

A microscopic image showing numerous circular cells with blue-stained nuclei. The cells are densely packed and vary slightly in size and shape, suggesting a biological sample like a tissue or a suspension of cells.

# Biology I

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LABORATORY MANUAL

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Laboratory Manual For SCI103  
Biology I at Roxbury Community College

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<sup>1</sup>[https://commons.wikimedia.org/wiki/File:Osmotic\\_pressure\\_on\\_blood\\_cells\\_diagram.svg](https://commons.wikimedia.org/wiki/File:Osmotic_pressure_on_blood_cells_diagram.svg)

<sup>2</sup>[https://commons.wikimedia.org/wiki/File:Turgor\\_pressure\\_on\\_plant\\_cells\\_diagram.svg](https://commons.wikimedia.org/wiki/File:Turgor_pressure_on_plant_cells_diagram.svg)

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<sup>3</sup>[https://commons.wikimedia.org/wiki/File:Linear\\_visible\\_spectrum.svg](https://commons.wikimedia.org/wiki/File:Linear_visible_spectrum.svg)

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# Acknowledgements

At RCC<sup>4</sup>, introductory (general) biology is split into two courses (SCI103, Biology I, and SCI104, Biology II) which are taught over two semesters. The two courses were originally developed in the 1970s, by Prof. Georgia Whitman essentially representing botany (Biology I) and zoology (Biology II). Some 20 years ago, Prof. Kyrsis Rodriguez who served as the biology course coordinator until her retirement reduced the botany content and introduced molecular aspects of biology.

In 2015, the Massachusetts Department of Higher Education with its MassTransfer Pathways<sup>5</sup> initiative prompted a statewide critical evaluation of the structure and content of foundational courses, such as introductory biology, in order to make these courses eligible for transfer across the Commonwealth amongst public higher education institutions. In 2016, we have been fortunate to receive a large grant from the Massachusetts Life Sciences Center<sup>6</sup> to upgrade and modernize our science labs. We have taken this opportunity to re-structure our courses along the lines of current practice of introductory biology teaching such that the Biology I course introduces the molecular and cellular basis of life, while the Biology II course is focused on the basics of organismal biology. Overall the proposed changes will expose our students to modernized (up-to-date) concepts of biology and thus make them more competitive.

This laboratory manual was inspired by “Encounters with Life<sup>7</sup>” by Larry J. Scott and Hans F. E. Wachtmeister published by the Morton Publishing Company in Englewood CO. We used that manual at RCC for many years. Progress in the biological sciences is, however, relentless and so over the years, our requirements changed, and we felt that it was best to put together a new manual specifically tailored to our needs.

The creation of this manual was greatly facilitated and owes a major debt to Wikipedia and its large number of voluntary contributors. I very liberally copied from many Wikipedia pages and then remixed, edited, adapted and added text. With your continued support and help this manual can only get better over time. I urge you to email me with your criticisms and suggestions at nsucher@rcc.mass.edu<sup>8</sup>. This manual is as open educational resource licensed under Creative Commons Attribution-Share Alike 3.0 Unported<sup>9</sup> United States License for others to do as I did and improve and adapt to specific requirements.

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<sup>4</sup><http://www.rcc.mass.edu>

<sup>5</sup><http://www.mass.edu/masstransfer/>

<sup>6</sup><http://www.masslifesciences.com>

<sup>7</sup><https://www.amazon.com/Encounters-Life-General-Biology-Laboratory/dp/0895826852>

<sup>8</sup><mailto:nsucher@rcc.mass.edu>

<sup>9</sup><https://creativecommons.org/licenses/by-sa/3.0/deed.en>



# Course Description

This course provides an introduction to the molecular and cellular basis of life, the theory of evolution and the diversity of microscopic organisms. Four hours of lecture and a two-hour lab session are required each week.

## Learning Objectives

- Identify the basic characteristics of life and outline the theories that attempt to explain the origin of life, as we know it and define it, on the planet Earth.
- Identify some basic chemical concepts and apply them to the structure and biological processes that occur in living cells.
- Identify cell parts, and demonstrate understanding of their related functions. Identify similarities and differences in the structure of viruses, prokaryotic cells, and eukaryotic cells
- Explain the significance of enzymes, coenzymes, and ATP, and to identify the main events, products and significance of the processes of cell respiration, fermentation, and photosynthesis
- Understand, explain, and contrast the processes of mitosis, meiosis, and binary fission in terms of their physical differences and their genetic and evolutionary significance. Explain the process of viral replication
- Identify the structural parts of DNA and RNA, and understand how DNA directs the activities of a cell through protein synthesis, including some examples of how this process is regulated
- Demonstrate understanding of how genes are passed from one generation to the next by completing representative genetic problems and explaining/applying genetic concepts
- Demonstrate understanding of the three Domains of Life (Archaea, Bacteria and Eukarya) and viruses. Identify some of the distinguishing characteristics of each domain and the viruses
- Learn and apply the laboratory skills associated with the objectives listed above
- Explain the basic concepts of biology in written and oral form
- Apply the concepts learned to better understand the biological world, and the problems that affect human society



# How to do well in this class

## Before the lab

- Know what's coming up. Each week, look at the schedule to know what lab is coming up.
- Be prepared. Read the chapter in the manual **before** you come to the lab.
- In your own words, explain to yourself or someone else what the upcoming lab is about.

## During the lab

- Read the lab instructions carefully.
- Follow the instructions carefully.
- Make sure that you know what you are doing and why you are doing it.
- Don't be afraid to ask your instructor for help or clarifications.

## After the lab

- Ask yourself: what was this lab about? What did we do? How did we do it?
- In your own words, tell yourself or someone else what you learned in the lab.



# Chapter 1

## Lab Safety

The laboratory classes are hands-on. Some classes require the use of hazardous chemicals and materials. Safety in the classroom is the #1 priority for students and faculty. To ensure a safe science laboratory, a list of rules must be followed at all times.

Please watch the RCC safety video<sup>1</sup>



Prior to your participation in the science lab course, you must read this safety document and sign and return the acknowledgment and agreement page.

### 1.1 General rules

1. NO FOOD, BEVERAGES, GUM, in the labs. Cell phone usage is also prohibited in the lab.
2. Conduct yourself in a responsible manner at all times in the lab. Horseplay, pranks and practical jokes are prohibited and will not be tolerated. If you participate in inappropriate behavior the INSTRUCTOR HAS THE RIGHT TO ASK YOU TO LEAVE THE lab.
3. Students cannot be in the lab without an instructor present.
4. Read all lab procedures, precautions, and equipment instructions thoroughly before each lab. Follow all written and verbal instructions carefully. Perform only those experiments authorized by the instructor. If during the lab you don't understand, stop and ask the instructor before proceeding. Never do anything in the lab that is outside of your instructors directions or that is not in your lab procedure.
5. Do not begin lab activities; touch any chemicals or equipment until you are instructed to do so.
6. Work areas should be kept organized and clean at all times. Only necessary items (lab notebook, worksheets, etc.) should be on your workbench. Backpacks and purses must be stored under the benches or against the walls. CLEAN ALL OF YOUR WORK SURFACES AND EQUIPMENT AT THE END OF THE EXPERIMENT. Safely dispose of waste in its proper container and place glassware in the grey bins by the sink. DO NOT STACK GLASSWARE. If the bin is full ask the instructor for another bin.
7. Keep aisles clear. Push lab stools under the lab benches when not in use.
8. Know where the safety equipment is and how to use it. This includes the first aid kit, eyewash station, safety shower, fire extinguisher, and fire blanket. Know the location of the fire alarm, and emergency phone. In the event of a fire drill during lab time containers must be closed, gas valves off, fume hood, and all electrical equipment must be turned off.

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<sup>1</sup><https://youtu.be/NxcsyTv7stQ>

9. NEVER DISPOSE OF ANYTHING IN THE SINK. All materials are to be disposed of in the proper hazardous waste containers with the assistance of the instructor. All waste containers must be closed and placed inside a secondary containment bin.
10. As classes in these labs use toxic chemicals, keep your hands away from your face, eyes and mouth while working in the lab. Always wash your hands thoroughly with warm water and soap before leaving the lab to prevent injury or illness. This is part of proper lab procedure.
11. Students are not permitted in the prep room areas (between the lab rooms).
12. Handle all living organisms used for lab experiments in a respectful, humane manner.
13. Microscopes must be properly cleaned, the electrical cords properly wrapped, and returned to their places with their protective covers.

Disposal of all hazardous waste is ONLY to be handled by the instructor and in a manner consistent with federal, state and local hazardous waste disposal regulations. Organic solvents are never to be disposed of down the sink; receptacles will be provided as needed for their collection. All hazardous chemical substances must be placed in the appropriate type of container and labeled with chemical, name and date, sealed and placed upright in a gray plastic bin.

## 1.2 Class dissections

14. Preserved biological specimens should be treated with respect and disposed of in a clear plastic bag and placed inside the hazardous waste drum located in the classroom. This container must be sealed at the end of each dissection.
15. When using sharp objects always carry the tips pointed down and away from you. When dissecting, cut away from your body. Grasp the instrument only by the handle. Never try to catch falling sharp instruments or glassware. When you are finished dissecting, wash and dry your instruments and dissecting pan before storing them in the proper location. Do not leave any instruments in the sink.

## 1.3 Clothing

16. Students must wear lab goggles when using chemicals, or using heat. NO EXCEPTIONS! Lab coats are mandatory for Anatomy and Physiology, Biology, Microbiology, Chemistry, and Biotechnology labs, except for when lecturing and working on dry lab (for example, looking at models or prepared slides) activities.
17. Gloves should be worn when handling solutions, solids, specimens, etc.
18. Proper dress should always be observed in the lab. Long hair must be tied back. Loose or baggy clothing (especially sleeves), dangling jewelry, hats, shorts, short skirts, bare mid riffs, high heels, sleeveless shirts, and open toed or open heeled shoes and sandals are prohibited in the lab. Failure to comply may result in expulsion from class.

## 1.4 Handling chemicals

18. Always work in a well-ventilated area. Use the fume hood when working with volatile substances or poisonous vapors, or any chemical with an odor.
19. Never smell a chemical by sniffing. Use your hand to wave the chemical towards your nose.
20. DO NOT TASTE, TOUCH or smell anything unless instructed to do so. You should wear a lab apron and gloves at all times. Your instructor will tell you the proper gloves to wear depending on the chemical being used.
21. CHECK EACH LABEL TWICE before removing any of its contents. Take only what is needed of each chemical. NEVER return unused chemicals to their original container; put it in the waste container.
22. Do not use your fingers to transfer solid chemicals. Use a scoop or spatula.

23. Use a rubber bulb, pipette, or pi-pump when transferring liquid chemicals. NEVER USE YOUR MOUTH TO PIPETTE!
24. When transferring reagents hold the containers away from your body while working on the bench.
25. Acids must be handled with extreme care. You will be shown the proper method for diluting strong acids. ALWAYS ADD ACID TO WATER, swirl or invert the solution and be careful of the heat produced particularly with sulfuric acid.
26. Handle hazardous liquids over a pan to contain spills.
27. Never handle flammable liquids anywhere near an open flame or heat source.
28. Be careful when transporting chemicals across the lab. Hold securely and walk carefully.
29. NEVER POUR CHEMICALS INTO SINK. Waste should be disposed of in the proper hazardous waste container provided.

## 1.5 Glassware

30. Never handle broken glass with bare hands. Use a brush and a dustpan to clean up broken glass. Place uncontaminated broken glass in the white and blue broken glass receptacles. Contaminated trash goes in the biohazard bin.
31. Fill wash bottles only with distilled water and use only as intended, e.g.; rinsing glassware, adding water to a container.
32. Never use chipped, cracked or dirty glassware to avoid shattering.
33. Never immerse hot glassware in cold water. It may shatter.
34. Never place dirty glassware with the clean glassware. All dirty glassware should be placed in gray wash bins. DO NOT STACK DIRTY GLASSWARE IN BINS.

## 1.6 Heating substances

35. Exercise extreme caution when using a gas burner. Be careful to keep hair, loose clothing and hands away from flames at all times. Wear safety goggles. Do not put any substances into the flame unless specifically instructed to do so. Never reach over an exposed flame. The instructor will provide a demonstration of the proper way to operate a Bunsen burner. Never leave a lighted burner or hot plate unattended. Always turn the burner or hot plate off when not in use.
36. Do not point the open end of a test tube being heated at yourself or anyone else. Never look into a container that's being heated.
37. Heated metals and glass remain hot for a very long time. They should be set aside to cool and picked up with caution. Use tongs or heat protective gloves.

## 1.7 Handling microbiology materials

38. Please be aware that micro labs include work with pathogenic organisms. Be alert. Conduct yourself in a responsible manner at all times.
39. If you spill anything notify your instructor immediately. There are special procedures to be followed for spills containing microorganisms.
40. A lab coat must be worn during lab activities. Lab coats/aprons may never leave the lab. If you must leave the lab during a class then your lab coat must be removed.
41. Gloves must be worn at all times when working with bacteria. Gloves need to be disposed of in the biohazard waste container.
42. All contaminated waste must be disposed of in the biohazard container. Do not overfill biohazard containers.

43. You must spray down your lab bench with Lysol after each lab. Do not wipe with paper towels. The bench surface must remain wet for at least five minutes for the Lysol to destroy any micro organisms.
44. Wash your hands thoroughly before and after each lab as well as before you leave the lab for any reason.
45. Dispose of contaminated broken glass in the biohazard bin. Please wrap the broken glass in paper towels before disposal so that the broken glass doesn't cut through the bag. Dispose of uncontaminated glass in the white and blue cardboard glass boxes.

## Chapter 2

# The Microscope

A microscope<sup>1</sup> (from the Ancient Greek: mikrós, “small” and skopeîn, “to look” or “see”) is an instrument used to see objects that are too small to be seen by the naked eye. Microscopic means invisible to the eye unless aided by a microscope.

There are many types of microscopes, and they may be grouped in different ways. One way is to describe the way the instruments interact with a sample to create images, either by sending a beam of light or electrons to a sample in its optical path, or by scanning across, and a short distance from, the surface of a sample using a probe. The most common microscope (and the first to be invented) is the optical microscope<sup>2</sup>, which uses light to pass through a sample to produce an image.

The objective lens of a microscope (Figure 2.1) is a cylinder containing one or more lenses that are typically made of glass. It is essentially a high-powered magnifying glass which is brought very close to the specimen being examined. The objective collects light from the sample so that it comes to a focus inside the microscope tube. This creates an enlarged image of the specimen.

The eyepieces, or ocular lenses (Figure 2.2), are the lenses that are closest to your eyes when you look through the microscope. The objective lens or mirror collects light and brings it to focus creating an image. The eyepiece is placed near the focal point of the objective to magnify this image. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By carefully focusing a brightly lit specimen, a highly enlarged image can be seen. It is this real image that is viewed by the eyepiece lens that provides further enlargement. The amount of magnification depends on the focal length of the eyepiece. The ocular in our microscopes have a  $0\times$  magnification.

Our microscopes have four objective lenses with different magnifications, screwed into the circular “nosepiece” which you rotate to select the required lens. These lenses are color coded for easier use. The least powerful lens is called the scanning objective lens and is a  $4\times$  objective. The second lens is referred to as the small objective lens and is  $10\times$  lens. The most powerful lens out of the four are referred to as the large objective lenses and are  $40\times$  and  $100\times$ . The  $100\times$  objective is an oil-immersion lens. This objective is specially designed for use with refractive index matching oil, which must fill the gap between the objective lens and the specimen.

The stage is a platform below the objective which supports the specimen being viewed. Adjustment knobs (on the left side of the microscope) move the stage up and down with separate adjustment for coarse and fine focusing (Figure 2.3). In the center of the stage is a hole through which light passes to illuminate the specimen (Figure 2.4). The stage has arms to hold slides (rectangular glass plates on which the specimen is mounted).

The stage moves up and down for focus. Always start with the lowest magnification in order to center

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<sup>1</sup><https://en.wikipedia.org/wiki/Microscope>

<sup>2</sup>[https://en.wikipedia.org/wiki/Optical\\_microscope](https://en.wikipedia.org/wiki/Optical_microscope)



**Figure 2.1:** The microscope objectives.



**Figure 2.2:** The oculars (eye pieces) of the microscope.



**Figure 2.3:** The coarse (big wheel) and fine (small wheel) focus adjustment knobs.



**Figure 2.4:** The stage with the slide holder and central opening showing the condenser lens.

the specimen on the stage. After moving to a higher magnification re-focus using the fine focus knob. You may also have to adjust the horizontal positions using the horizontal stage and slide holder adjustment knobs hanging down on the right side of the stage (Figure 2.5). Our microscopes, an adjustable LEDs light source (knob on the right side). The condenser is a lens designed to focus light from the illumination source onto the sample. The light source and condenser also each include a diaphragm to influence the quality and intensity of the illumination. For our purposes, the diaphragms should always be completely open. Adjust the light intensity only using the knob on the right side of the frame of the microscope (the black knob below the green switch in Figure 2.4).

We have prepared a number of videos that introduce our microscopes to you and also demonstrate how you can capture images of the slides that you are viewing and transfer them to your own equipment.

## 2.1 How to turn on the microscope

The tablets and microscopes must be turned on in a specific order:

1. Microscope must be plugged in. If already plugged in, proceed to step 3.
2. Motic logo will appear on tablet. After a few seconds, the charging symbol appears.
3. Press and hold the power button on the tablet for 6 seconds.
4. Motic symbol will appear again and tablet will start up.
5. Turn on microscope using the switch on the lower right side.

## 2.2 View Prepared Slides

1. Get a white slide box.
2. Clean all of the exposed lenses with special lens paper. Do not use paper towels, Kimwipes®, or cloth as this will scratch the lenses. If the view through the microscope becomes blurred, additional cleaning with lens paper may be necessary. Use alcohol pads if necessary.



**Figure 2.5:** The condenser (below the stage), the horizontal stage and slide holder adjustment knobs (hanging down from the stage), the light on switch and light intensity adjustment knob (in the back).

3. Make sure that the low power objective is clicked into position.
4. Move the oculars as far apart from each other as possible then look through them with both eyes open. You will see two non-overlapping regions of light. Push the oculars slowly towards each other until you see one circle of light.
5. Always use both eyes when you look at slides. This will avoid eye strain and headaches.
6. Get the slide labelled “Letter e” (Figure 2.6) from the slide box.
7. Place the slide (coverslip up) on the stage and center the specimen over the opening in the stage.
8. Always start with the low power ( $4\times$ ) objective in place.
9. While looking through the ocular, use the coarse adjustment knob to slowly move the stage upward until the specimen comes into focus. If it does not, check to see that the material is centered on the stage, lower the stage, and try again.
10. Using the fine adjustment knob, obtain a sharp focus.
11. To increase the magnification, be sure the area you wish to examine specifically is in the center of the field; then, watching from the side to be sure that the objective clears the slide, turn the nose-piece until the next higher power objective clicks into position. The material now should be in view and should require only slight focusing with the fine adjustment. Never focus with the coarse adjustment under high power.
12. What happens when you increase the magnification?
13. Before removing the slide, always return the microscope to low power and turn the coarse adjustment knob until the stage is moved all the way down.
14. View the computer chip slide (Figure 2.7).
15. View the colored threads slide (Figure 2.8).
16. Which thread is at the bottom, in the middle, on top?
17. Depth perception requires that a slightly different angle of an object is seen by the left and right eye. This happens because of the horizontal separation parallax of the eyes. If an object is far away, the disparity of that image falling on both retinas will be small. If the object is close or near, the disparity will be large. The microscope presents the same view to both eyes. Therefore, the only way to answer the above question is to change the focus of the image and observe what happens: when you move the stage upwards, to bring the slide closer to the objective, the thread that is on top will come into focus



**Figure 2.6:** A printed letter.

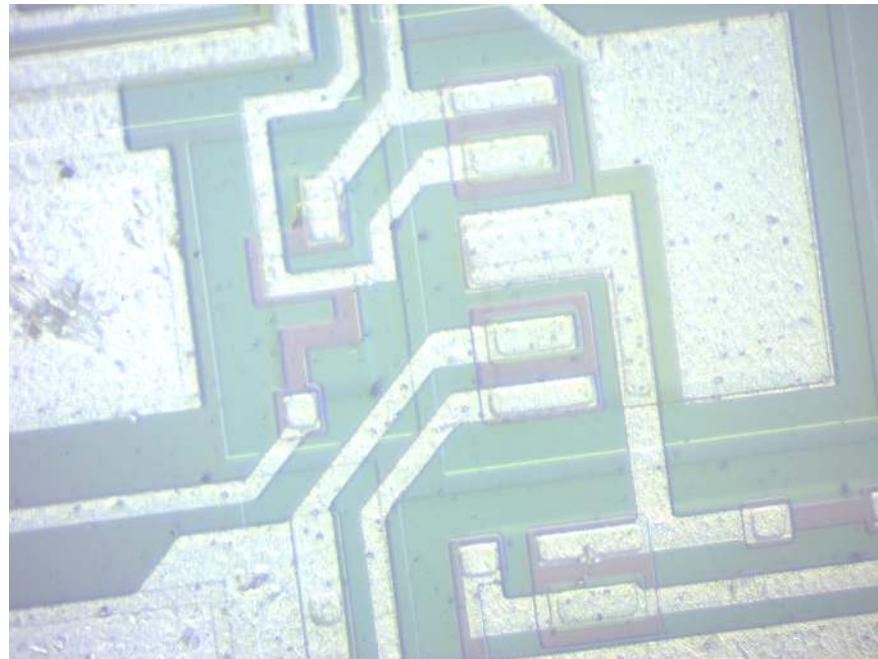
first, the middle one second and the bottom one last. Try it and write down your answer!

