn about the concepts of blind spots, after images, color blindness, and attention. Note that all files necessary for this station can be found on all of the student laptops in the lab (as well as on Canvas).

Blind Spot

Open the blind spot activity PowerPoint. Cover your left eye and keep your right eye focused on the black dot closest to the left edge of the window. Press start on the animation.

☐ What do you perceive as the right dot moves across the screen? Do you notice a moment where you cannot see the moving dot?

☐ What is a blind spot? Why is this area of the retina "blind"?

☐ Why don't you normally notice your blind spot in day-to-day life?

2. After-Images

Go through the after-image PowerPoint.

☐ What is an after-image?

Once you are ready, start the PowerPoint with your group and follow the directions as told in the PowerPoint. Answer these questions as you go. Think about what you learned about rods and cones in the previous section to help you answer the questions below.

☐ What color after-image did you see after looking at the red cross? Why did you see that color?

☐ What color after-image did you see after looking at the blue square? Why did you see that color?

☐ What color after-image did you see after looking at the green arrow? Why did you see that color?

☐ What does it mean for cones to be bleached?

3. Color Blindness

In this station you will explore color blindness. Look at the color blindness tests available at the station. Take the tests yourself and answer the questions below.

☐ What structures of the eye allow you to see colors?

☐ Red-green color blindness is the most common type of color blindness. What would happen if you were missing red cones? What would happen if you were missing green cones? Why would red and green look the same in both cases but blue would still be distinguishable?

☐ Complete the tests for color blindness. Assess yourself for color blindness.

4. Optical Illusions

In this station you will check out the Magic Eye optical illusion books and the Where's Waldo books. After you go through the provided books, answer the questions below.

☐ What is an optical illusion?

☐ Why is it sometimes difficult to find Waldo in the Where's Waldo books?

5. Attention Activity

Watch the attention video on one of the lab laptops.

☐ How good were you at paying attention during the video?

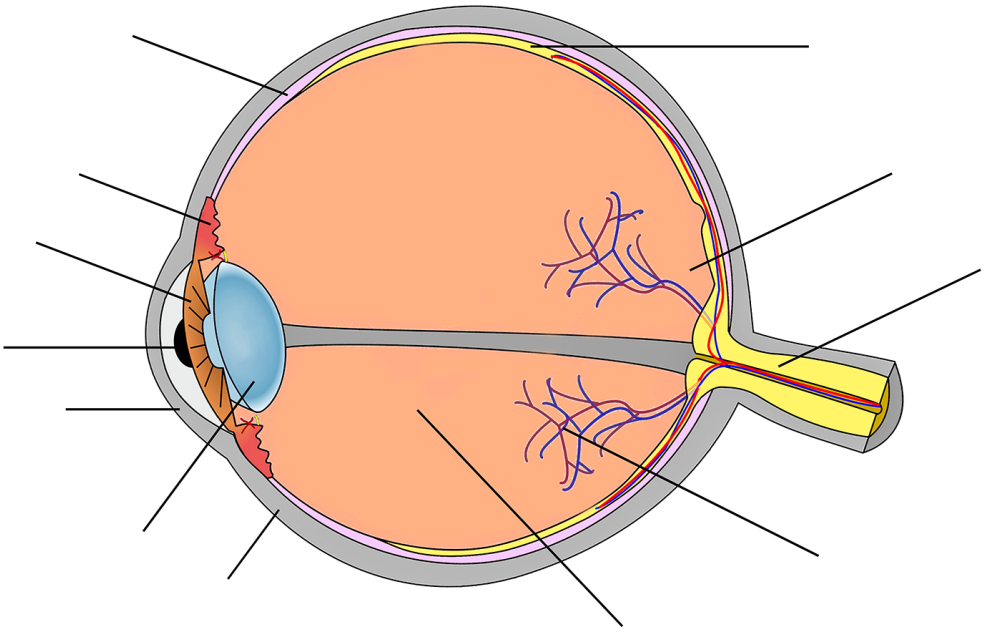
☐ Look up what "inattentive blindness" means. Do you think there is a benefit to this in your day-to-day life?