- Bottom thread: \_\_\_\_\_
  - Middle thread: \_\_\_\_\_
  - Top thread: \_\_\_\_\_
18. View the stage micrometer slide (Figure 2.9).
  19. What is the unit of this scale?
  20. What is the distance between 1.0 and 1.5 in meters?
  21. How many subdivisions can you distinguish between 0 and 0.1?
  22. What is the distance in meters between the smallest subdivision?
  23. View the blood smear slide (Figure 2.10).
  24. Which cells are red blood cells? Mark with an arrow and label it “RBC”.
  25. Which cells are white blood cells? Mark with an arrow and label it “WBC”.
  26. Return the slides to the slide boxes and the slide boxes to the bench where you picked it up.

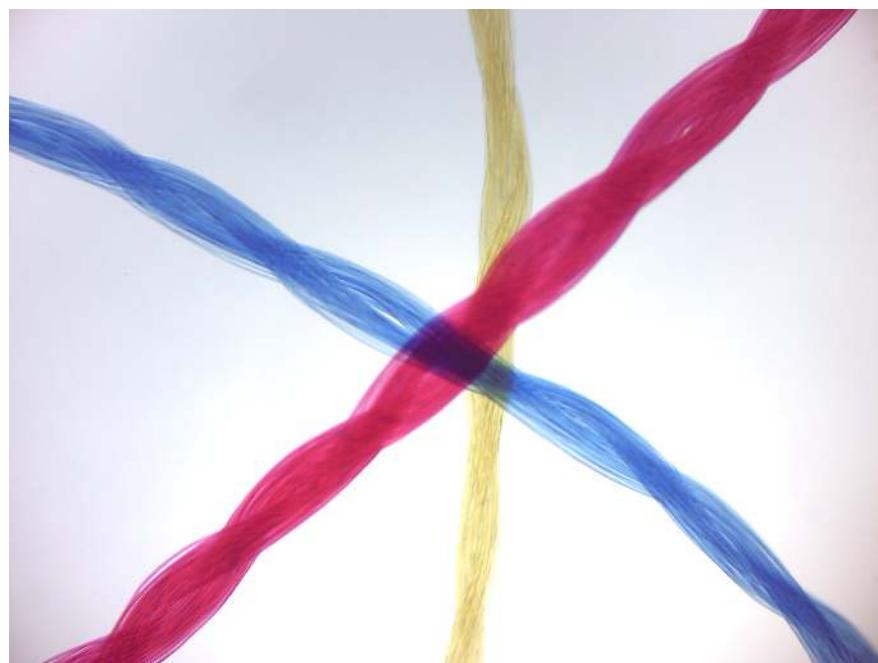
### 2.3 Elodea Leaf Wet Mount

*Elodea canadensis*<sup>3</sup> (American or Canadian waterweed or pondweed) is a perennial aquatic plant, or submergent macrophyte, native to most of North America. It grows rapidly in favorable conditions and can choke shallow ponds, canals, and the margins of some slow-flowing rivers. It requires summer water temperatures of 10-25 °C and moderate to bright lighting. Young plants initially start with a seedling stem with roots growing in mud at the bottom of the water; further adventitious roots are produced at intervals along the stem, which may hang free in the water or anchor into the bottom. It grows indefinitely at the stem tips, and single specimens may reach lengths of 3 m or more. The leaves are bright green, translucent, oblong, 6-17 mm long and 1-4 mm broad, borne in whorls of three (rarely two or four) round the stem. It lives entirely underwater, the only exception being the small white or pale purple flowers which float at the surface and are attached to the plant by delicate stalks. It is dioecious, with male and female flowers on different plants. The flowers have three small white petals; male flowers have 4.5-5 mm petals and nine stamens,

<sup>3</sup>[https://en.wikipedia.org/wiki/Elodea\\_canadensis](https://en.wikipedia.org/wiki/Elodea_canadensis)



**Figure 2.7:** Close-up view of an electronic chip.



**Figure 2.8:** Which thread is on top, in the middle, at the bottom?



**Figure 2.9:** A microscopic scale.



**Figure 2.10:** A human blood smear.



**Figure 2.11:** Elodea leaf wet mount (4 $\times$  objective).

female flowers have 2-3 mm petals and three fused carpels. The fruit is an ovoid capsule, about 6 mm long containing several seeds that ripen underwater. The seeds are 4-5 mm long, fusiform, glabrous (round), and narrowly cylindrical. It flowers from May to October.

### 2.3.1 Experimental procedures

1. Get a single leaf from the Elodea plant and mount it on a slide, cover it with a drop of water and a cover slip.
2. Place the slide onto the microscope stage and observe at the leaf under the microscope.
3. These leaves are two cells thick, so you should be able to focus up and down to see that the cells in one layer are larger than those in the other. When one layer is in focus, you may be able to see the shadowy outlines of cell walls in the other layer.
4. Notice that the cells are clearly delineated by the cell wall.
5. Inside the cells are large oval-shaped green bodies, the chloroplasts.
6. As the cells warm, you can see the chloroplasts carried by the moving cytoplasm around the nearly transparent nucleus in the center of the cell.
7. Make a drawing of what you see at 400 $\times$  magnification.

## 2.4 How to turn off the microscope

The tablets and microscopes must be turned off in a specific order:

1. Turn off microscope using the switch on the lower right side.
2. While the microscope is still plugged in, turn off the tablet by holding the power button down for a few seconds. Select power off.
3. Motic logo will appear on tablet.
4. Wait till charging symbol will appear.

5. Leave microscopes kept on the student bench plugged in after turning them off and place the cover over the microscope.

## 2.5 Review Questions

1. Why do biologists use microscopes?
2. What is the function of the microscope objectives?
3. What are the magnification factors of the objectives of our microscopes?
4. What is the name of the lenses that are close to your eyes when you look through the microscope?
5. What is the magnifying power of these lenses?
6. What is the total magnification of the image that you observe if you use the  $40\times$  objective?
7. What is the difference between the action of the coarse and the fine focus knobs?
8. Which part of the microscope moves when you turn the focus knobs?
9. What is the field of view?
10. How does it change when you switch from a lower to a higher power objective?

## Chapter 3

# Chemical Aspects of Life

Everything that we can bump into, touch or squeeze including living things are composed of atoms.<sup>1</sup> Chemical elements<sup>2</sup> are pure substances of one type of atom<sup>3</sup>. Atoms combine to form molecules<sup>4</sup>. Molecules combined of more than one element are called compounds.

Commonly people distinguish between organic and inorganic compounds. However, there is no clear or universally agreed-upon distinction between organic and inorganic compounds. Organic chemists traditionally and generally refer to any molecule containing carbon as an organic compound and by default this means that inorganic chemistry deals with molecules lacking carbon. As many minerals are of biological origin, biologists may distinguish organic from inorganic compounds in a different way that does not hinge on the presence of a carbon atom. Pools of organic matter, for example, that have been metabolically incorporated into living tissues persist in decomposing tissues, but as molecules become oxidized into the open environment, such as atmospheric CO<sub>2</sub>, this creates a separate pool of inorganic compounds. The International Union of Pure and Applied Chemistry (IUPAC), an agency widely recognized for defining chemical terms, does not offer definitions of inorganic or organic compounds. Hence, the definition for an inorganic versus an organic compound in a multidisciplinary context spans the division between organic life living (or animate) and inorganic non-living (or inanimate) matter. In broader speech, the term commonly referred to compounds synthesized by purely geological systems, in contrast to those with a biological component in their origin.

Cells consist mostly of water (70%-90%). The bulk of their dry weight consists of compounds containing the elements carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and phosphorus (P). The four major types of organic biomolecules are carbohydrates, lipids, proteins and nucleic acids. The more complex members of these categories (biomacromolecules) are made up of chains of smaller molecules (monomers) strung together more or less like beads in a necklace. These complex molecules are called polymers. In living organisms, polymers are made by dehydration synthesis, the loss of a water molecule between each pair of monomers. Conversely, polymers can be digested (broken up into monomers) by the addition of a molecule of water between each pair of monomers. This process is known as hydrolysis.

Carbohydrates are biomolecules consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen to oxygen atom ratio of 2:1 (as in water) with the formula C<sub>n</sub>(H<sub>2</sub>O)<sub>n</sub>.

Lipids are substances of biological origin that are insoluble in water. Lipids comprise a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, and phospholipids. The main biological functions of lipids include storing energy, signaling, and acting as structural components of cell membranes.

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<sup>1</sup><https://en.wikipedia.org/wiki/Matter>

<sup>2</sup>[https://en.wikipedia.org/wiki/Chemical\\_element](https://en.wikipedia.org/wiki/Chemical_element)

<sup>3</sup><https://en.wikipedia.org/wiki/Atom>

<sup>4</sup><https://en.wikipedia.org/wiki/Molecule>

Proteins are large biomolecules consisting of one or more long chains of amino acids linked by peptide bonds. Proteins perform a vast array of functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in protein folding into a specific three-dimensional structure that determines its activity.

## 3.1 Test for Reducing sugars

A reducing sugar<sup>5</sup> is one that reduces another compound and is itself oxidized; that is, the carbonyl carbon of the sugar is oxidized to a carboxyl group. A reducing sugar has a free aldehyde group or a free ketone group. All monosaccharides are reducing sugars, along with some disaccharides, oligosaccharides, and polysaccharides. Monosaccharides which contain an aldehyde group are known as aldoses, and those with a ketone group are known as ketoses. The aldehyde can be oxidized via a redox reaction in which another compound is reduced. Thus, a reducing sugar is one that reduces certain chemicals. Sugars with ketone groups in their open chain form are capable of isomerizing via a series of tautomeric shifts to produce an aldehyde group in solution. Therefore, ketone-bearing sugars like fructose are considered reducing sugars but it is the isomer containing an aldehyde group which is reducing since ketones cannot be oxidized without decomposition of the sugar. This type of isomerization is catalyzed by the base present in solutions which test for the presence of aldehydes. Aldoses or aldehyde-bearing sugars are reducing also because during oxidation of aldoses, there are certain oxidizing agents that are reduced. The common dietary monosaccharides galactose, glucose and fructose are all reducing sugars. Many disaccharides, like lactose and maltose, also have a reducing form, as one of the two units may have an open-chain form with an aldehyde group. However, sucrose is a non-reducing disaccharide since neither of the rings is capable of opening. Benedict's reagent ( $\text{Cu}^{2+}$  in aqueous sodium citrate) is used as a qualitative test to detect the presence of reducing sugars. The reducing sugar reduces the copper(II) ions to copper(I), which then forms a brick red copper(I) oxide precipitate.

### 3.1.1 Experimental procedures

1. Turn on the 65 °C heat block (Figure 3.1) by pushing the little black switch on the lower right side of the to “high”. Rotate the white “high temperature adjust” button to number 2 to 3.
2. Turn on the 37 °C heat block by pushing the little black switch on the lower right side of the to “low”. Rotate the white “low temperature adjust” button to number 3 to 4.
3. Obtain a bottle containing deionized water (Figure 3.2).
4. Obtain 7 plastic test tubes (Figure 4.1) and a test tube rack (Figure 3.4).
5. Using a wax pencil, label each tube with a number (1 to 7).
6. Place the tubes from left (tube #1) to right (tube #7) in the first row of a test tube rack.
7. Add the test material each tube as indicated in Table 3.1.
8. Add 2 ml of Benedict's reagent to each tube using a plastic transfer pipette.
9. Mix well.
10. Check the that the temperature of the heat block is ~65 °C and then place the tubes in the heat block. Set a timer and incubate the tubes for 15 minutes. Begin setting up Experiment 2 (below) while the tubes are incubating.
11. After 15 minutes, remove the tubes using a test tube holder (be careful, they are hot!), place them in the tube rack and record the color (in your own words) in Table 3.1.

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<sup>5</sup>[https://en.wikipedia.org/wiki/Reducing\\_sugar](https://en.wikipedia.org/wiki/Reducing_sugar)



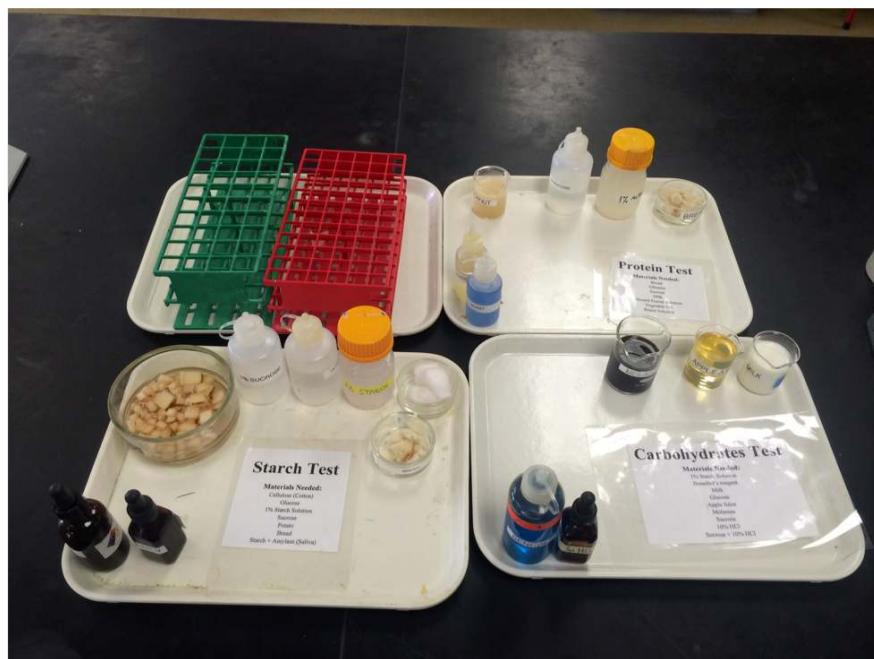
**Figure 3.1:** Heat block ("dry bath").



**Figure 3.2:** Bottle containing deionized water for use in the experiments.



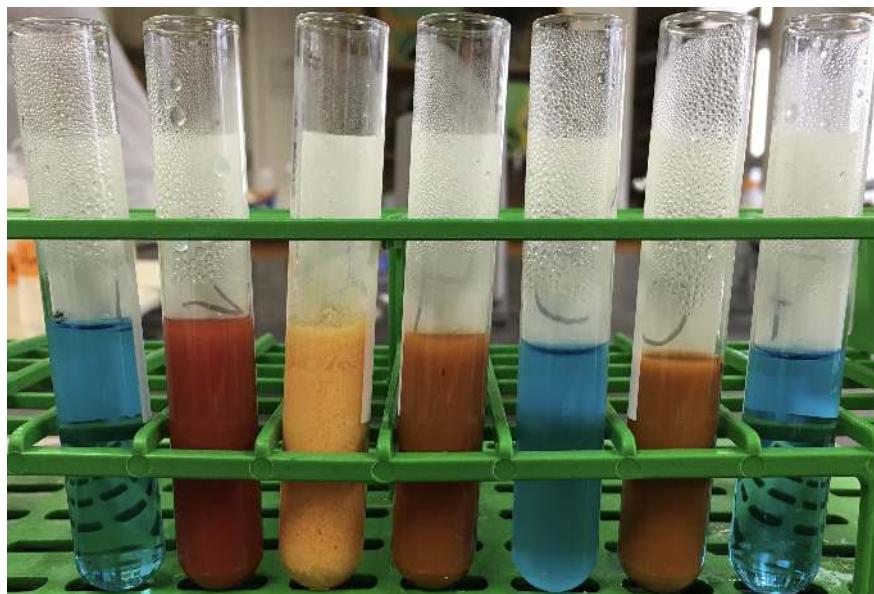
**Figure 3.3:** Additional experimental materials for this lab.



**Figure 3.4:** Experimental materials for this lab provided on your lab bench.



**Figure 3.5:** Plastic transfer pipette (2 ml capacity).



**Figure 3.6:** Results from experiment 1. Compare to your results!

**Table 3.1:** Test for reducing sugars.

Tube #	Test material	Benedict's	Observed color	Test Result (+ or -)
1	2 ml H <sub>2</sub> O	2 ml		
2	2 ml glucose	2 ml		
3	2 ml milk	2 ml		
4	2 ml apple juice	2 ml		
5	2 ml starch	2 ml		
6	2 ml molasses	2 ml		
7	2 ml sucrose	2 ml		

## 3.2 Test for Starch

Starch is a polymeric carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants as energy storage. It is the most common carbohydrate in human diets and is contained in large amounts in staple foods like potatoes, wheat, maize (corn), rice, and cassava. Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of two types of molecules the linear and helical amylose and the branched amylopectin.

An amylase<sup>6</sup> is an enzyme that catalyzes the hydrolysis of starch into maltose and glucose. Amylase is present in the saliva of humans, where it begins the chemical process of digestion. It is also produced by the

3. Place the tubes from left (tube #1) to right (tube #9) in the first row of a test tube rack.
4. Add the test material to tubes 1 to 8 as indicated in the table below. Leave tube 9 empty.
5. Check the that the temperature of the heat block is ~37 °C and then place tube #8 in the heat block. Set a timer and incubate the tubes for 15 minutes.
6. Add 5 drops of Iodine solution to the test tubes #1 to #7. Mix well.
7. Record the color (in your own words) in in Table 3.2.
8. After 15 minutes, remove test tube 8 from the heat block and transfer 2 ml (half of the contents of test tube 8) to tube 9.
9. Add 5 drops of iodine to tube 8 and record your observation in Table 3.2.
10. Add 2 ml of Benedict's solution to tube #9.
11. Place tube 9 in the ~65 °C heat block. Set a timer and incubate the tube for 15 minutes.
12. After 15 minutes, remove tube 9 using a test tube holder (be careful, it is hot!), place it in the tube rack and record the color (in your own words) in the table.

**Table 3.2:** Test for starch.

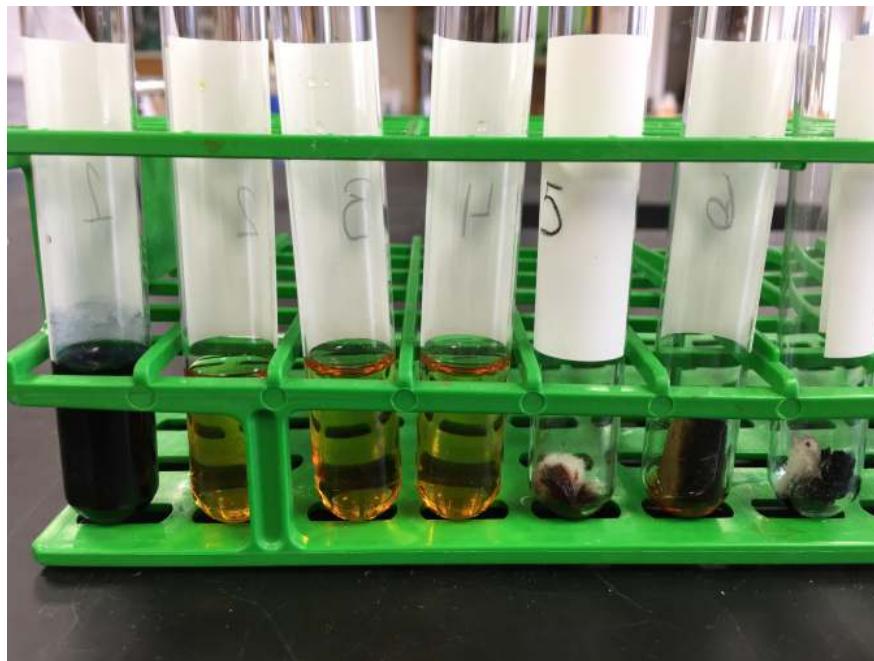
Tube #	Test material	Iodine	Observed color	Test Result (+ or -)
1	2 ml starch	5 drops		
2	2 ml glucose	5 drops		
3	2 ml H <sub>2</sub> O	5 drops		
4	2 ml sucrose	5 drops		
5	cotton (cellulose)	5 drops		
6	a small piece of bread	5 drops		
7	a small piece of potato	5 drops		
8	2 ml of starch plus 2 ml of amylase and then place the tube in the 37 °C heat block			
9	Leave empty and follow step 8 above			

### 3.3 Test for Proteins

Proteins<sup>7</sup> are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. Proteins perform a vast array of functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli, and transporting molecules from one location to another. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in protein folding into a specific three-dimensional structure that determines its activity. A linear chain of amino acid residues is called a polypeptide. A protein contains at least one long polypeptide. Short polypeptides, containing less than 20-30 residues, are rarely considered to be proteins and are commonly called peptides, or sometimes oligopeptides. The individual amino acid residues are bonded together by peptide bonds and adjacent amino acid residues. The sequence of amino acid residues in a protein is defined by the sequence of a gene, which is encoded in the genetic code. In general, the genetic code specifies 20 standard amino acids; however, in certain organisms the genetic code can include selenocysteine and-in certain archaea-pyrrolysine.

The biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper(II) ion forms violet-colored coordination complexes in an alkaline solution. The biuret reaction can be used to assess the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color is directly proportional to the protein

<sup>7</sup><https://en.wikipedia.org/wiki/Protein>



**Figure 3.7:** Results from experiment 2. Compare to your results!

concentration. Despite its name, the reagent does not in fact contain biuret ( $(H_2N-CO)_2NH$ ). The test is named so because it also gives a positive reaction to the peptide-like bonds in the biuret molecule. In this assay, the copper(II) binds with nitrogen present in the peptides of proteins. In a secondary reaction, the copper(II) is reduced to copper(I). Due to its insensitivity and little interference by free amino acids, this assay is most useful for whole tissue samples and other sources with high protein concentration.

### 3.3.1 Experimental procedures

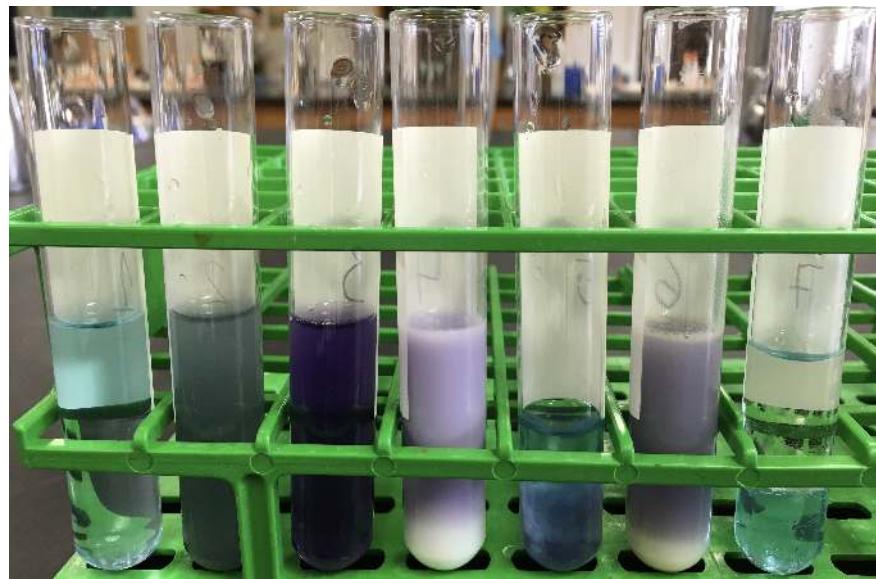
1. Obtain 7 plastic test tubes and a test tube rack.
2. Using a wax pencil, label each tube with a number (1 to 7).
3. Place the tubes from left (tube #1) to right (tube #7) in the first row of a test tube rack.
4. Add the materials to these tubes as indicated in Table 3.3 and mix well. No heating is needed to produce a reaction.
5. Wait 2 minutes and then record your observations in the table below. Base your conclusion only on the presence or absence of the violet color.

**Table 3.3:** Test for protein.

Tube #	Test material	Biuret	Observation	Test
1	2 ml $H_2O$	2 ml		
2	2 ml sucrose	2 ml		
3	2 ml albumin	2 ml		
4	2 ml milk	2 ml		
5	a small piece of bread	2 ml		
6	2 ml soy	2 ml		
7	2 ml vegetable oil	2 ml		

### 3.4 Cleaning up

1. Empty the contents of all plastic tubes into the labeled waste container (brown bottle) in the chemical fume hood (Figure 3.9).
2. Discard the empty tubes in the regular waste basket.



**Figure 3.8:** Results from experiment 3. Compare to your results!



**Figure 3.9:** Waste containers for sharps, liquids and broken glass in the fume hood.

### 3.5.1 Experimental procedures

1. Obtain a small glass tube.
2. Add 2 ml of water into the glass tube.
3. Add 6 drops of vegetable oil to on top.
4. Shake thoroughly and observe the way the oil is dispersed only temporarily. This is an emulsion a mixture of two liquids, each insoluble in the other.
5. Now add 3 drops of lipid-specific red Sudan stain and mix again.
6. Add 2 ml of a liquid detergent to the tube and shake again.
7. Allow the tube to stand and note that the two phases (oil and water) are no longer distinctly separated. Detergent is often termed an emulsifier. Its molecules are water-soluble on one end and lipid-soluble on the other. These surround small oil droplets, water-soluble end out, and allow the droplets to stay suspended in the water.

## 3.6 Cleaning up

Empty the contents of the glass tube into the labeled waste container (brown bottle) in the chemical fume hood. Discard the glass tube in the plastic container labeled “broken glass” in the chemical fume hood (Figure 3.9).

## 3.7 Test for organic and inorganic compounds (Demonstration)

### 3.7.1 Experimental procedures

1. The instructor will use a Bunsen burner to heat a number of substances. Organic substances will burn, inorganic substances will remain unchanged.
2. Record your observation in Table 3.4.

**Table 3.4:** Test for organic and inorganic compounds.

Substance	Organic	Inorganic
sugar		
table salt		
baking soda		
unknown		

## 3.8 Strawberry DNA extraction

We will use strawberries for DNA<sup>9</sup> isolation. Wild strawberries (*Fragaria vesca*) are “diploid” organisms (two sets of chromosomes; 14 total), just like us, but the at a size of 240 million base the strawberry genome (which has been fully sequenced) is much smaller than ours (3 billion bases). The cultivated strawberry, *Fragaria ananassa*, were generated only about 250 years ago are thus a very young crop species. Botanically, it is neither a berry nor a true fruit. Strawberry “fruits” consist of dry seeds that dot the surface of a fleshy modified shoot tip (the receptacle). Genomically, *Fragaria ananassa* is among the most complex of crop plants they harbor eight sets of chromosomes ( $2n = 8x = 56$ ), which are originally derived from as many as four different diploid ancestors. Thus, *Fragaria ananassa*, is an octoploid organism, with 4 times more DNA

<sup>9</sup><https://en.wikipedia.org/wiki/DNA>

in each cell than wild strawberries. This property makes this fruit a great choice for demonstrating DNA extraction. In addition, strawberries are soft and easy to homogenize and ripe.

The first step of DNA extraction from live or dead cells is to lyse or break open the cell. After the cells have broken open, a salt solution such as sodium chloride (NaCl) and a detergent solution containing the compound SDS (sodium dodecyl sulfate) is added. This solution breaks down and emulsifies the fat & proteins that make up the cell membrane. Finally, ice cold ethanol is added. The alcohol and salt cause the DNA to precipitate, or settle out of the solution, leaving behind all the cellular components that aren't soluble in alcohol. The DNA can be spooled (wound) on a stirring rod and pulled from the solution at this point.

It is important to mention that the procedure for DNA extraction we are using is really a procedure for total nucleic acid extraction. However, much of the RNA extracted is cut by ribonucleases (enzymes that cut RNA) that come in contact with the RNA when the cells are broken open.

### 3.8.1 Experimental procedures

1. Place 2 to 4 strawberries in a zip lock bag. Gently, mash up the fruit by pushing the bags with your hand against the lab bench. This will mechanically breakdown the cell wall.
2. Add 15 ml of the extraction buffer to the bag. Mix and mash again for one minute (chemical portion of the extraction procedure to lyse the cell membranes to release the DNA).
3. Filter the homogenate using cheesecloth (Figure 3.10). This step removes cell organelles, broken cell walls, membrane fragments, and other cell debris.
4. Collect the filtrate; squeeze the cheesecloth to get the as much of the lysate as possible.
5. Pour the filtrate into a plastic tube. Fill the tube to about half the volume
6. Use the transfer pipet to drip alcohol slowly down the sides of the tube (about 5 ml of ice cold alcohol), while holding the tube at approximately an angle of 45°. Try to make a clear and undisturbed layer of alcohol to float on the lysate. The line between the two layers is called the interface.
7. At the interface, you will see the DNA precipitate out of solution and float to the top. Spool the DNA on your glass rod (Figure 3.11).

## 3.9 Cleaning up

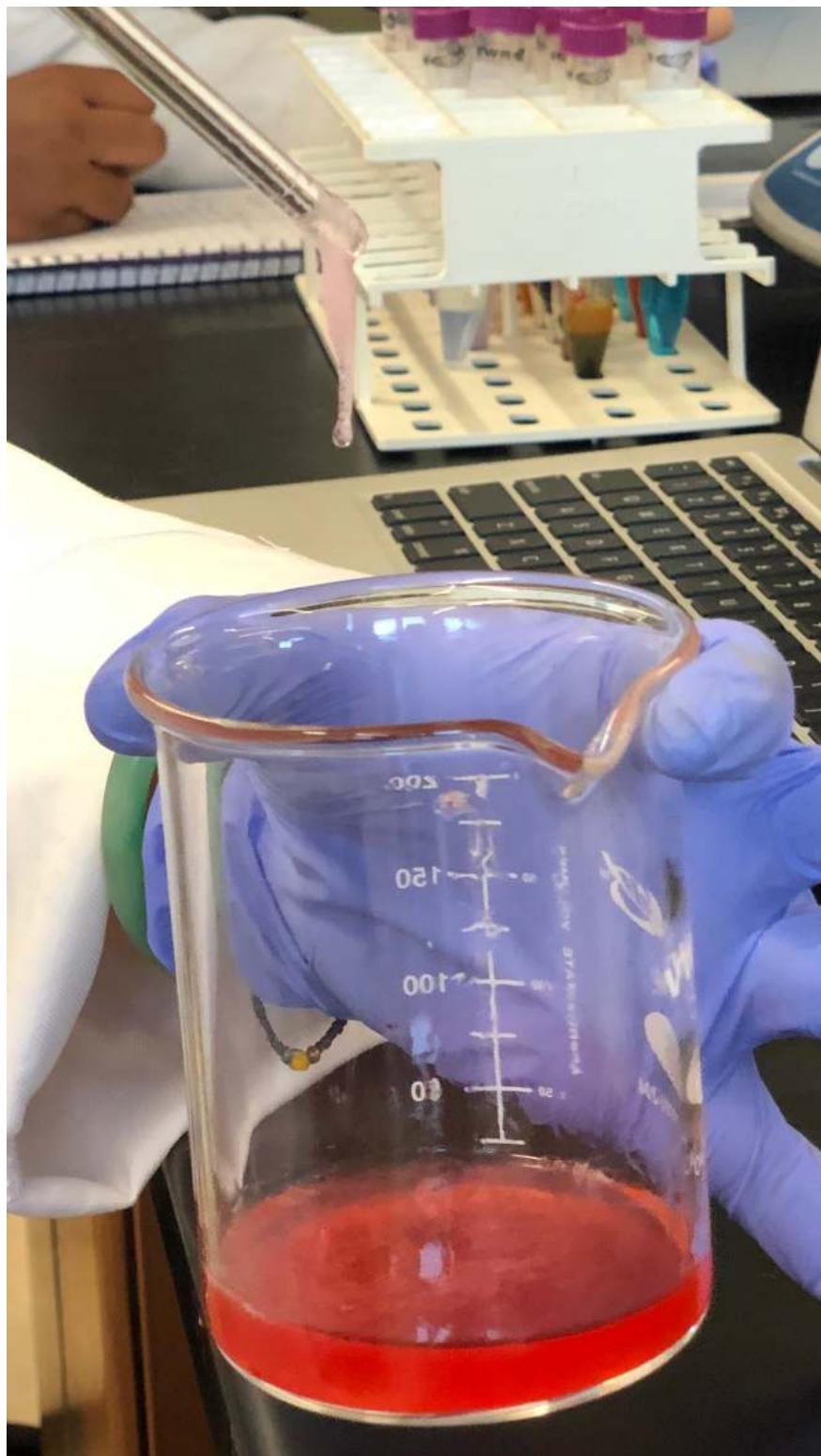
1. Empty the contents of the plastic tube into the labeled waste container (brown bottle) in the chemical fume hood.
2. Discard the empty tubes and other waste in the regular waste basket.
3. Rinse the glass rod and glassware with water and detergent.
4. Return the glass rod and glass ware to the trays on your bench where you originally found them.

## 3.10 Review Questions

1. What are organic compounds?
2. Which are the five most common elements in living organisms?
3. What are the four major groups of biomacromolecules?
4. What are the building blocks (monomers) of DNA?
5. What are lipids?
6. What are proteins?
7. What is a peptide bond?
8. What is chemical reduction?
9. What is chemical oxidation?



**Figure 3.10:** Filtration of the strawberry homogenate.



**Figure 3.11:** Precipitated strawberry DNA sticking to the tip of the glassrod.

10. What are detergents?
11. Why do we use the detergent in the DNA extraction experiment?

# Chapter 4

## Cell structure

The smallest unit of life is the cell<sup>1</sup>. In this lab, we will look at eukaryotic (plant and animal) cells under the microscope.

### 4.1 Elodea cells

#### 4.1.1 Experimental procedures

1. Get a single leaf from the Elodea plant and mount it on a slide, cover it with a drop of water and a cover slip.
2. Look at the leaf under the microscope (Figure 4.2).
3. Notice that the cells are clearly delineated by the cell wall. Inside the cells are large oval-shaped green bodies, the chloroplasts.

### 4.2 Onion leaf epidermal cells

#### 4.2.1 Experimental procedures

1. Peel a very thin layer from the concave side of a piece of onion, and mount in water. Make sure the membrane lays very flat and is not wrinkled or folded on your slide.
2. Dim the light and find the nucleus, which is much easier to see here because there are no chloroplasts (Figure 4.3). Close examination should reveal one or more nucleoli in the nucleus.
3. Note the movement of small particles in the nearly trans-parent cytoplasm. This is Brownian movement, which we will study in more detail in another exercise.

### 4.3 Carrot root cells

#### 4.3.1 Experimental procedures

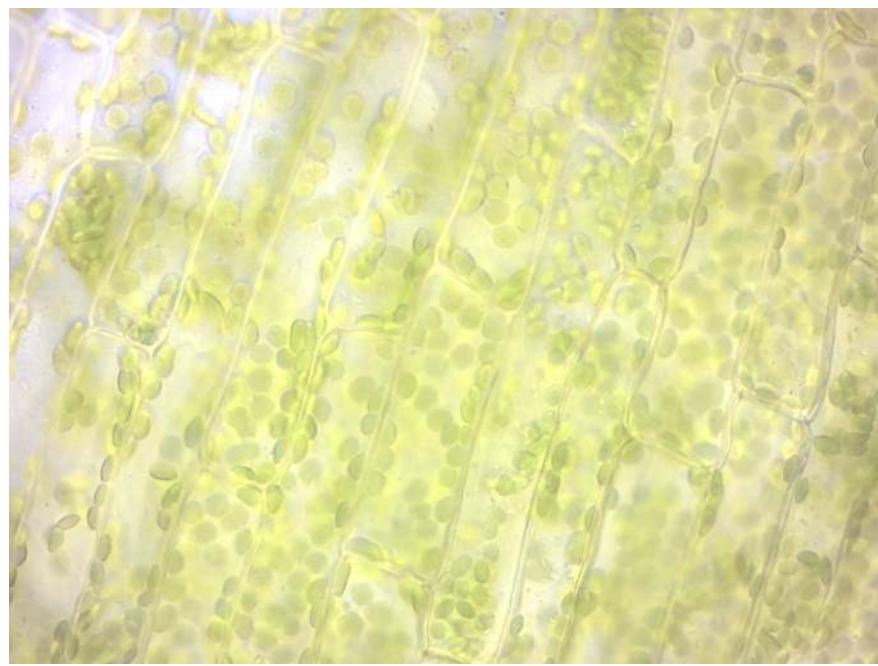
1. Use a razor blade and shave a very thin (almost translucent) slice of carrot onto a slide and prepare a wet mount of it (Figure 4.4).

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<sup>1</sup>[https://en.wikipedia.org/wiki/Cell\\_\(biology\)](https://en.wikipedia.org/wiki/Cell_(biology))



**Figure 4.1:** Experimemtal materials



**Figure 4.2:** Elodea wet mount (100 $\times$  oil immersion objective).



**Figure 4.3:** Onion epidermis.

2. Observe the orange-yellow bodies called chromoplasts, another type of plastid. Chromoplasts are enriched in pigments that give flowers, fruits, and fall leaves their colors, and carrots their orange color.

## 4.4 Potato cells

Potatoes are stems full of starch that is stored within the cells in colorless plastids called amyloplasts.

### 4.4.1 Experimental procedures

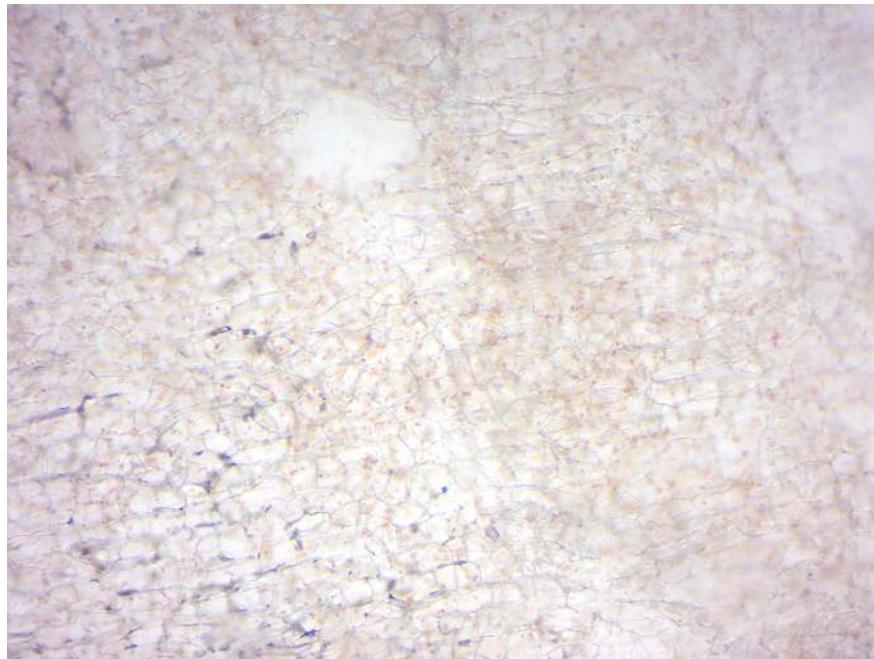
1. Cut a very thin wedge-shaped sliver of potato.
2. Place it on a microscope slide.
3. Add a drop of iodine on top of the slice of potator.
4. Place a coverslip on top.
5. Observe the potato slice under the microscope.
6. Iodine stains starch a purple or blue-black color.

## 4.5 Cleaning up

Discard the used glass slides in the container marked “Broken glass” in the fume hood.

## 4.6 Human cheek cells

The outer and inner surface of our body is formed by epithelial cells.



**Figure 4.4:** Carrot wet mount.

#### 4.6.1 Experimental procedures

1. Rub the inner side of your cheek with a toothpick to pick up some cells.
2. Scrape the cloudy (cell-containing) fluid onto a slide, add a drop of dilute methylene blue, and observe under the microscope (Figure 4.5). Note that bacteria and possibly fungi on top and around your cheek cells.

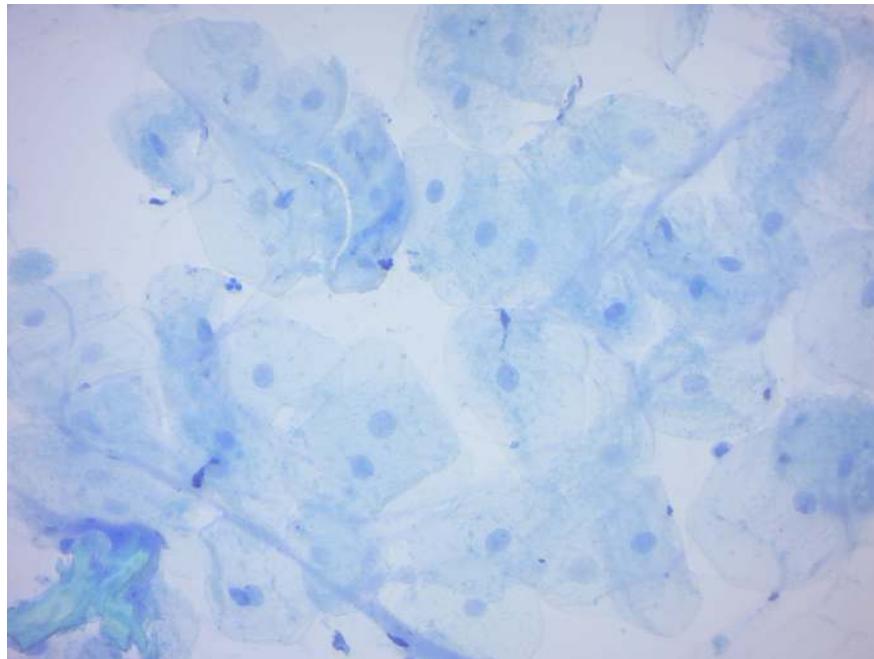
### 4.7 Cleaning up

Dispose of the toothpicks and the slide with the cheek cells in the beakers containing (green looking) disinfectant liquid.