Study Material

The following material will not be collected or graded but is provided to further test your understanding of the material in the lab. These will be valuable assets for quiz and practical studying.

Review Activity 13.1

Test your knowledge of the structures of the eye by filling in the missing labels on the diagram of a mammalian eye below.



Review Figure 13.1. Image Source: adapted from "Human Eye Transverse Cut" by Andrew Meyerson and Soerfm, CC BY SA 3.0.

LABORATORY 14.

MUSCULAR AND SKELETAL SYSTEMS

Objectives

Following this week's lab, student's will be able to:

- Identify the structures and describe the function of the mammalian muscular system
- Identify the structures and describe the function of the mammalian skeletal system
- Describe similarities and differences between the skeletal systems of different vertebrates based on their locomotory behavior
- Describe similarities and differences between the hydrostatic, exo-, and endo-skeletons
- Use PowerLab and Chart software to measure muscle summation, tetany, and recruitment and describe what those terms mean in a physiological context

Contribution Points:

Consult with your TA to receive a stamp at the end of your lab period.

I have completed the necessary tasks required during this week's lab to earn Contribution Points. I am aware that I may have point(s) deducted from my Contribution Points if my workspace is not appropriately clean at the conclusion of lab.

Resources

- Fetal pig dissection guide, Smith and Schenk 2011 (provided in binders for use in lab)
- Biological Science, Freeman *et al.* 2024 (8th edition)
  - Muscular and Skeletal System (Chapter 45)
- Canvas resources

☐ This icon represents a question meant to test your understanding. Answering these questions in the space provided as you go through the lab will help you better understand the topic and study more effectively. Use your text or e-book, pig dissection guide, and the internet to help you.

REMINDER

Printed instructions for the PowerLab Activity can be found at each PowerLab station in the laboratory. They can also be located on Canvas. Students should follow the printed instructions to complete the activity. As the activity is completed in lab, students should answer the related questions in their lab manual.

PART 1: Muscle Physiology PowerLab Activity

WARNING

These exercises involve application of electrical stimulation to muscle through electrodes placed on the skin. The PowerLab limits the stimulus current to levels that are safe. **If you have a cardiac pacemaker or suffer from neurological or cardiac disorders do not volunteer for these exercises.** If the volunteer feels major discomfort during the exercises, discontinue the exercise, and consult your TA.

Exercise 2: Twitch Response and Recruitment

Complete Exercise 2 and fill in the data table below.

Table 14.1. Effect of Stimulus Amplitude on muscle contraction.

Stimulus	Response (mV.s)	Stimulus	Response (mV.s)
0.0 mA		11.0 mA	
1.0 mA		12.0 mA	
2.0 mA		13.0 mA	
3.0 mA		14.0 mA	
4.0 mA		15.0 mA	
5.0 mA		16.0 mA	
6.0 mA		17.0 mA	
7.0 mA		18.0 mA	
8.0 mA		19.0 mA	
9.0 mA		20.0 mA	
10.0 mA			

Motor Unit Recruitment Questions.

Muscles are composed of many **motor units**. Each motor unit consists of a group of muscle cells all controlled by a single motor neuron, and the muscle cells in each motor unit contract in an all-or- none fashion. As additional motor units are stimulated the force of contraction increases (a process known as **recruitment**).

a. What happened to muscle contraction force as you increased the amplitude (in milliamps) of the electrical current stimulus you delivered to the median nerve? Describe what you see in the data you collected.

b. Why is there a threshold? Or in other words, a minimum stimulus current below which no contraction occurred? Why was there no contraction below the threshold stimulus current?

c. Why does contraction force increase with stimulus amplitude beyond this threshold?

d. Why does contraction force reach a maximum, even though stimulus amplitude continues to increase?

Summation and Tetanus

In this exercise, you will keep stimulus amplitude constant at the value that produced a maximal contraction in the preceding experiment, and examine the effects of changing the frequency with which the muscle is stimulated. With increasing frequency of stimulation, the muscle may not relax completely before the next stimulus arrives. As a result, a new contraction begins in a muscle fiber that is already partly contracted.