### 4.8 Human blood cells

Blood<sup>2</sup> is a body fluid in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. In vertebrates, it is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissolved proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood. The blood cells are mainly red blood cells (also called RBCs or erythrocytes), white blood cells (also called WBCs or leukocytes) and platelets (also called thrombocytes). The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates oxygen transport by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is mostly transported extracellularly as bicarbonate ion transported in plasma. Vertebrate blood is

<sup>2</sup><https://en.wikipedia.org/wiki/Blood>



**Figure 4.5:** Human cheek epidermal cells.

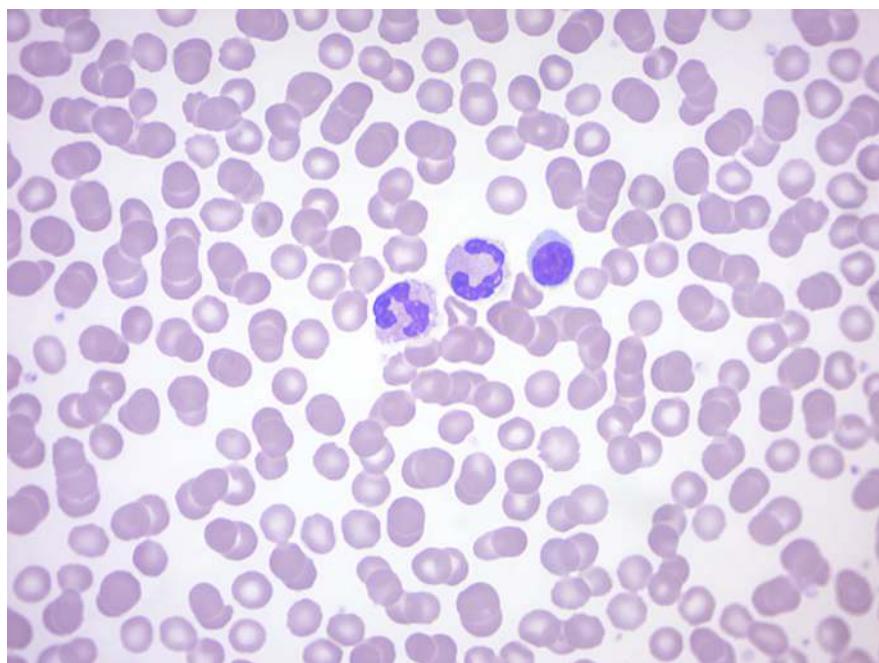
bright red when its hemoglobin is oxygenated and dark red when it is deoxygenated. Some animals, such as crustaceans and mollusks, use hemocyanin to carry oxygen, instead of hemoglobin. Insects and some mollusks use a fluid called hemolymph instead of blood, the difference being that hemolymph is not contained in a closed circulatory system. In most insects, this “blood” does not contain oxygen-carrying molecules such as hemoglobin because their bodies are small enough for their tracheal system to suffice for supplying oxygen. Jawed vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled.

#### 4.8.1 Experimental procedures

1. Look at the prepared slide containing a human blood stain (Figure 4.6).
2. Can you distinguish multiple types of white blood cells?
3. Return the slide to the white slide box.

### 4.9 Review Questions

1. What are the main structural features of eukaryotic cells?
2. What distinguishes animal and plant cells?
3. What are mitochondria?
4. What are chloroplasts?



**Figure 4.6:** Human blood smear. Note red and two types of white blood cells.

# Chapter 5

## Exchange Between Cells and Their Environment

### 5.1 Diffusion

Diffusion<sup>1</sup> is the net movement of molecules or atoms from a region of high concentration (or high chemical potential) to a region of low concentration (or low chemical potential) as a result of random motion of the molecules or atoms. The word diffusion derives from the Latin word, diffundere, which means “to spread way out”.

Diffusion is driven by a gradient in chemical potential of the diffusing species. A gradient is the change in the value of a quantity e.g. concentration, pressure, or temperature with the change in another variable, usually distance. A change in concentration over a distance is called a concentration gradient, a change in pressure over a distance is called a pressure gradient, and a change in temperature over a distance is a called a temperature gradient.

A distinguishing feature of diffusion is that it depends on particle random walk, and results in mixing or mass transport without requiring directed bulk motion.

#### 5.1.1 Diffusion in air

#### 5.1.2 Experimental procedures

1. The instructor will open a bottle containing a fragrant substance.
2. Raise your hand when you smell the odor.

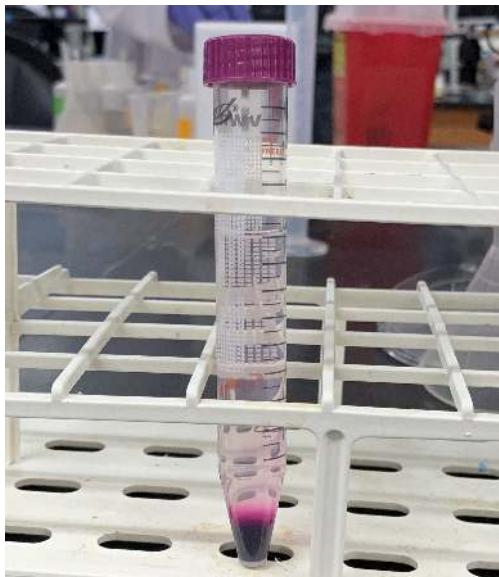
#### 5.1.3 Diffusion in water

#### 5.1.4 Experimental procedures

1. Add 10 mL of water to a plastic test tube.
2. Drop a crystal of purple KMnO<sub>4</sub> (potassium permanganate) into it.
3. Put the tube in a test tube rack and place it where it will not be disturbed for the remainder of the lab period
4. From time to time, observe the how the KMnO<sub>4</sub> diffuses through the water (Figure 5.1).

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<sup>1</sup><https://en.wikipedia.org/wiki/Diffusion>



**Figure 5.1:** Diffusion of a solid ( $\text{KMnO}_4$ ) in a liquid ( $\text{H}_2\text{O}$ ).

## 5.2 Diffusion in a solid

### 5.2.1 Experimental procedures

1. Place a few crystals of  $\text{KMnO}_4$  onto a Petri dish with agarose gel.
2. Place a few crystals of malachite green about 2 centimeters from the potassium permanganate crystal (Figure 5.2).
3. Place a few specks of carmine red about 2 cm from each of the other crystals.
4. Note the rate of diffusion.
5. Find the molecular weights of each compound from Wikipedia<sup>2</sup>.

## 5.3 Diffusion Through a Selectively Permeable Membrane

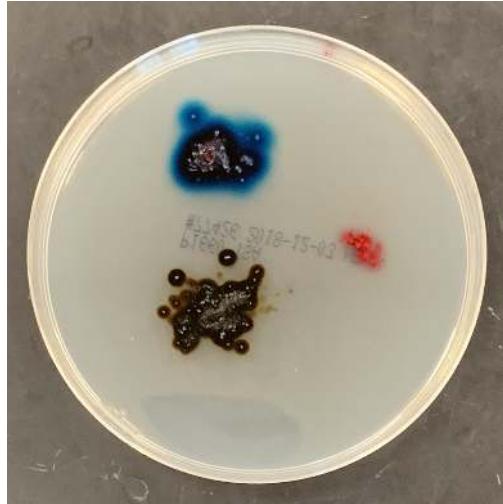
A selectively permeable membrane is a type of biological or synthetic, polymeric membrane that will allow certain molecules or ions to pass through it by diffusion—or in the case of biological membranes (e.g. the cell membrane) by more specialized processes such as facilitated diffusion and active transport. In this experiment we will use a dialysis or semipermeable membrane<sup>3</sup>—a selectively permeable membrane that allows particles below a certain size through but excludes any particles that are larger.

### 5.3.1 Experimental procedures

1. Fill a 500-ml beaker with 300 ml of water.
2. Get a ~10 cm long piece of dialysis tubing and submerge it for ~10 seconds in the water.
3. Remove the dialysis tubing and clamp one end with the yellow clamp.
4. Rub the other end between the tips of your thumb and index fingers to open the dialysis membrane.
5. Get the starch solution and shake it well.
6. Fill the dialysis tubing with ~10 ml of starch solution.

<sup>2</sup><https://www.wikipedia.org>

<sup>3</sup>[https://en.wikipedia.org/wiki/Semipermeable\\_membrane](https://en.wikipedia.org/wiki/Semipermeable_membrane)



**Figure 5.2:** Diffusion of three different solids through agarose.

7. Clamp off the open end.
8. Rinse with tap water.
9. Add iodine solution to the beaker with water until the solution becomes dark orange.
10. Add the dialysis tube containing the starch to the beaker and submerge completely in the water.
11. After about 15 minutes, observe the bag and surrounding liquid for any color change.
12. When starch molecules touch iodine, a blue or purplish color appears (Figure 5.3).
13. Start setting up Experiment 3 while you wait.

## 5.4 Brownian motion

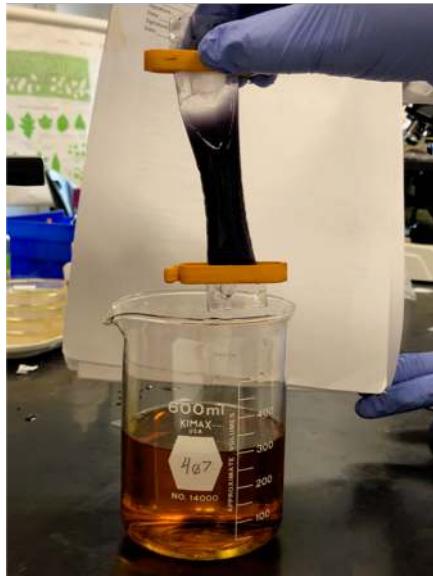
Brownian motion<sup>4</sup> is the random motion of particles suspended in a fluid (a liquid or a gas) resulting from their collision with the fast-moving molecules in the fluid. This motion is named after Robert Brown<sup>5</sup> (botanist, born 1773). In 1827, while looking through a microscope at particles trapped in cavities inside pollen grains in water, he noted that the particles moved through the water; but he was not able to determine the mechanisms that caused this motion. Atoms and molecules had long been theorized as the constituents of matter, and Albert Einstein published a paper in 1905 that explained in precise detail how the motion that Brown had observed was a result of the pollen being moved by individual water molecules, making one of his first big contributions to science. This explanation of Brownian motion served as convincing evidence that atoms and molecules exist and was further verified experimentally by Jean Perrin in 1908. Perrin was awarded the Nobel Prize in Physics in 1926 “for his work on the discontinuous structure of matter”. The direction of the force of atomic bombardment is constantly changing, and at different times the particle is hit more on one side than another, leading to the seemingly random nature of the motion.

### 5.4.1 Experimental procedures

1. Put a drop of water on a microscope slide.
2. Take a toothpick and pick up a tiny bit of powdered carmine from the carmine container.
3. Hold the toothpick over the drop of water on the slide and tap it so that small specs of carmine fall into the water on the slide.
4. Add a coverslip on top of the drop of water with the carmine on the slide.

<sup>4</sup>[https://en.wikipedia.org/wiki/Brownian\\_motion](https://en.wikipedia.org/wiki/Brownian_motion)

<sup>5</sup>[https://en.wikipedia.org/wiki/Robert\\_Brown\\_\(botanist,\\_born\\_1773\)](https://en.wikipedia.org/wiki/Robert_Brown_(botanist,_born_1773))



**Figure 5.3:** Result of the dialysis experiment.

5. Put the slide under the microscope.
6. Observe the quivering motion of the particles under high power. A mass movement of particles in any one direction may result if the microscope is not quite level, or you are using only one stage clip, or the coverslip is caught under the stage clips. This is not what you are looking for.

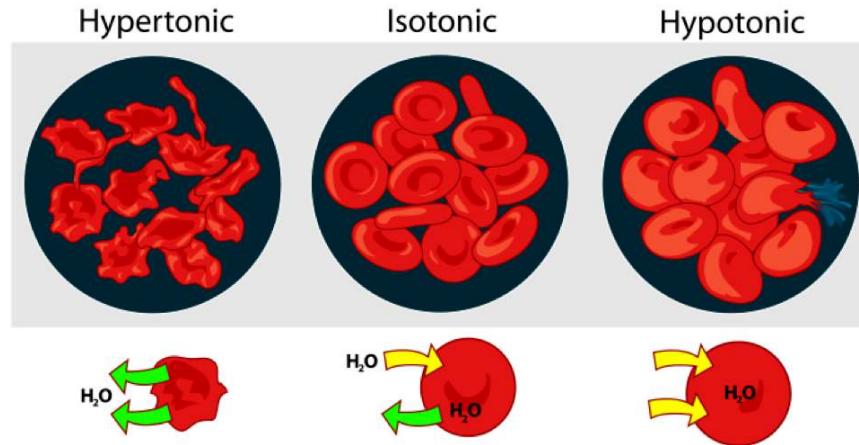
## 5.5 Osmosis in Animal and Plant Cells

Osmosis<sup>6</sup> is the spontaneous net movement of solvent molecules through a selectively-permeable membrane into a region of higher solute concentration, in the direction that tends to equalize the solute concentrations on the two sides. A selectively permeable membrane is a membrane that is permeable for some but not other molecules. Osmotic pressure is defined as the external pressure required to be applied so that there is no net movement of solvent across the membrane. Osmotic pressure is a colligative property, meaning that the osmotic pressure depends on the molar concentration of the solute but not on its identity. Osmosis is a vital process in biological systems, as biological membranes are selectively permeable. In general, these membranes are impermeable to large and polar molecules, such as ions, proteins, and polysaccharides, while being permeable to non-polar or hydrophobic molecules like lipids as well as to small molecules like oxygen, carbon dioxide, nitrogen, and nitric oxide. Permeability depends on solubility, charge, or chemistry, as well as solute size. Water molecules travel through the plasma membrane, tonoplast membrane (vacuole) or protoplast by diffusing across the phospholipid bilayer via aquaporins (small transmembrane proteins similar to those responsible for facilitated diffusion and ion channels). Osmosis provides the primary means by which water is transported into and out of cells. The turgor pressure of a cell is largely maintained by osmosis across the cell membrane between the cell interior and its relatively hypotonic environment.

In unusual environments, osmosis can be very harmful to organisms. For example, freshwater and saltwater aquarium fish placed in water of a different salinity than that to which they are adapted to will die quickly, and in the case of saltwater fish, dramatically. Another example of a harmful osmotic effect is the use of table salt to kill leeches and slugs. Suppose an animal or a cell is placed in a solution of sugar or salt in water. 1. If the medium is hypotonic relative to the cell cytoplasm - the cell will gain water through osmosis. 2. If the medium is isotonic - there will be no net movement of water across the cell membrane. 3. If the medium is hypertonic relative to the cell cytoplasm - the cell will lose water by osmosis. Essentially,

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<sup>6</sup><https://en.wikipedia.org/wiki/Osmosis>



**Figure 5.4:** Osmosis in red blood cells.<sup>8</sup>

this means that if a cell is put in a solution which has a solute concentration higher than its own, it will shrivel, and if it is put in a solution with a lower solute concentration than its own, the cell will swell and may even burst.

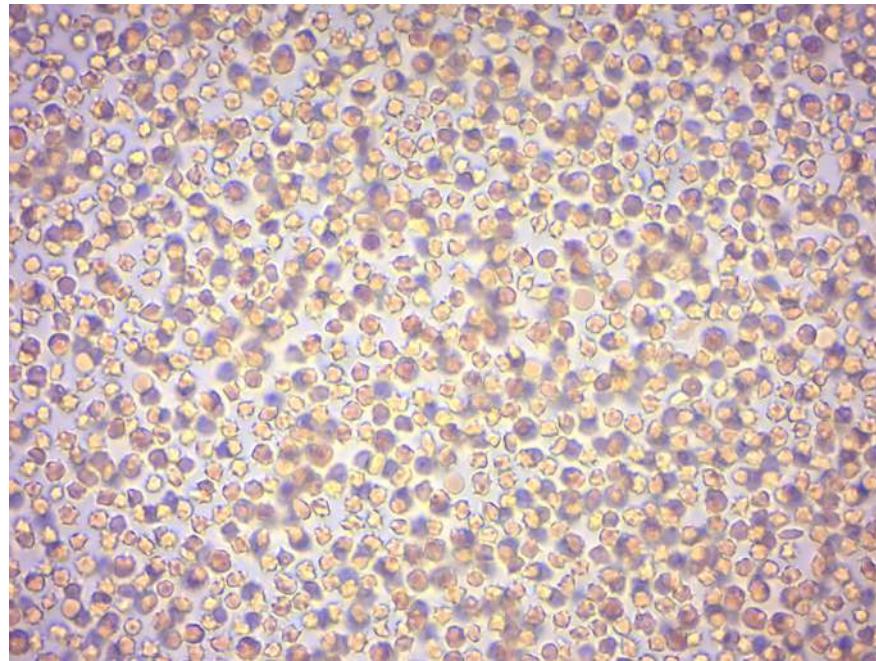
## 5.6 Crenation and Hemolysis of Red Blood Cells

Crenation<sup>7</sup> (Figure 5.4) is used to describe blood cells that look as if they have projections extending from a smaller central area, like a spiked ball. Hemolysis or haemolysis is the rupturing (lysis) of red blood cells (erythrocytes) and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma).

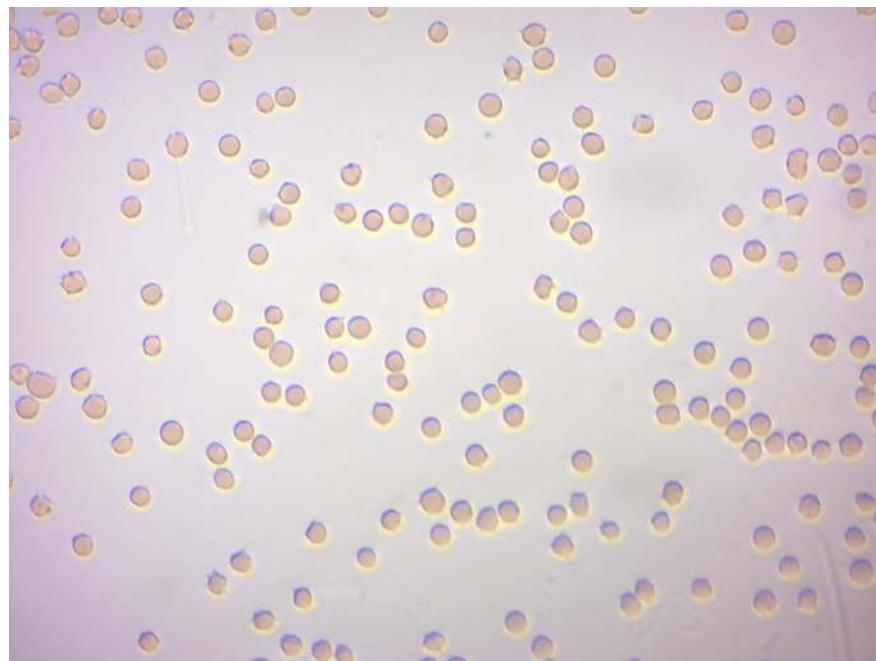
### 5.6.1 Experimental procedures

1. Work in groups of three students
2. Get three clean slides.
3. With a wax pencil, mark the first slide “5%”, the second “0.85%”, and the third “0%”.
4. Using a plastic transfer pipette, place a drop of 5% NaCl solution just slightly off center on the first slide (labelled “5%”), then place a drop of 0.85% NaCl solution just slightly off center on the second slide (labelled “0.85%”) and finally place a drop of distilled water just slightly off center on the last slide (labelled “0%”).
5. To each of the three slides, add a small drop of sheep’s blood beside the drop of saline or water.
6. Put a coverslip on top of the adjacent drops of liquid and blood on each slide. Raise and lower the coverslip a couple of times to mix the blood and saline.
7. Place each slide on the stage of each of three microscopes that are side by side.
8. Using the 4× objective, focus on the blood and choose an area where the cells are thinly distributed.
9. Switch to the 10× objective. Refocus on the blood using the fine focus knob. Then switch to the 40× objective, refocus using the fine focus knob and observe the blood cells under 400× magnification.
10. Compare your observation with Figure 5.5, Figure 5.6 and Figure 5.7.