☐ What effect do you think this might have on the force of contraction of that muscle? State this as a hypothesis:

Follow the printed instructions to complete the activity for Summation and Tetanus. Fill in the data tables below. Then answer the questions on the following page.

Exercise 3: Summation and Tetanus

Table 14.2. Summation data table

Stimulation Frequency (Hz)	Stimulus Interval(s)	Amplitude of First Response (mV.s)	Amplitude of Second Response (mV.s)
1			
2			
5			
10			
20			

Table 14.3. Tetanus data table

Stimulus Frequency (Hz)	Stimulus Interval(s)	Number of Pulses	Amplitude of Response (mV.s)
20		3	
20		4	

Temporal Summation and Tetany. Answer **each** of the questions (A through D) below.

a. When you stimulated your subject's muscles with bursts of 4 stimuli at different frequencies, you should have seen results similar to those pictured in figure included in Step 8 above. What happened to the force of contraction as stimulation frequency increased? Describe the collected data.

b. Was there a frequency at which you saw a marked increase in muscle force from the peak force seen in the first experiment exploring recruitment? If so, what was the threshold frequency for your test subject?

c. What chemical process within your muscle cells could account for this increase of maximum muscle force at tetany? What role does Calcium play?

d. How do you know that the results of this experiment do not just represent recruitment of additional motor units?

PART 2: Muscle and Skeletal System Stations

Station 1: Microscopic Anatomy of the Neuromuscular Junction and Muscle Tissue

☐ Look at the prepared slide of the neuromuscular junction under a compound microscope. Identify **muscle cells**, **axons of motor neurons**, and the **synapses** between motor neurons and muscle cells, called **neuromuscular junctions** and label them on ***Figure 14.1*** below.

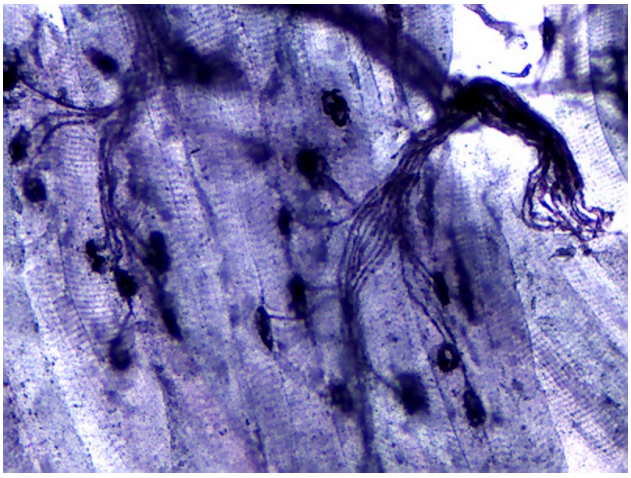


Figure 14.1. Microscopic view of the neuromuscular junction. Image source: "Hlj55567516" via Wikimedia Commons, CC BY SA 3.0.

☐ What is a neuromuscular junction?

☐ How many neuromuscular junctions are there in each muscle cell?

☐ With how many muscle cells does a single neuron synapse?

☐ Are muscle cells controlled individually, or in groups?

Look at the slides available of the three different muscle types: smooth, cardiac, and skeletal. Sketch what you see in ***Table 14.3*** and describe the characteristics of each type. Think about how the structure is related to the function of each muscle type.

Table 14.3. Student worktable to describe differences between muscle cell types.

Muscle Cell Type	Sketch	Function and Characteristics
Smooth		
Cardiac		
Skeletal		

Station 2: Exoskeletons, Endoskeletons, and Hydrostatic Skeletons

There are many different types of skeletons in the animal kingdom. First check out the exoskeleton mini-station that includes the crayfish and lubber grasshopper.

☐ What is an exoskeleton, and how can you tell that these animals have exoskeletons?

☐ What material makes up the exoskeleton of insects? How is it different from bone?

☐ How do animals with exoskeletons grow in size?

Compare the exoskeletons to the endoskeletons. Take some time to observe the specimens available and answer the following questions.

☐ What is an endoskeleton?

☐ How do animals with endoskeletons differ from those with exoskeletons in terms of development, growth, and ability to support their body weight?