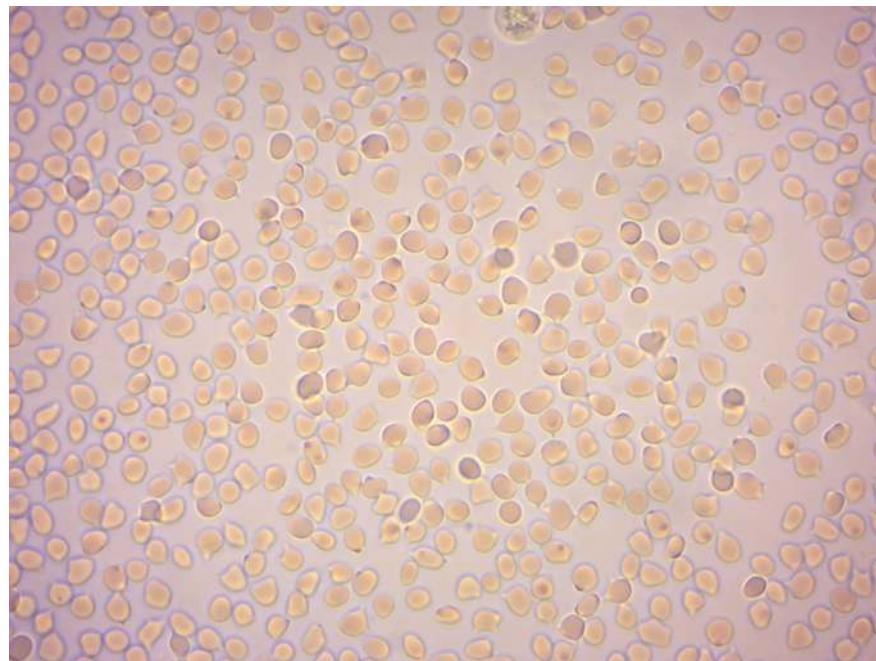
<sup>7</sup><https://en.wikipedia.org/wiki/Crenation>



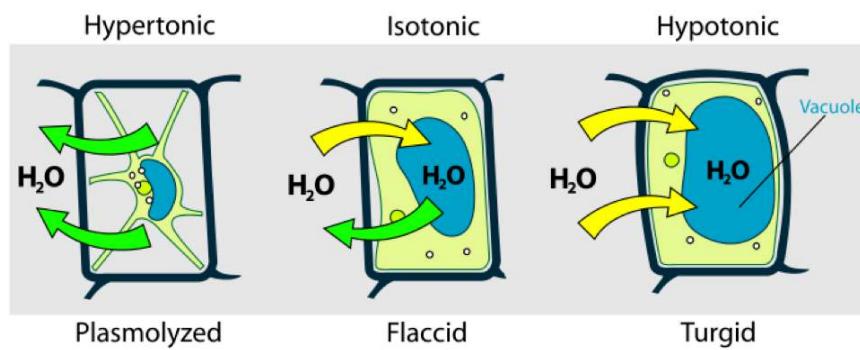
**Figure 5.5:** Red blood cells from sheep in hypertonic saline solution (5% NaCl).



**Figure 5.6:** Red blood cells from sheep in isotonic saline solution (0.85% NaCl).



**Figure 5.7:** Red blood cells from sheep in distilled water.



**Figure 5.8:** Osmosis in plant cells.<sup>9</sup>

## 5.7 Turgor and Plasmolysis

Osmotic pressure is the main cause of support in many plants. The osmotic entry of water raises the turgor pressure exerted against the cell wall, until it equals the osmotic pressure, creating a steady state. When a plant cell is placed in a solution that is hypertonic relative to the cytoplasm, water moves out of the cell and the cell shrinks (Figure 5.8). In doing so, the cell becomes flaccid. In extreme cases, the cell becomes plasmolyzed - the cell membrane disengages with the cell wall due to lack of water pressure on it. When a plant cell is placed in a solution that is hypotonic relative to the cytoplasm, water moves into the cell and the cell swells to become turgid. Osmosis is responsible for the ability of plant roots to draw water from the soil. Plants concentrate solutes in their root cells by active transport, and water enters the roots by osmosis. Osmosis is also responsible for controlling the movement of guard cells.

### 5.7.1 Experimental procedures

1. Mount a leaf of Elodea in water on a slide and observe.



**Figure 5.9:** Elodea leaf in hypertonic saline solution (7% NaCl).

2. Blot off the water and add salt solution (7% NaCl) by adding the salt at one side of the coverslip and drawing it under by using a paper towel to absorb the fluid from the opposite side of the coverslip.
3. Let it sit for a minute or two and observe again. The cell contains a vacuole, which holds most of the cell's water, similar to a water bag inside the cell. Note the clear space forming between the cell wall and the cell membrane (Figure 5.9).
4. Rinse the salt solution from the leaf and remount in water.
5. Observe periodically for about 5 minutes. How is the cell changing?

## 5.8 Review Questions

1. What is and what causes Brownian motion?
2. What is the definition of concentration?
3. What is a concentration gradient?
4. What is diffusion?
5. What is a selectively permeable membrane?
6. What is osmosis?
7. What is osmotic pressure?

# Chapter 6

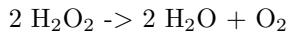
## Enzymes

Enzymes<sup>1</sup> are macromolecular biological catalysts. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life. Metabolic pathways depend upon enzymes to catalyze individual steps. Enzymes are known to catalyze more than 5,000 biochemical reaction types. Most enzymes are proteins, although a few are catalytic RNA molecules. The latter are called ribozymes. Enzymes' specificity comes from their unique three-dimensional structures.

Like all catalysts, enzymes increase the reaction rate by lowering its activation energy. Some enzymes can make their conversion of substrate to product occur many millions of times faster. An extreme example is orotidine 5'-phosphate decarboxylase, which allows a reaction that would otherwise take millions of years to occur in milliseconds. Chemically, enzymes are like any catalyst and are not consumed in chemical reactions, nor do they alter the equilibrium of a reaction. Enzymes differ from most other catalysts by being much more specific. Enzyme activity can be affected by other molecules: inhibitors are molecules that decrease enzyme activity, and activators are molecules that increase activity. Many therapeutic drugs and poisons are enzyme inhibitors. An enzyme's activity decreases markedly outside its optimal temperature and pH.

Some enzymes are used commercially, for example, in the synthesis of antibiotics. Some household products use enzymes to speed up chemical reactions: enzymes in biological washing powders break down protein, starch or fat stains on clothes, and enzymes in meat tenderizer break down proteins into smaller molecules, making the meat easier to chew.

In this laboratory, we will study the effect of temperature, concentration and pH and on the activity of the enzyme catalase. Catalase speeds up the following reaction:



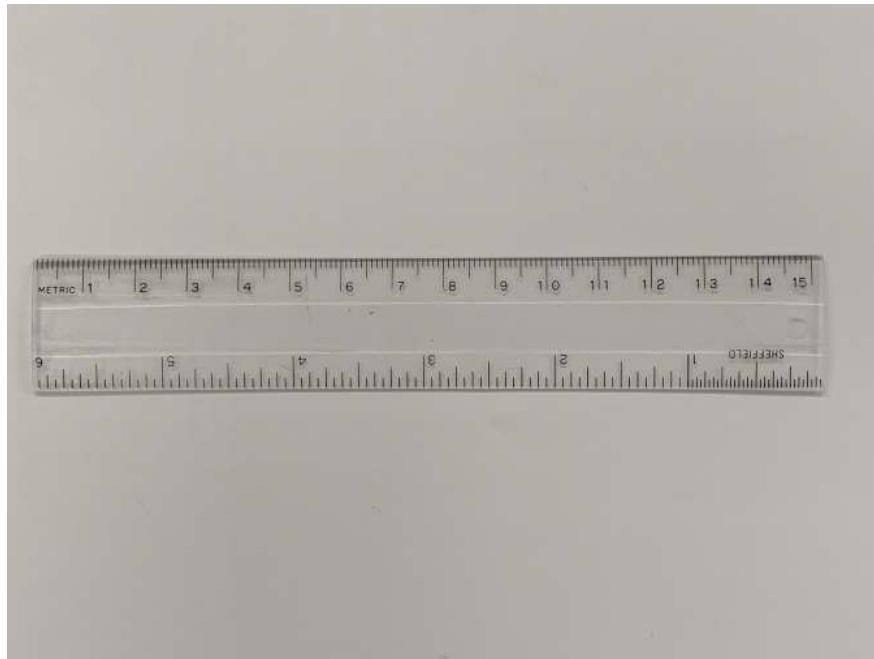
Hydrogen peroxide is toxic. Cells therefore use catalase to protect themselves. In these experiments, we will use catalase enzyme from potato.

The first experiment will establish that our catalase works (positive control) and that our reagents are not contaminated (negative control).

Hydrogen peroxide will not spontaneously degrade at room temperature in the absence of enzyme. When catalase is added to hydrogen peroxide, the reaction will take place and the oxygen produced will lead to the formation of bubbles in the solution. The height of the bubbles above the solution will be our measure of enzyme activity (Figure 6.2).

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<sup>1</sup><https://en.wikipedia.org/wiki/Enzyme>



**Figure 6.1:** A ruler with metric (cm) and imperial (inch) scales.

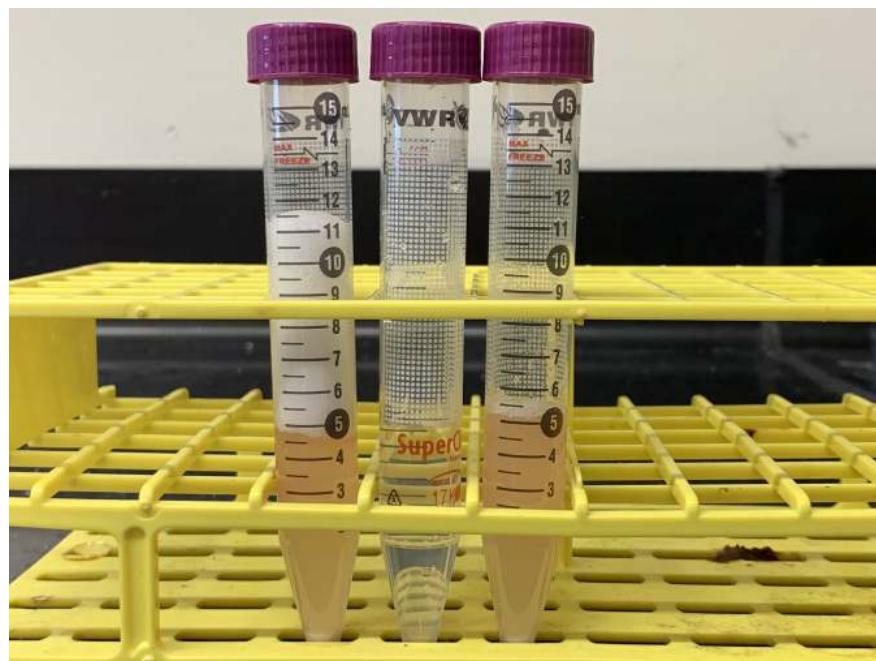
## 6.1 Positive and negative controls (Experiment 1)

### 6.1.1 Experimental procedures

1. Obtain and label three 15 ml conical plastic reaction tubes.
2. Then to **Tube 1**
  - Add 1 ml of potato juice (catalase) to tube 1 (use a plastic transfer pipette).
  - Add 4 ml of hydrogen peroxide to tube 1. Swirl well to mix and wait at least 20 seconds for bubbling to develop.
  - Use a ruler (Figure 6.1) and measure the height of the bubble column above the liquid (in millimeters; use the centimeter scale of the ruler) and record the result in Table 6.1.
3. Then to **Tube 2**
  - Add 1 ml of water.
  - Add 4 ml of hydrogen peroxide. Swirl well to mix and wait at least 20 seconds.
  - Measure the height of the bubble column (in millimeters) and record the result in Table 6.1.
4. Then to **Tube 3**
  - Add 1 ml of potato juice (catalase).
  - Add 4 ml of sucrose solution. Swirl well to mix; wait 20 seconds.
  - Measure the height of the bubble column and record the result in Table 6.1.

**Table 6.1:** Positive and negative controls.

Tube #	Height of bubbles (mm)
1	
2	
3	



**Figure 6.2:** Results from experiment 1. Compare with your results!

## 6.2 Effect of temperature on enzyme activity (Experiment 2)

### 6.2.1 Experimental procedures

1. Obtain and label three tubes.
2. Add 1 ml of potato juice (catalase) to each tube.
3. Place tube 1 in the refrigerator, tube 2 in a 37 °C (Celsius) heat block, and tube 3 in a 97 °C heat block for 15 minutes.
4. Remove the tubes with the potato juice (catalase) from the refrigerator and heat blocks and immediately add 4 ml hydrogen peroxide to each tube.
5. Swirl well to mix and wait 20 seconds.
6. Measure the height of the bubble column (in millimeters) in each tube and record your observations in Table 6.2.

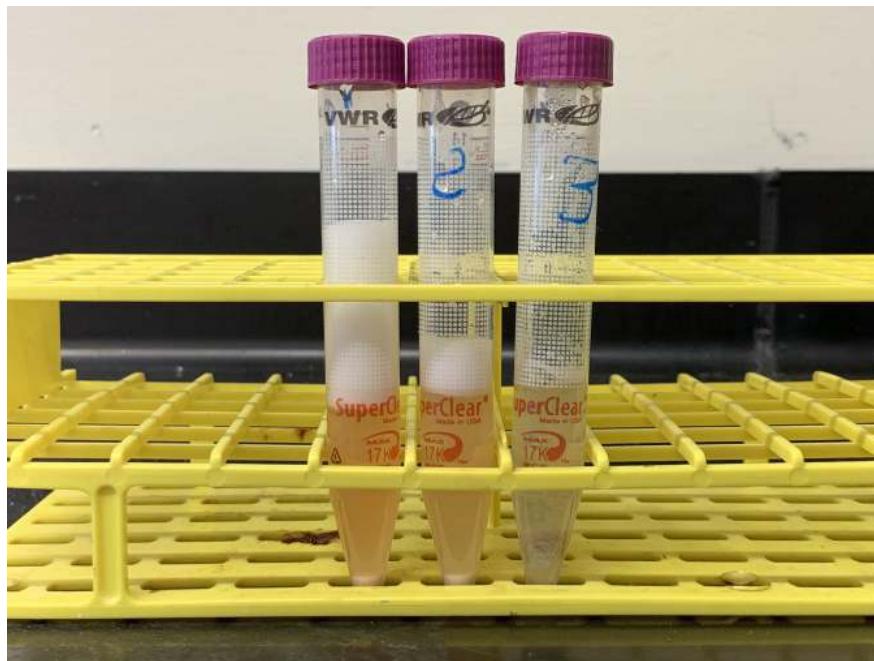
**Table 6.2:** Effect of temperature on enzyme activity.

Tube #	Height of bubbles (mm)
1	
2	
3	

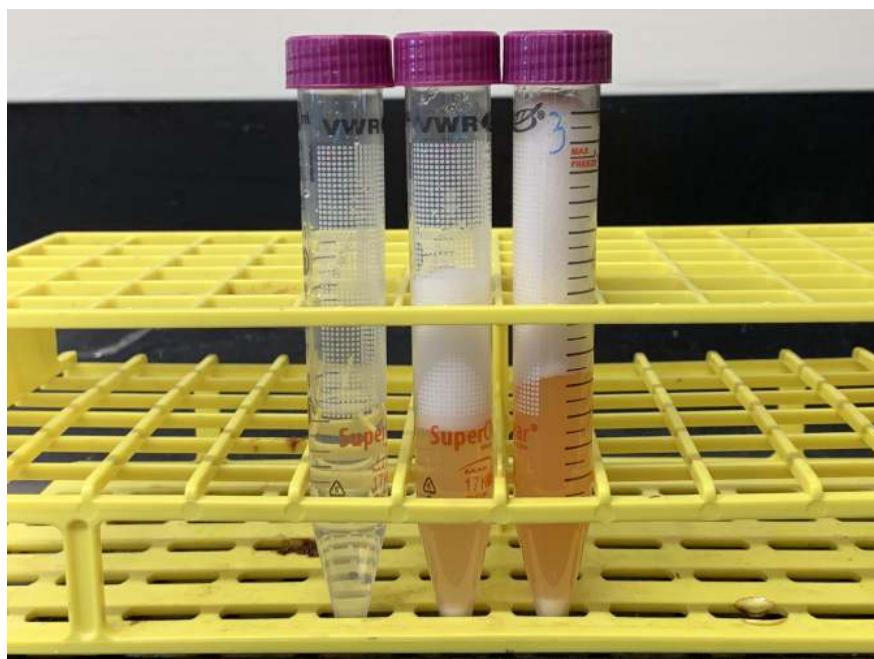
## 6.3 Effect of concentration on enzyme activity (Experiment 3)

### 6.3.1 Experimental procedures

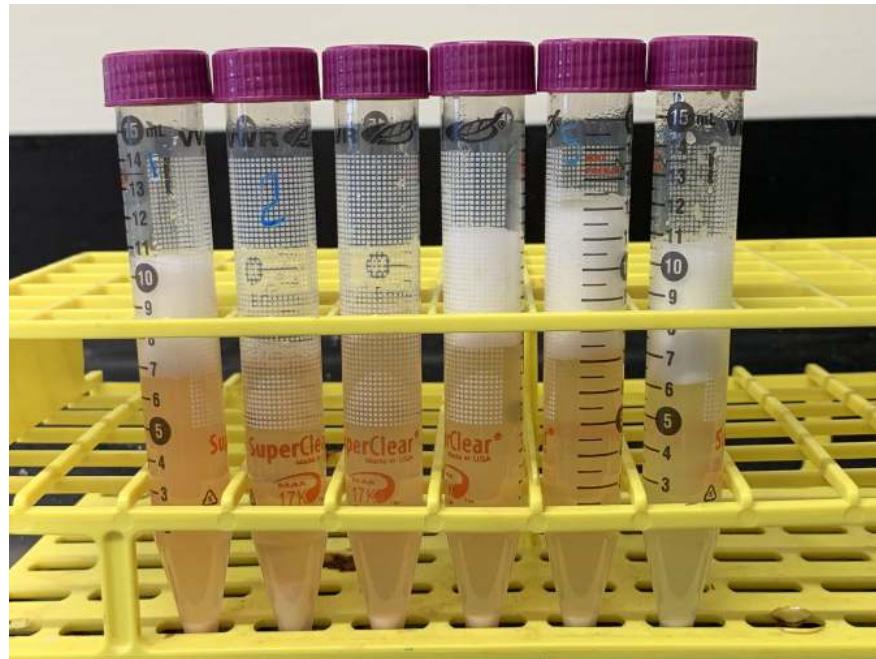
1. Obtain and label three tubes.
2. Then to **Tube 1**
  - Add 1 ml of water.
  - Add 4 ml of hydrogen peroxide. Swirl well to mix and wait at least 20 seconds.
  - Measure the height of the bubble column (in millimeters) and record your observations in Table 6.3.
3. Then to **Tube 2**
  - Add 1 ml of potato juice (catalase).



**Figure 6.3:** Results from experiment 2. Compare with your results!



**Figure 6.4:** Results from experiment 3. Compare with your results!



**Figure 6.5:** Results from experiment 4. Compare with your results!

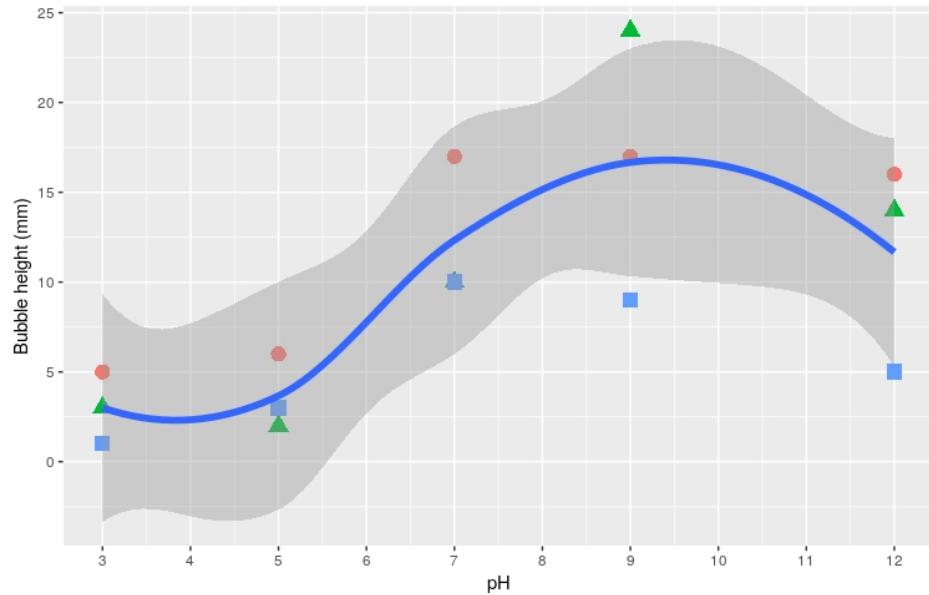
## 6.4 Effect of pH on enzyme activity (Experiment 4)

### 6.4.1 Experimental procedures

1. Obtain 6 tubes and label each tube with a number from 1 to 6.
2. Place the tubes from left (tube #1) to right (tube #6) in the first row of a test tube rack.
3. Add to 1 ml of potato juice (catalase) to each tube.
4. Add 2 ml of water to tube 1.
5. Add 2 ml of pH buffer 3 to tube 2.
6. Add 2 ml of pH buffer 5 to tube 3.
7. Add 2 ml of pH buffer 7 to tube 4.
8. Add 2 ml of pH buffer 9 to tube 5.
9. Add 2 ml of pH buffer 12 to tube 6.
10. Add 4 ml of hydrogen peroxide to each of the six tubes.
11. Swirl each tube well to mix and wait at least 20 seconds.
12. Measure the height of the bubble column (in millimeters) in each tube and record your observations in Table 6.4.