Compare the exoskeletons and endoskeletons to the hydrostatic skeletons. Take some time to observe the specimens available and answer the following questions.

☐ What is a hydrostatic skeleton? How does a hydrostatic skeleton work? What kinds of organisms have a hydrostatic skeleton?

Station 3: Anatomy of Bones

Look at the sectioned femur supplied at station 3 in the lab. Using ***Figure 14.2*** below as a reference, identify the following structures in the sectioned bone: **diaphysis, epiphysis, articular cartilage, compact bone, spongy bone,** and **marrow.**

☐ What is a growth plate, and why is it important? In adults, it becomes an epiphyseal line. Why is this?

☐ Where would you find spongy bone and compact bone? How do they differ in structure and function?

☐ What are the functions of the red and yellow bone marrow?

☐ On display at this station are a variety of different kinds of bones from various animals. Make a list of the bones you can identify at this station below.

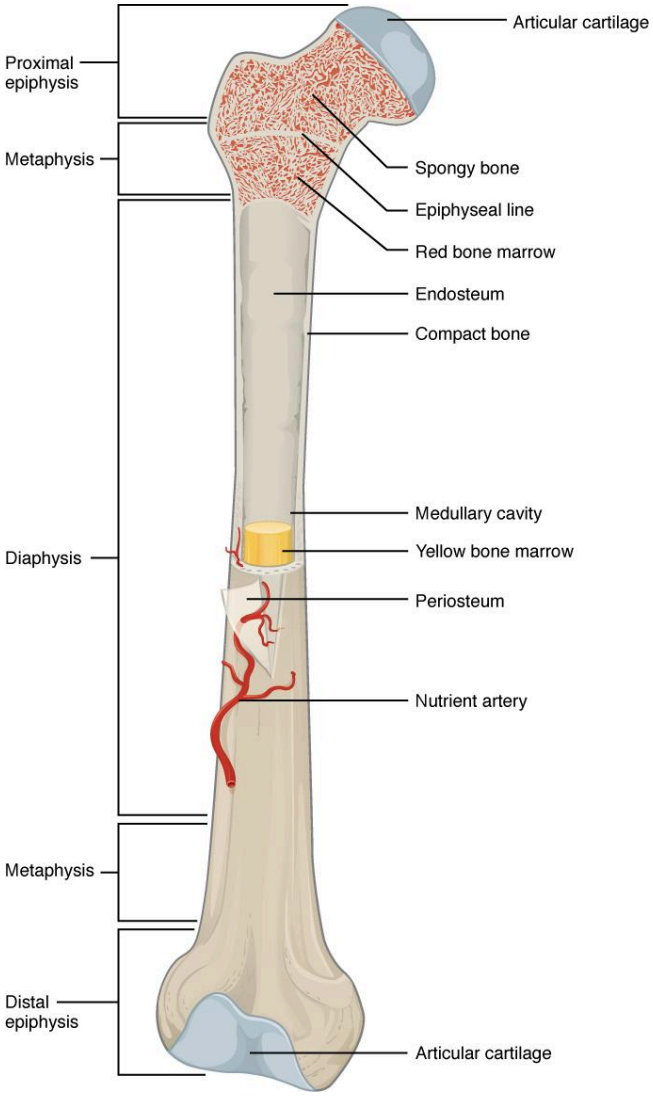


Figure 14.2. Illustration of a human femur.

Station 4: Bone Materials

Look at the ground bone prepared slide. Make sure to identify the **Haversian (Central) canals**, **lacunae,** and **extracellular matrix.** Label ***Figure 14.3*** with these structures.