**Table 6.4:** Effect of pH on enzyme activity.

Tube #	Height of bubbles (mm)
1	
2	
3	
4	
5	
6	



**Figure 6.6:** Catalase activity is dependent on pH. The data shown in this figure were obtained by three groups of students during a previous laboratory session. The green triangles represent the data from the experimental results shown in Figure 6.5.

## 6.5 Cleaning up

1. Empty the contents of the plastic tubes into the labeled waste container (brown bottle) in the chemical fume hood.
2. Discard the empty tubes and other waste in the regular waste basket.
3. Rinse the glass rod and glassware with water and detergent.
4. Return the glass ware to the trays on your bench where you originally found them.

## 6.6 Review Questions

1. What is a catalyst?
2. What are enzymes?
3. What is the name of the enzyme that we studied in this laboratory session?
4. What is an enzyme substrate?
5. What is the substrate of the enzyme that we used in this laboratory session?
6. What are the products of the reaction that was catalyzed by the enzyme that we studied in this laboratory session?
7. What is the active site of an enzyme?
8. What is the purpose of the negative and positive controls?
9. State the hypothesis that was tested in experiment 2?
10. State the hypothesis that was tested in experiment 3?
11. State the hypothesis that was tested in experiment 4?
12. The enzyme from potato appeared to work better at 4 °C than at 37 °C. Would you expect the same if we had used the equivalent human enzyme? Justify your answer.
13. Why did heating the enzyme at high temperature (> 65 °C) result in loss of activity?

# Chapter 7

## Photosynthesis

In this lab, we will study the effect of light intensity and quality (wave length - color) on photosynthesis<sup>1</sup>. As a measure of the rate of photosynthesis, we will monitor the rate of oxygen production. When plants that spend their life submerged in water release oxygen it forms bubbles, which we can count over a period of time to determine photosynthesis rate.

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

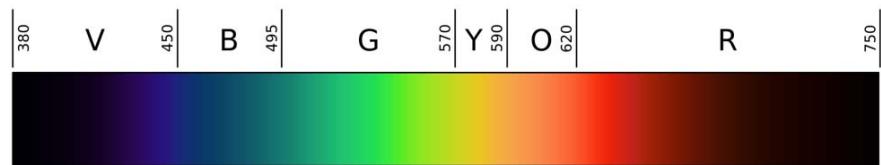
Although photosynthesis is performed differently by different species, the process always begins when energy from light is absorbed by proteins called reaction centers that contain green chlorophyll pigments. In plants, these proteins are held inside organelles called chloroplasts, which are most abundant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

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

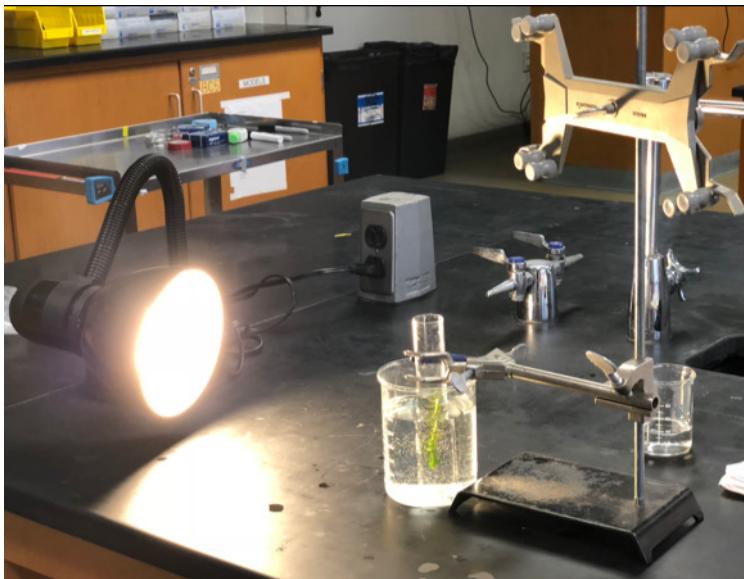
The first photosynthetic organisms probably evolved early in the evolutionary history of life and most likely used reducing agents such as hydrogen or hydrogen sulfide, rather than water, as sources of electrons. Cyanobacteria appeared later; the excess oxygen they produced contributed directly to the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

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<sup>1</sup><https://en.wikipedia.org/wiki/Photosynthesis>



**Figure 7.1:** Spectrum of light. V, violet; B, blue; G, green Y, yellow; O, orange; R, red<sup>3</sup>



**Figure 7.2:** Setup for photosynthesis experiment.

The main source of light on Earth is the Sun. Sunlight provides the energy that green plants use to create sugars mostly in the form of starches, which release energy into the living things that digest them. This process of photosynthesis provides virtually all the energy used by living things. The primary properties of visible light are intensity, propagation direction, frequency or wavelength spectrum, and polarization, while its speed in a vacuum, 299,792,458 meters per second, is one of the fundamental constants of nature. Visible light, as with all types of electromagnetic radiation (EMR), is experimentally found to always move at this speed in a vacuum.

## 7.1 Intensity of light

Light<sup>2</sup> is electromagnetic radiation within a certain portion of the electromagnetic spectrum (Figure 7.1). The word usually refers to visible light, which is visible to the human eye and is responsible for the sense of sight. Visible light is usually defined as having wavelengths in the range of 400-700 nanometres (nm), or  $400 \times 10^{-9}$  to  $700 \times 10^{-9}$  m, between the infrared (with longer wavelengths) and the ultraviolet (with shorter wavelengths). This wavelength means a frequency range of roughly 430-750 terahertz (THz).

In this experiment (Figure 7.2), we will study the effect of light intensity on the photosynthetic activity of *Elodea canadensis*. We will vary the light intensity by changing the distance between the light source and the plant. We will count the emerging oxygen bubbles as an indicator of the photosynthetic activity of the plant.

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<sup>2</sup><https://en.wikipedia.org/wiki/Light>

### 7.1.1 Experimental procedures

1. Select a fresh, crisp sprig of Elodea about 15 cm in length.
2. While the plant is still submerged, cut 2-3 mm from its base.
3. Place the sprig upside down in a test tube filled with 0.25% sodium bicarbonate. This is a buffer to absorb toxic materials evolved (i.e., that are released). Keeping the plant submerged, position a light source 1 foot away and adjust so the light shines directly on the plant.
4. Place the test tube in a beaker of water as shown in Fig. @ref(fig:photosynthesis) to prevent overheating the plant. Allow the system to stand 7-10 minutes, or until bubbles begin to appear regularly.
5. Count the bubbles produced each minute for a 5-minute period and average them. Record your findings in the table.
6. Move the light back 2 feet from the plant, wait 5 minutes, and repeat counting. Record your findings in Table 7.1.
7. Move the light back to 3 feet from the plant and repeat counting the bubbles.

**Table 7.1:** Intensity of light.

Distance of light source/Bubbles per minute	1	2	3	4	5	Average
1 foot						
2 feet						
3 feet						

## 7.2 Color of light

In this experiment, we will study the effect of the color of light on the photosynthetic activity of *Elodea canadensis*. We will use filter to expose the plant to light of only a limited range of wavelengths. We will again count the emerging oxygen bubbles as an indicator of the photosynthetic activity of the plant.

### 7.2.1 Experimental procedures

1. Select a fresh, crisp sprig of Elodea about 15 cm in length.
2. While the plant is still submerged, cut 2-3 mm from its base.
3. Place the sprig upside down in a test tube filled with 0.25% sodium bicarbonate. This is a buffer to absorb toxic materials evolved (i.e., that are released). Keeping the plant submerged, position a light source 1 foot away and adjust so the light shines directly on the plant.
4. Place a colored filter between the test tube and the heat shield beaker and allow it to sit for 5 minutes.
5. Count bubbles for 5 minutes as in the previous experiment. Record your findings in Table 7.2. Repeat steps 1 and 2 for each color filter available.

**Table 7.2:** Color of light.

Color of filter/Bubbles per minute	1	2	3	4	5	Average
blue						
orange						
red						



**Figure 7.3:** Appearance of bubbles indicates active photosynthesis.



**Figure 7.4:** Spectrophotometer and cuvettes with dye solutions.

## 7.3 Determination of the light absorption spectrum of dye solutions

In this experiment, we will use a spectrophotometer<sup>4</sup> to measure the differential absorption of light of different wavelength by water stained with food dyes.

### 7.3.1 Experimental procedures

1. Take six cuvettes.
2. Fill one cuvette with water.
3. Fill each of the remaining five cuvettes with one of the color solutions listed in Table 7.3.
4. Insert the cuvette with water into the slot marked “B”.
5. Insert the other cuvettes into the slots marked 1 to 5 and write down which color is in which slot.
6. Following the instructions posted on the spectrophotometer, program the machine to take absorption measurements at wavelengths between 380-740 nm in 20 nm steps.
7. Once the measurements are completed, write down the absorption number for each dye and wavelength.
8. Use a spreadsheet program to graph your results.
9. Compare your curves with the data shown in Figure 7.6.

**Table 7.3:** ( Determination of the light absorption spectrum of dye solutions.

Wavelength (nm)	Purple	Blue	Green	Yellow	Red
380					
400					
420					
...					

<sup>4</sup><https://en.wikipedia.org/wiki/Spectrophotometry>

Wavelength (nm)	Purple	Blue	Green	Yellow	Red
720					
740					

## 7.4 Chromatography

Chromatography<sup>5</sup> is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation. Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture.

In this experiment, we separate a mixture of food dyes (dark greenish liquid. The mobile phase (separation buffer) is 1% NaCl in water, the stationary phase is chromatography paper.

### 7.4.1 Experimental procedures

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

## 7.5 Review Questions

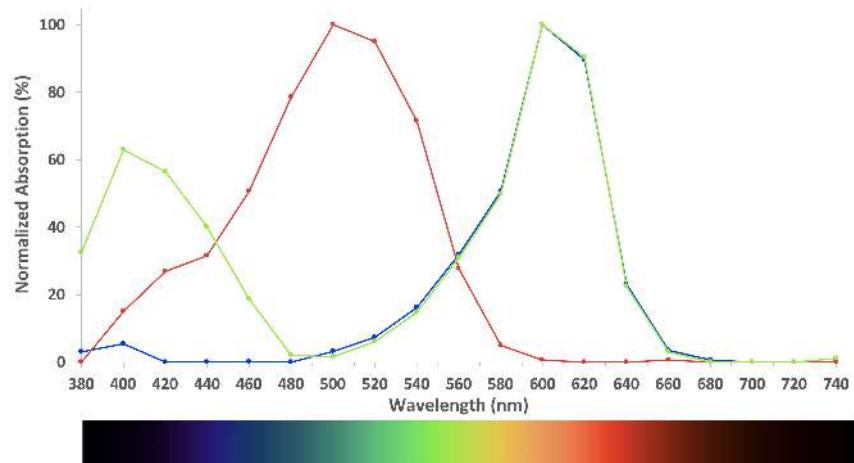
1. What is light?
2. In your own words, describe the endproducts of photosynthesis.
3. In your own words, describe what happens in photosynthesis.
4. What is chlorophyll and what does it do?
5. Where inside of plant cells does photosynthesis happen?
6. What is chromatography and what is it used for?

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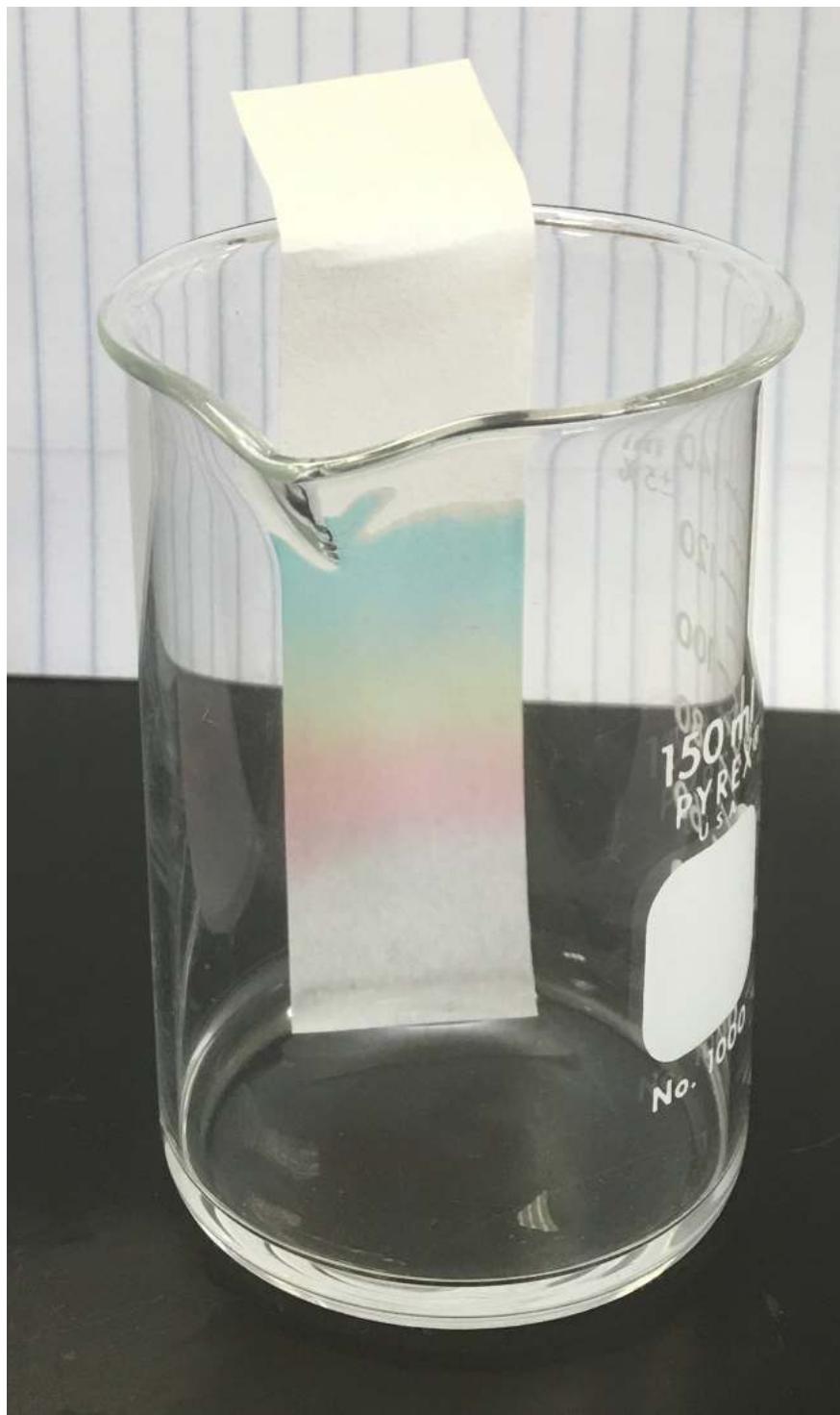
<sup>5</sup><https://en.wikipedia.org/wiki/Chromatography>



**Figure 7.5:** Cuvettes placed in the spectrophotometer.



**Figure 7.6:** Normalized absorption of red, green and blue dye solutions. Compare these data with your own results.



**Figure 7.7:** Result of the Chromatography experiment.



# Chapter 8

## Mitosis and Meiosis

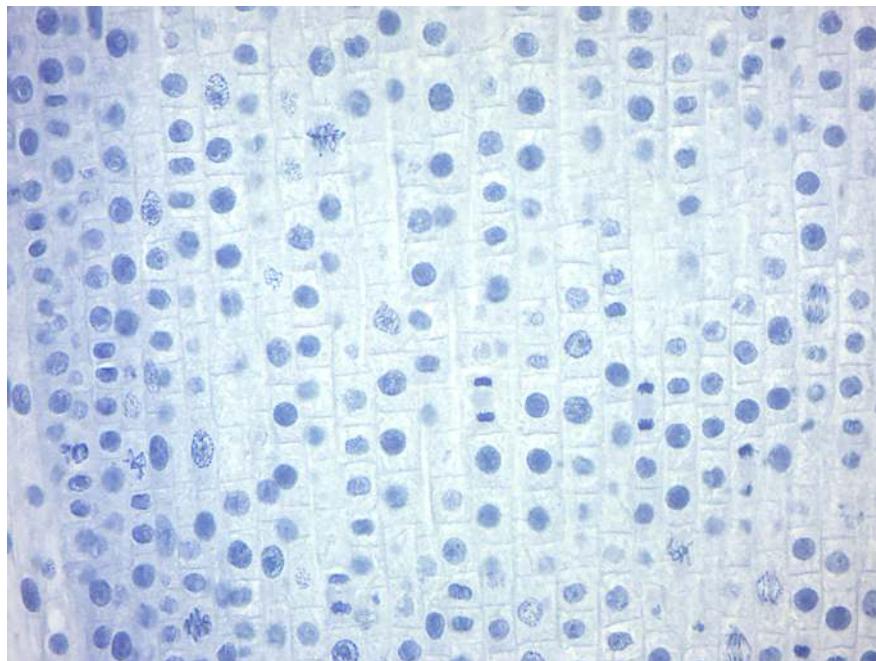
Mitosis<sup>1</sup> is the part of the cell cycle when replicated chromosomes are separated into two new nuclei. In general, mitosis (division of the nucleus) is preceded by the S stage of interphase (during which the DNA is replicated) and is often accompanied or followed by cytokinesis, which divides the cytoplasm, organelles and cell membrane into two new cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the mitotic (M) phase of an animal cell cycle (the division of the mother cell into two daughter cells genetically identical to each other). The process of mitosis is divided into stages corresponding to the completion of one set of activities and the start of the next. These stages are prophase, pro-metaphase, metaphase, anaphase, and telophase.

Meiosis<sup>2</sup> is a specialized type of cell division that reduces the chromosome number by half, creating four haploid cells, each genetically distinct from the parent cell that gave rise to them. This process occurs in all sexually reproducing single-celled and multicellular eukaryotes, including animals, plants, and fungi. Errors in meiosis resulting in aneuploidy are the leading known cause of miscarriage and the most frequent genetic cause of developmental disabilities. In meiosis, DNA replication is followed by two rounds of cell division to produce four daughter cells, each with half the number of chromosomes as the original parent cell. The two meiotic divisions are known as Meiosis I and Meiosis II. Before meiosis begins, during S phase of the cell cycle, the DNA of each chromosome is replicated so that it consists of two identical sister chromatids, which remain held together through sister chromatid cohesion. This S-phase can be referred to as “premeiotic S-phase” or “meiotic S-phase”. Immediately following DNA replication, meiotic cells enter a prolonged G2-like stage known as meiotic prophase. During this time, homologous chromosomes pair with each other and undergo genetic recombination, a programmed process in which DNA is cut and then repaired, which allows them to exchange some of their genetic information. A subset of recombination events results in crossovers, which create physical links known as chiasmata (singular: chiasma, for the Greek letter Chi (X)) between the homologous chromosomes. In most organisms, these links are essential to direct each pair of homologous chromosomes to segregate away from each other during Meiosis I, resulting in two haploid cells that have half the number of chromosomes as the parent cell. During Meiosis II, the cohesion between sister chromatids is released and they segregate from one another, as during mitosis. In some cases, all four of the meiotic products form gametes such as sperm, spores, or pollen. In female animals, three of the four meiotic products are typically eliminated by extrusion into polar bodies, and only one cell develops to produce an ovum. Because the number of chromosomes is halved during meiosis, gametes can fuse (i.e. fertilization) to form a diploid zygote that contains two copies of each chromosome, one from each parent. Thus, alternating cycles of meiosis and fertilization enable sexual reproduction, with successive generations maintaining the same number of chromosomes. For example, diploid human cells contain 23 pairs of chromosomes including 1 pair of sex chromosomes (46 total), half of maternal origin and half of paternal origin. Meiosis produces haploid gametes (ova or sperm) that contain one set of 23 chromosomes. When two gametes (an egg and a sperm) fuse, the resulting zygote is once again diploid, with the mother and father each contributing 23

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<sup>1</sup><https://en.wikipedia.org/wiki/Mitosis>

<sup>2</sup><https://en.wikipedia.org/wiki/Meiosis>



**Figure 8.1:** Onion root tip

chromosomes. This same pattern, but not the same number of chromosomes, occurs in all organisms that utilize meiosis.