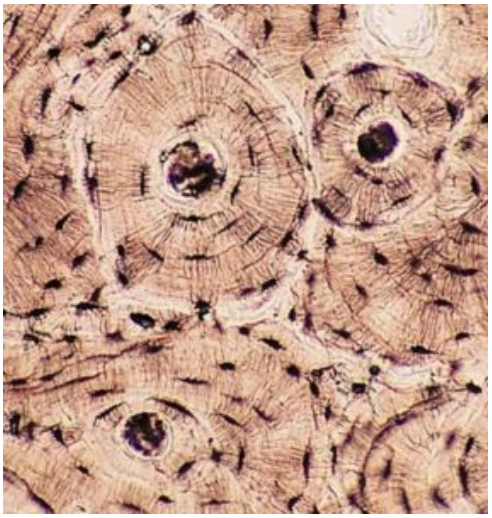


Figure 14.3. Microscopic anatomy of human bone. Image source: "Bone connective tissue" by Darshani Kansara, CC BY SA 4.0

☐ Are bones living or dead in an adult animal? Is your answer consistent with the observation that broken bones can heal?

☐ What is a Haversian canal, and what would you find in the canal space in a living animal?

Observe the decalcified bones. These vertebrate limb bones have been treated with dilute acid to dissolve the bone mineral **hydroxyapatite**. Use forceps to remove the decalcified bones to observe them. Put them back when you are done.

☐ What is the material that makes bone hard? What material makes bone somewhat flexible?

☐ How do bone and cartilage differ in both structure and function?

Station 5: Vertebrate Skeletal Structure and Function

In this station, you will compare the skeletal anatomy of different vertebrates. Be sure to think about how the skeleton of each animal is related to its locomotion and feeding. Some of the skeletons available in lab are: **frog, turtle, rat, bat, snake, human,** and **bird (other skeletons may also be available).**

As you observe each skeleton, locate the following bones: **vertebrae, skull, sternum, clavicle, humerus, radius, ulna, carpals, metacarpals, ileum, ischium, pubic bone, femur, tibia, fibula, tarsals,** and **metatarsals.**

☐ In the frog, how do the leg bones (tibia/fibula) differ from those in humans and what is the significance of that difference to the frog?

☐ How are the forelimbs (front legs/arms) of a frog and bat similar; what features do they share? How are they different and how do the differences relate to the mode of locomotion?

☐ What adaptations for flight can you see in the skeletons of birds and bats?

☐ There are strong similarities in the patterning and structure of the skeletons of humans, birds, bats, turtles, frogs, etc. For example, in the forelimb (arm) of these animals, they all have a single humerus, paired radius and ulna, many small carpal bones, and digits, despite differences in locomotion mode and function. What does this suggest about the evolutionary origins of the skeletons of mammals, reptiles, birds, and amphibians?

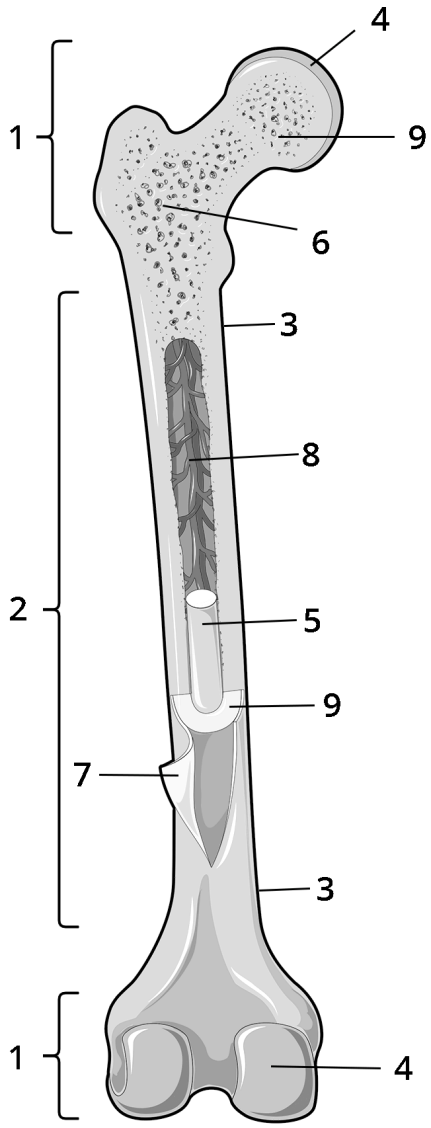
Study Material

The following material will not be collected or graded but is provided to further test your understanding of the material in the lab. These will be valuable assets for quiz and practical studying.