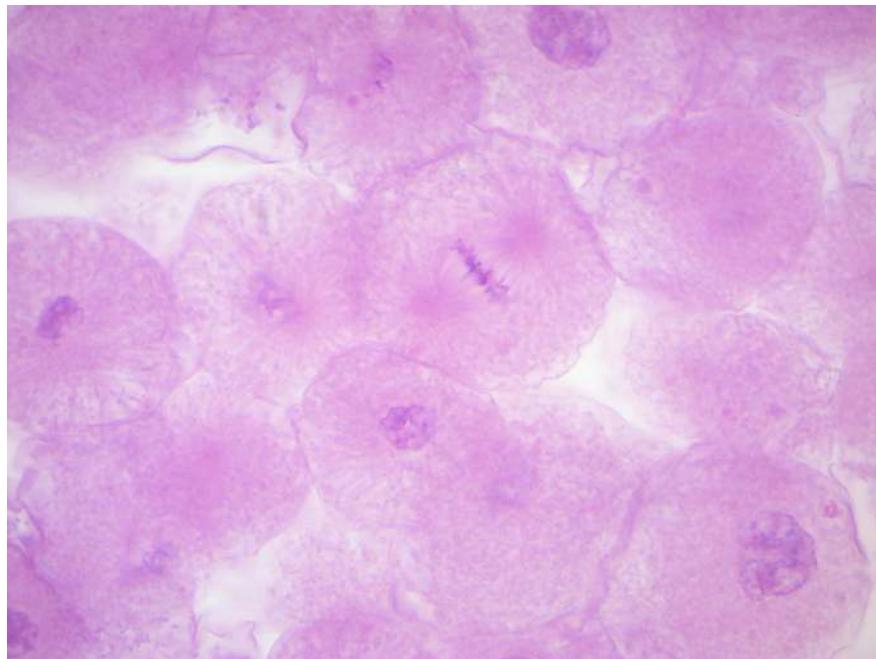
## 8.1 View Prepared Slides

1. View the onion root tip and observe the different stages of mitosis (Figure 8.1).
2. View the fish blastodisc and observe the different stages of mitosis (Figure 8.2).

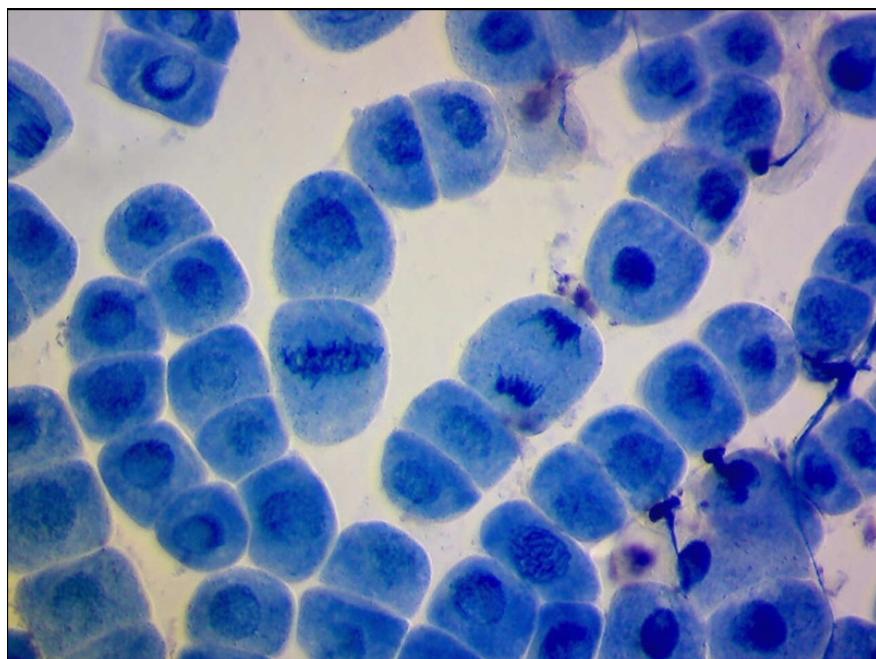
## 8.2 Preparing an Onion root tip squash

### 8.2.1 Experimental procedures

1. Obtain an onion bulb that shows some roots.
2. Cut off a root tip and place it on a clean slide.
3. Cut off 1mm to 2mm of the root tip and throw away the upper portion of the root.
4. Cover the root tip with four drops of 1 N HCl and warm the slide over an alcohol burner flame for 1 minute. Do not boil.
5. Blot off the excess HCl and cover the root tip with 0.5% aqueous toluidine blue.
6. Again, pass the slide through the alcohol burner flame for 1 minute without boiling.
7. Blot off the excess stain, add a drop of fresh stain, and apply a coverslip.
8. Cover the slide with a paper towel and carefully squash the coverslip firmly with your thumb.
9. Examine the slide for the stages of mitosis as well as interphase and cytokinesis.



**Figure 8.2:** Fish blastodisc



**Figure 8.3:** Several different phases of mitosis are visible in this onion root tip spread.

### 8.3 Review Questions

1. What is mitosis and what is its outcome?
2. What is meiosis and what is its outcome?
3. What is homologous recombination and what is its outcome?
4. What do the terms haploid and diploid mean?
5. Are you a haploid or a diploid organism?
6. What are gametes?
7. What is a zygote?

# Chapter 9

## Mendelian Genetics

In this experiment, we will use maize *Zea mays* subsp. *mays*, from Spanish: maíz after Taíno mahiz), also known as corn to study Mendelian inheritance<sup>1</sup>. This cereal grain was first domesticated by indigenous peoples in southern Mexico about 10,000 years ago. The leafy stalk of the plant produces separate pollen and ovuliferous inflorescences or ears, which are fruits, yielding kernels or seeds. Maize has become a staple food in many parts of the world, with total production surpassing that of wheat or rice. However, not all of this maize is consumed directly by humans. Some of the maize production is used for corn ethanol, animal feed and other maize products, such as corn starch and corn syrup. The six major types of corn are dent corn, flint corn, pod corn, popcorn, flour corn, and sweet corn.

The principles of Mendelian inheritance were named for and first derived by Gregor Johann Mendel<sup>2</sup>, a nineteenth-century Moravian monk who formulated his ideas after conducting simple hybridisation experiments with pea plants (*Pisum sativum*) he had planted in the garden of his monastery. Between 1856 and 1863, Mendel cultivated and tested some 5,000 pea plants. From these experiments, he induced two generalizations which later became known as Mendel's Principles of Heredity or Mendelian inheritance (Table 9.1). He described these principles in a two-part paper, *Versuche über Pflanzen-Hybriden* (Experiments on Plant Hybridization), that he read to the Natural History Society of Brno on 8 February and 8 March 1865, and which was published in 1866. Mendel's conclusions were largely ignored by the vast majority of scientists at the time. In 1900, however, his work was "re-discovered" by three European scientists, Hugo de Vries, Carl Correns, and Erich von Tschermak.

Mendel discovered that, when he crossed purebred white flower and purple flower pea plants (the parental or P generation), the result was not a blend. Rather than being a mix of the two, the offspring (known as the F1 generation) was purple-flowered. When Mendel self-fertilized the F1 generation pea plants, he obtained a purple flower to white flower ratio in the F2 generation of 3 to 1. In the first experiment, we will examine the F2 generation resulting from the F1 generation obtained from a parental generation of yellow and purple corn.

He then conceived the idea of heredity units, which he called "factors". Mendel found that there are alternative forms of factors—now called genes—that account for variations in inherited characteristics. For example, the gene for flower color in pea plants exists in two forms, one for purple and the other for white. The alternative "forms" are now called alleles. For each biological trait, an organism inherits two alleles, one from each parent. These alleles may be the same or different. An organism that has two identical alleles for a gene is said to be homozygous for that gene (and is called a homozygote). An organism that has two different alleles for a gene is said be heterozygous for that gene (and is called a heterozygote).

Mendel hypothesized that allele pairs separate randomly, or segregate, from each other during the production of gametes: egg and sperm. Because allele pairs separate during gamete production, a sperm or egg

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<sup>1</sup>[https://en.wikipedia.org/wiki/Mendelian\\_inheritance](https://en.wikipedia.org/wiki/Mendelian_inheritance)

<sup>2</sup>[https://en.wikipedia.org/wiki/Gregor\\_Mendel](https://en.wikipedia.org/wiki/Gregor_Mendel)

carries only one allele for each inherited trait. When sperm and egg unite at fertilization, each contributes its allele, restoring the paired condition in the offspring. This is called the Law of Segregation. Mendel also found that each pair of alleles segregates independently of the other pairs of alleles during gamete formation. This is known as the Law of Independent Assortment. In the second experiment, we will observe this law exemplified by a dihybrid cross of corn.

The genotype of an individual is made up of the many alleles it possesses. An individual's physical appearance, or phenotype, is determined by its alleles as well as by its environment. The presence of an allele does not mean that the trait will be expressed in the individual that possesses it. If the two alleles of an inherited pair differ (the heterozygous condition), then one determines the organism's appearance and is called the dominant allele; the other has no noticeable effect on the organism's appearance and is called the recessive allele. Thus, in the example above the dominant purple flower allele will hide the phenotypic effects of the recessive white flower allele. This is known as the Law of Dominance but it is not a transmission law: it concerns the expression of the genotype. The upper case letters are used to represent dominant alleles whereas the lowercase letters are used to represent recessive alleles.

**Table 9.1:** Mendel's laws of inheritance.

Law	Definition
Law of segregation	During gamete formation, the alleles for each gene segregate from each other so that each gamete carries only one allele for each gene.
Law of independent assortment	Genes for different traits can segregate independently during the formation of gametes.
Law of dominance	Some alleles are dominant while others are recessive; an organism with at least one dominant allele will display the effect of the dominant allele.

## 9.1 Punnett square

The Punnett square<sup>3</sup> (Figures 9.1 and 9.2) is a visual representation of Mendelian inheritance and used to predict an outcome of a particular cross or breeding experiment. It is named after Reginald C. Punnett<sup>4</sup>, who devised the approach. In our first experiment, both parents are homozygous, one carrying two copies of the dominant allele (R), the other two copies of the recessive (r) allele. Each parent can only make gametes that have either the R (purple) or r (yellow) allele. The Punnett square for the parental cross is shown in Figure 9.1

The squares containing the single letters represent the possible gametes. The squares with two letters represent the zygotes resulting from the combination of the respective gametes. It can be easily seen that all offspring will be heterozygous (Rr) and therefore purple. The Punnett square for the F1 cross is depicted in Figure 9.2

## 9.2 Monohybrid cross (Experiment 1)

A monohybrid cross<sup>5</sup> is a mating between two individuals with different variations at one genetic trait of interest. The character(s) being studied in a monohybrid cross are governed by two or multiple variations for a single locus. A cross between two parents possessing a pair of contrasting characters is known as monohybrid cross. To carry out such a cross, each parent is chosen to be homozygous or true breeding for

<sup>3</sup>[https://en.wikipedia.org/wiki/Punnett\\_square](https://en.wikipedia.org/wiki/Punnett_square)

<sup>4</sup>[https://en.wikipedia.org/wiki/Reginald\\_Punnett](https://en.wikipedia.org/wiki/Reginald_Punnett)

<sup>5</sup>[https://en.wikipedia.org/wiki/Monohybrid\\_cross](https://en.wikipedia.org/wiki/Monohybrid_cross)

	Yellow	
Purple	r	r
R	Rr	Rr
R	Rr	Rr

Figure 9.1: Punnett square for homozygous cross.

	Maternal gametes	
Paternal gametes	R	r
R	RR	Rr
r	Rr	rr

Figure 9.2: Punnett square for heterozygous cross.



**Figure 9.3:** Monohybrid cross

a given trait (locus). When a cross satisfies the conditions for a monohybrid cross, it is usually detected by a characteristic distribution of second-generation (F2) offspring that is sometimes called the monohybrid ratio.

Generally, the monohybrid cross is used to determine the dominance relationship between two alleles. The cross begins with the parental (P) generation. One parent is homozygous for one allele, and the other parent is homozygous for the other allele. The offspring make up the first filial (F1) generation. Every member of the F1 generation is heterozygous and the phenotype of the F1 generation expresses the dominant trait. Crossing two members of the F1 generation produces the second filial (F2) generation. Probability theory predicts that three quarters of the F2 generation will have the dominant allele's phenotype. And the remaining quarter of the F2s will have the recessive allele's phenotype. This predicted 3:1 phenotypic ratio assumes Mendelian inheritance.

In the first experiment, we will study the result obtained from a monohybrid cross. A strain of corn producing pure purple kernels (RR) is crossed with a strain producing pure yellow kernels (rr). Purple is dominant with the resulting F1 ears all bearing purple kernels. These plants that are heterozygous for a single trait are called monohybrids. When the F1 is self-pollinated, the resulting F2 ears bear both purple and yellow kernels (Figure 9.3).

### 9.2.1 Experimental procedures

1. Count the number of purple and yellow kernels on one row of the F2 ear without removing the kernels.
2. Determine the ratio of purple to yellow.
3. Now tabulate the numbers obtained by each of your class mates in Table 9.2 and add these figures to get a total.
4. Using the total numbers, determine a ratio of purple to yellow.

**Table 9.2:** Monohybrid cross.

Row #	purple	yellow	ratio
1			
2			
3			
...			
7			
8			
9			
Total			

**Figure 9.4:** Dihybrid cross

### 9.3 Dihybrid cross (Experiment 2)

In the second experiment, we will study the result obtained from a dihybrid cross<sup>6</sup>. A dihybrid cross is a cross between two different lines (varieties, strains) that differ in two observed traits. In the name “Dihybrid cross”, the “di” indicates that there are two traits involved (in our example designated R and Su), the “hybrid” means that each trait has two different alleles (in our example R and r, or Su and su), and “cross” means that there are two individuals who are combining or “crossing” their genetic information. In our example, a pure strain of corn producing purple-starchy kernels (RR SuSu) is crossed with a pure strain producing yellow-sweet (rr susu). The starchy seeds are smooth, the sweet seeds are wrinkled. The resulting F1 ears all bear purple-starchy (smooth) kernels. Plants that are heterozygous for two traits are called dihybrids. When the F1 is self-pollinated, the resulting F2 generation contains various combinations (Figure 9.4).

The rules of meiosis, as they apply to the dihybrid, are codified in Mendel’s first law and Mendel’s second law, which are also called the Law of Segregation and the Law of Independent Assortment, respectively (Table 9.1). For genes on separate chromosomes, each allele pair showed independent segregation. If the first filial generation (F1 generation) produces four identical offspring, the second filial generation, which occurs by crossing the members of the first filial generation, shows a phenotypic (appearance) ratio of **9:3:3:1**, where:

- the **9** represents the proportion of individuals displaying both dominant traits
- the first **3** represents the individuals displaying the first dominant trait and the second recessive trait
- the second **3** represents those displaying the first recessive trait and second dominant trait
- the **1** represents the homozygous, displaying both recessive traits.

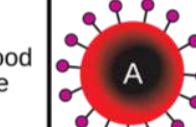
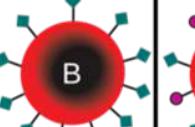
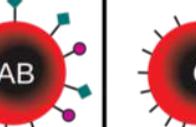
#### 9.3.1 Experimental procedures

1. Carefully count the number of kernels of each phenotype appearing on a row of F2 ear. Tabulate the results and determine the totals and total ratios in Table 9.3.

**Table 9.3:** Dihybrid cross.

Row #	purple and starchy (smooth)	purple and sweet (wrinkled)	yellow and starchy (smooth)	yellow and sweet (wrinkled)	ratio
1					
2					
3					
...					
7					
8					

<sup>6</sup>[https://en.wikipedia.org/wiki/Dihybrid\\_cross](https://en.wikipedia.org/wiki/Dihybrid_cross)

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma			None	
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

**Figure 9.5:** Blood type (or blood group) is determined, in part, by the ABO blood group antigens present on red blood cells.

Row #	purple and starchy (smooth)	purple and sweet (wrinkled)	yellow and starchy (smooth)	yellow and sweet (wrinkled)	ratio
9					
Total					

## 9.4 Blood Typing (Experiment 3)

In this experiment, we will determine the ABO properties of four (artificial) blood samples.

A blood type<sup>7</sup> (also called a blood group) (Figure 9.5<sup>8</sup>) is a classification of blood based on the presence and absence of antibodies and also based on the presence or absence of inherited antigenic substances on the surface of red blood cells (RBCs). These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Blood types are inherited and represent contributions from both parents. A total of 35 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT). The two most important ones are ABO and the RhD (“Rhesus”) antigen; they determine someone’s blood type (A, B, AB and O, with +, - or Null denoting RhD status).

<sup>7</sup>[https://en.wikipedia.org/wiki/Blood\\_type](https://en.wikipedia.org/wiki/Blood_type)

<sup>8</sup>[https://commons.wikimedia.org/wiki/File:ABO\\_blood\\_type.svg](https://commons.wikimedia.org/wiki/File:ABO_blood_type.svg)

### 9.4.1 Experimental procedures

1. Using the dropper vial, place a drop of the first synthetic blood sample in each well of the blood-typing slide (Figure 9.6). Replace the cap on the dropper vial. Always replace the cap on one vial before opening the next vial, to prevent cross-contamination.
2. Add a drop of synthetic anti-A (blue) to the well labeled A. Replace the cap.
3. Add a drop of synthetic anti-B serum (yellow) to the well labeled B. Replace the cap.
4. Add a drop of synthetic anti-D (Rh) serum (clear) to the well labeled Rh. Replace the cap.
5. Using a mixing stick of a different color for each well (blue for anti-A, yellow for anti-B, white for anti-Rh), gently stir the synthetic blood and anti-serum drops for 30 seconds. Remember to discard each mixing stick after a single use to avoid contamination of your samples.
6. Carefully examine the thin films of liquid mixture left behind:
  - If a film remains uniform in appearance, there is no agglutination.
  - If the sample appears granular, agglutination has occurred.
7. Answer yes or no as to whether agglutination occurred in each sample. A positive agglutination reaction indicates the blood type.
8. Record the results for the first blood sample in Table 9.4.
9. Determine the blood type of the sample using the results that you entered in Table 9.4.
10. Thoroughly rinse the blood-typing slide and repeat steps 1 through 7 for synthetic blood samples 2, 3, and 4, recording the results of each test as you go (and rinsing the slide after each sample).

**Table 9.4:** Blood Typing.

	Sample 1	Sample 2	Sample 3	Sample 4
Anti-A				
Anti-B				
Anti-D (Rh) + or -				
Blood Type				



**Figure 9.6:** Blood typing result

## 9.5 Review Questions

1. What is a gene?
2. What is an allele?
3. What are dominant and recessive alleles?
4. What is the genotype of an organism?
5. What is a trait?
6. What is the phenotype of an organism?
7. What is the genotype of the F<sub>1</sub> generation of the monohybrid cross?
8. What is the phenotype of the F<sub>1</sub> generation monohybrid cross?
9. What are the possible maternal and paternal genotypes of the F<sub>1</sub> gametes monohybrid cross?
10. What is the genotype of the parents of the dihybrid cross?
11. What are the phenotypes of the parents of the dihybrid cross?
12. What are the possible genotypes of the parent gametes of the dihybrid cross?
13. What is the genotype of the F<sub>1</sub> generation of the dihybrid cross?
14. What is the phenotype of the F<sub>1</sub> generation dihybrid cross?
15. What are the possible maternal and paternal genotypes of the F<sub>1</sub> gametes dihybrid cross?



# Chapter 10

## Molecular Biology

Molecular biology<sup>1</sup> concerns the molecular basis of biological activity between biomolecules in the various systems of a cell, including the interactions between DNA, RNA, and proteins and their biosynthesis, as well as the regulation of these interactions.