Review Activity 14.1

Help yourself visualize the mammalian femur. Using crayons, colored pencils, and/or markers, fill in the name of each structure with a specific color and using that same color fill in the corresponding structure. Use different colors for different parts.

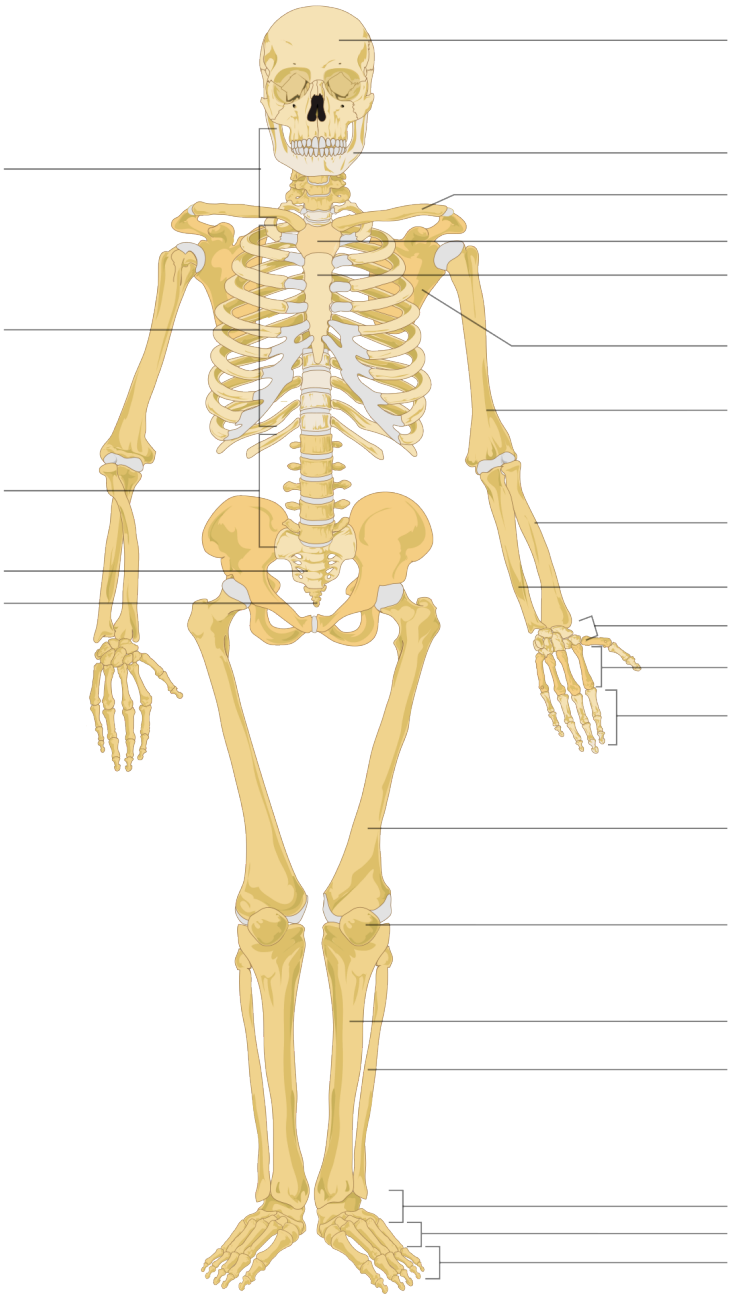
EPIPHYSIS **1**
DIAPHYSIS **2**
PERIOSTEUM **3**
ARTICULATE CARTILAGE **4**
YELLOW MARROW **5**
SPONGY BONE **6**
COMPACT BONE **7**
BLOOD VESSEL **8**
RED MARROW **9**



Review Figure 14.1. Image Source: Smart Servier via Wikimedia Commons, CC BY SA 3.0. Adapted by Abbey Elder.

Review Activity 14.2

Test your knowledge of the bones in the human body filling in the missing labels on the diagram of the skeleton below.



Review Figure 14.2. Skeletal Structure. Image Source: Smart Servier via Wikimedia Commons, CC BY SA 3.0.