One of the most basic techniques of molecular biology to study protein function is molecular cloning. In this technique, DNA coding for a protein of interest is cloned using polymerase chain reaction (PCR), and/or restriction enzymes into a plasmid (expression vector). A vector has 3 distinctive features: an origin of replication, a multiple cloning site (MCS), and a selective marker usually antibiotic resistance. Located upstream of the multiple cloning site are the promoter regions and the transcription start site which regulate the expression of cloned gene. This plasmid can be inserted into either bacterial or animal cells. Introducing DNA into bacterial cells can be done by transformation via uptake of naked DNA, conjugation via cell-cell contact or by transduction via viral vector. Introducing DNA into eukaryotic cells, such as animal cells, by physical or chemical means is called transfection. Several different transfection techniques are available, such as calcium phosphate transfection, electroporation, microinjection and liposome transfection. The plasmid may be integrated into the genome, resulting in a stable transfection, or may remain independent of the genome, called transient transfection.

DNA coding for a protein of interest is now inside a cell, and the protein can now be expressed. A variety of systems, such as inducible promoters and specific cell-signaling factors, are available to help express the protein of interest at high levels. Large quantities of a protein can then be extracted from the bacterial or eukaryotic cell. The protein can be tested for enzymatic activity under a variety of situations, the protein may be crystallized so its tertiary structure can be studied, or, in the pharmaceutical industry, the activity of new drugs against the protein can be studied.

### 10.1 DNA restriction digest

Lambda DNA<sup>2</sup> comes from a virus (bacteriophage<sup>3</sup>) that infects bacteria. This virus does not infect humans and is therefore safe source to work with. Lambda DNA is approximately 48,000 base pairs long.

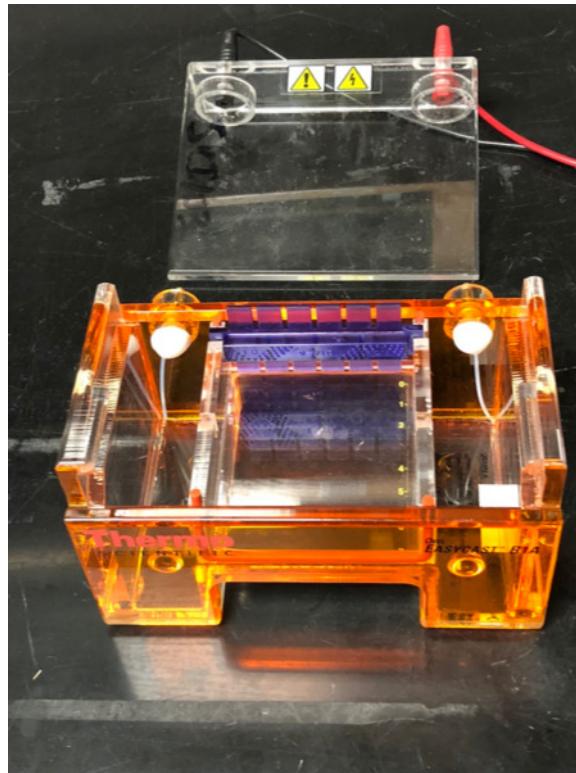
When restriction enzymes are used to cut DNA fragments of varying sizes are produced. Cut DNA can be separated using a process known as agarose gel electrophoresis<sup>4</sup>. Agarose gel electrophoresis separates DNA fragments by molecular weight. DNA fragments are loaded into an agarose gel slab, which is placed into a chamber filled with a conductive buffer solution. A direct current is passed between positive (red)

<sup>1</sup>[https://en.wikipedia.org/wiki/Molecular\\_biology](https://en.wikipedia.org/wiki/Molecular_biology)

<sup>2</sup>[https://en.wikipedia.org/wiki/Lambda\\_phage](https://en.wikipedia.org/wiki/Lambda_phage)

<sup>3</sup><https://en.wikipedia.org/wiki/Bacteriophage>

<sup>4</sup>[https://en.wikipedia.org/wiki/Agarose\\_gel\\_electrophoresis](https://en.wikipedia.org/wiki/Agarose_gel_electrophoresis)



**Figure 10.1:** Agarose gel box with comb in place, ready for gel to be poured.

and negative (black) wire electrodes at each end of the chamber. DNA fragments are negatively charged, and when placed in an electric field will be drawn toward the positive pole (red). The matrix of the agarose gel acts as a molecular sieve through which smaller DNA fragments can move more easily than larger ones. Therefore, the distance and rate at which DNA fragments migrate through the gel is inversely proportional to its molecular weight. Over a period of time smaller fragments will travel farther than larger ones. Fragments of the same size stay together and migrate in discrete “bands”.

## 10.2 Preparing a gel for agarose gel electrophoresis

We will use gel electrophoresis to separate the DNA fragments obtained from the restriction digest (Figure 10.1). Before setting up the digest, we will pour agarose gel because it will take about half an hour for the gel to harden.

### 10.2.1 Experimental procedures

2. Get the Erlenmeyer flask containing 0.5 g of agarose powder
3. Add 50 ml of 1× TAE (Tris base, acetic acid, EDTA) running buffer.
4. Add 5  $\mu$ l of SybrGreen<sup>TM</sup> dye (10,000× stock solution).
5. Heat in the microwave at full power for 1 minute.
6. Swirl to make sure that all powder has dissolved, and the solution is clear.
7. Add the comb into the comb slot.
8. Pour the solution onto the gel tray in the gel box.

## 10.3 Setting up the restriction digest reactions

In this experiment, we will use three restriction enzymes<sup>5</sup> (EcoRI, Hind III, and Pst I) to cut Lambda DNA.

### 10.3.1 Experimental procedures

1. Digest DNA: microtubes that contain the enzyme stock solution, Lambda DNA, and restriction buffer are provided on ice (in an ice bucket).
2. Get 4 new microtubes and label each as follows:
  - Tube 1: L = Lambda DNA
  - Tube 2: P = Pst I digest
  - Tube 3: E = EcoRI digest
  - Tube4: H = Hind III digest
3. Use a new pipette tip for each transfer and pipet the reagents (from the stock solutions kept on ice) into each tube according to Table 10.1.
4. Mix the components by gently flicking the tube with your finger. Pulse spin the tubes in the microcentrifuge to collect all the liquid to the bottom of the tube.
5. Place the tubes in the heat block and incubate for 30 minutes at 37 °C.

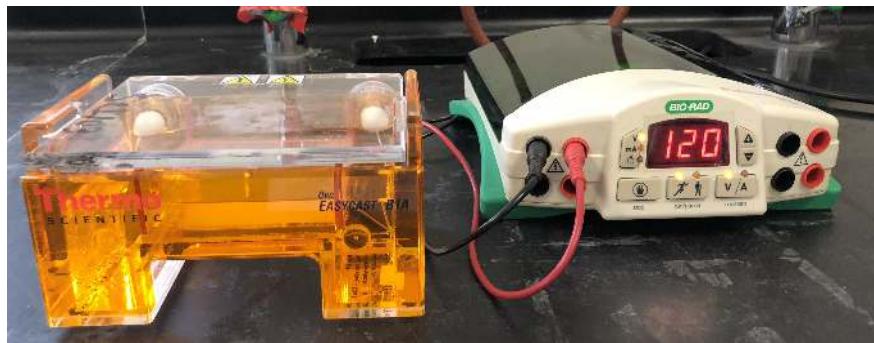
**Table 10.1:** DNA digestion.

Tube	DNA	buffer	Pst I	EcoR I	Hind III
L	4 µl	6 µl	—	—	—
P	4 µl	5 µl	1 µl	—	—
E	4 µl	5 µl	—	1 µl	—
H	4 µl	5 µl	—	—	1 µl

## 10.4 Loading the DNA samples on the agarose gel and agarose gel electrophoresis

1. Remove the digested DNA samples from the heat block.
2. Pulse spin the tubes in the centrifuge to bring all of the liquid to the bottom of the tube.
3. Add 2 µl of sample loading dye into each tube. Mix the contents by flicking the tube with your finger.
4. Fill the electrophoresis chamber and cover the gel with 1× TAE running buffer (this will require about 275 ml of buffer).
5. Check that the wells of the agarose gels are near the black (-) electrode and the base of the gel is near the red (+) electrode.
6. Load 12 µl of each sample into separate wells in the gel chamber in the following order:
  - Lane 1: L
  - Lane 2: P
  - Lane 3: E
  - Lane 4: H
  - Lane 5: DNA size marker
7. Place the lid on the electrophoresis chamber. Connect the electrical leads into the power supply, red to red and black to black.
8. Turn on the power and run the gel at 120 V for 35 minutes (Figure 10.2).

<sup>5</sup>[https://en.wikipedia.org/wiki/Restriction\\_enzyme](https://en.wikipedia.org/wiki/Restriction_enzyme)



**Figure 10.2:** Gel electrophoresis box and power supply.



**Figure 10.3:** Gel documentation system with UV light source.

## 10.5 Visualizing the DNA fragments from the restriction digest

We added a non-toxic green fluorescent dye to the agarose before we poured the gel. The inclusion of this dye will allow us to visualize the separated DNA fragments by exposing the gel to UV light source in the UV light box (Figure 10.3).

### 10.5.1 Experimental procedures

1. Visualize cut DNA using the UV light box (Figure 10.3).
2. Print out a picture of the gel.

## 10.6 Review Questions

1. What is DNA made of?

2. What is a restriction enzyme?
3. What is a DNA ligase?
4. What is a DNA polymerase?
5. What is molecular cloning?
6. What is gel electrophoresis?
7. In an electric field, DNA moves from the \_\_\_\_\_ to the \_\_\_\_\_ pole.
8. Smaller fragments of DNA move \_\_\_\_\_ than larger fragments.



# Chapter 11

## Archaea and Bacteria

In biological taxonomy<sup>1</sup>, a domain is the highest taxonomic rank of organisms in the three-domain system<sup>2</sup> of taxonomy designed by Carl Woese, an American microbiologist and biophysicist. According to the Woese system, introduced in 1990, the tree of life consists of three domains: Archaea, Bacteria, and Eukarya. The first two are all prokaryotic microorganisms, or single-celled organisms whose cells have no nucleus. All life that has a nucleus and membrane-bound organelles, and multicellular organisms, is included in the Eukarya.

### 11.1 Bacteria

Bacteria<sup>3</sup> (singular: bacterium) are prokaryotic microorganisms. Typically, a few micrometers in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust. Bacteria also live in symbiotic and parasitic relationships with plants and animals. Most bacteria have not been characterized, and only about half of the bacterial phyla have species that can be grown in the laboratory. The study of bacteria is known as bacteriology, a branch of microbiology. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water. There are approximately  $5 \times 10^{30}$  bacteria on Earth forming a biomass which exceeds that of all plants and animals. Bacteria are vital in many stages of the nutrient cycle by recycling nutrients such as the fixation of nitrogen from the atmosphere. The nutrient cycle includes the decomposition of dead bodies and bacteria are responsible for the putrefaction stage in this process. In the biological communities surrounding hydrothermal vents and cold seeps, extremophile bacteria provide the nutrients needed to sustain life by converting dissolved compounds, such as hydrogen sulfide and methane, to energy. In March 2013, data reported by researchers in October 2012, was published. It was suggested that bacteria thrive in the Mariana Trench, which with a depth of up to 11 kilometers is the deepest known part of the oceans. Other researchers reported related studies that microbes thrive inside rocks up to 580 meters below the sea floor under 2.6 kilometers of ocean off the coast of the northwestern United States. The largest number of bacteria in humans exist in the gut flora, and a large number on the skin. The vast majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, though many are beneficial particularly in the gut flora. However, several species of bacteria are pathogenic and cause infectious diseases. The most common fatal bacterial diseases are respiratory infections, with tuberculosis alone killing about 2 million people per year, mostly in sub-Saharan Africa. In developed countries, antibiotics are used to treat bacterial infections and are also used in farming, making antibiotic resistance a growing problem. In industry, bacteria are important in sewage treatment and the breakdown of oil spills, the production of cheese and yogurt through fermentation, and the recovery of gold, palladium,

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<sup>1</sup>[https://en.wikipedia.org/wiki/Taxonomy\\_\(biology\)](https://en.wikipedia.org/wiki/Taxonomy_(biology))

<sup>2</sup>[https://en.wikipedia.org/wiki/Three-domain\\_system](https://en.wikipedia.org/wiki/Three-domain_system)

<sup>3</sup><https://en.wikipedia.org/wiki/Bacteria>

copper and other metals in the mining sector, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.

## 11.2 Archaea

Archaea<sup>4</sup> have unique properties separating them from the other two domains of life, Bacteria and Eukaryota. The Archaea are further divided into multiple recognized phyla. Classification is difficult because the majority have not been isolated in the laboratory and have only been detected by analysis of their nucleic acids in samples from their environment. Archaea and bacteria are generally similar in size and shape, although a few archaea have very strange shapes. Despite this morphological similarity to bacteria, archaea possess genes and several metabolic pathways that are more closely related to those of eukaryotes, notably the enzymes involved in transcription and translation. Other aspects of archaeal biochemistry are unique, such as their reliance on ether lipids in their cell membranes. Archaea use more energy sources than eukaryotes: these range from organic compounds, such as sugars, to ammonia, metal ions or even hydrogen gas. Salt-tolerant archaea use sunlight as an energy source, and other species of archaea fix carbon; however, unlike plants and cyanobacteria, no known species of archaea does both. Archaea reproduce asexually by binary fission, fragmentation, or budding; unlike bacteria and eukaryotes, no known species forms spores. Archaea were initially viewed as extremophiles living in harsh environments, such as hot springs and salt lakes, but they have since been found in a broad range of habitats, including soils, oceans, and marshlands. They are also part of the human microbiota, found in the colon, oral cavity, and skin. Archaea are particularly numerous in the oceans, and the archaea in plankton may be one of the most abundant groups of organisms on the planet. Archaea are a major part of Earth's life and may play roles in both the carbon cycle and the nitrogen cycle. No clear examples of archaeal pathogens or parasites are known, but they are often mutualists or commensals. One example is the methanogens that inhabit human and ruminant guts, where their vast numbers aid digestion. Methanogens are also used in biogas production and sewage treatment, and biotechnology exploits enzymes from extremophile archaea that can endure high temperatures and organic solvents.

## 11.3 Eukarya

Members of the domain Eukarya are called eukaryotes<sup>5</sup>. A eukaryote is any organism whose cells have a cell nucleus and other organelles enclosed within membranes) can be unicellular or multicellular organisms. The defining feature that sets eukaryotic cells apart from prokaryotic cells (Bacteria and Archaea) is that they have membrane-bound organelles, especially the nucleus, which contains the genetic material enclosed by the nuclear membrane. The presence of a nucleus gives eukaryotes their name, which comes from the Greek eu, “well” or “true” and karyon, “nut” or “kernel”. Eukaryotic cells also contain other membrane-bound organelles such as mitochondria, endoplasmic reticulum and the Golgi apparatus. In addition, plants and algae contain chloroplasts. Unlike unicellular archaea and bacteria, eukaryotes may also be multicellular and include organisms consisting of many kinds of tissue and cell types. Eukaryotes can reproduce both asexually through mitosis and sexually through meiosis and gamete fusion. In mitosis, one cell divides to produce two genetically identical cells. In meiosis, DNA replication is followed by two rounds of cell division to produce four haploid daughter cells. These act as sex cells (gametes). Each gamete has just one set of chromosomes, each a unique mix of the corresponding pair of parental chromosomes resulting from genetic recombination during meiosis. Eukaryotes evolved approximately 1.6-2.1 billion years ago, during the Proterozoic eon.

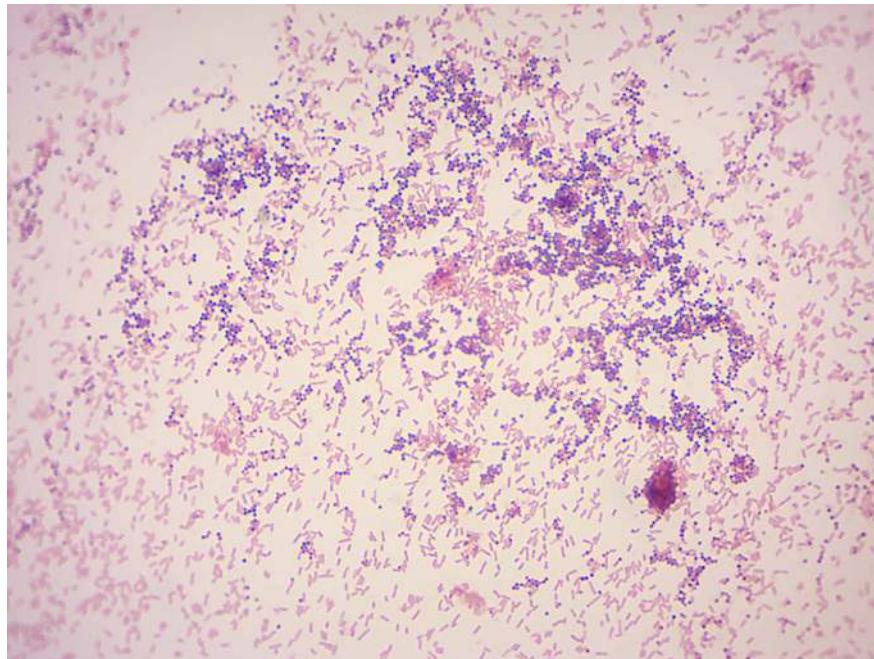
Viruses<sup>6</sup> are not part of the three-domain system.

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<sup>4</sup><https://en.wikipedia.org/wiki/Archaea>

<sup>5</sup><https://en.wikipedia.org/wiki/Eukaryote>

<sup>6</sup><https://en.wikipedia.org/wiki/Virus>



**Figure 11.1:** Gram stained bacteria.

## 11.4 Gram stain

Gram stain<sup>7</sup> or Gram staining (Figure 11.1), also called Gram's method, is a method of staining used to distinguish and classify bacterial species into two large groups (gram-positive and gram-negative). The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in the cell wall of Gram-positive bacteria. Gram-negative cells also contain peptidoglycan, but a very small layer of it that is dissolved when the alcohol is added. This is why the cell loses its initial color from the primary stain. Gram-positive bacteria retain the crystal violet dye, and thus are stained violet, while the Gram-negative bacteria do not; after washing, a counterstain is added (safranin) that will stain these Gram-negative bacteria a pink color. Both Gram-positive bacteria and Gram-negative bacteria pick up the counterstain. The counterstain, however, is unseen on Gram-positive bacteria because of the darker crystal violet stain.

### 11.4.1 Experimental procedures

1. Use a bacterial loop to pick up bacteria (*Staphylococcus aureus* and *Escherichia coli*) from the culture plate and streak out on a slide so that both samples partially overlap in the middle of the slide. Heat fix the sample to the slide by carefully passing the slide three times through a Bunsen burner flame.
2. Add crystal violet to the slide and incubate for 1 minute. Rinse slide with a gentle stream of water to remove unbound crystal violet.
3. Add Gram's iodine for 1 minute- this is the mordant, an agent that fixes the crystal violet to the bacterial cell wall.
4. Rinse slide with decolorizer until no more dye is running off. Rinse with a gentle stream of water.
5. Add safranin to the slide and incubate for 1 minute. Wash with a gentle stream of water. Gram positive bacteria it will retain crystal violet and stain purple. Gram negative will lose the primary stain and take the secondary stain causing it to appear pink when viewed under a microscope.

<sup>7</sup>[https://en.wikipedia.org/wiki/Gram\\_stain](https://en.wikipedia.org/wiki/Gram_stain)



**Figure 11.2:** *Oscillatoria*.

6. View under the microscope.

## 11.5 View living organisms

### 11.5.1 Oscillatoria

*Oscillatoria*<sup>8</sup> is a genus of filamentous cyanobacterium which is named after the oscillation in its movement (Figure 11.2). Filaments in the colonies can slide back and forth against each other until the whole mass is reoriented to its light source. It is commonly found in watering-troughs waters, and is mainly blue-green or brown-green. *Oscillatoria* is an organism that reproduces by fragmentation. *Oscillatoria* forms long filaments of cells which can break into fragments called hormogonia. The hormogonia can grow into a new, longer filament. Breaks in the filament usually occur where dead cells (necridia) are present. *Oscillatoria* uses photosynthesis to survive and reproduce. Each filament of *oscillatoria* consists of trichome which is made up of rows of cells. The tip of the trichome oscillates like a pendulum.

### 11.5.2 Nostoc

*Nostoc*<sup>9</sup> is a genus of cyanobacteria found in various environments that forms colonies composed of filaments of moniliform cells in a gelatinous sheath (Figure 11.10). *Nostoc* can be found in soil, on moist rocks, at the bottom of lakes and springs (both fresh- and saltwater), and rarely in marine habitats. It may also grow symbiotically within the tissues of plants, such as the evolutionarily ancient angiosperm Gunnera and the hornworts (a group of bryophytes), providing nitrogen to its host through the action of terminally differentiated cells known as heterocysts. These bacteria contain photosynthetic pigments in their cytoplasm to perform photosynthesis.

<sup>8</sup><https://en.wikipedia.org/wiki/Oscillatoria>

<sup>9</sup><https://en.wikipedia.org/wiki/Nostoc>



**Figure 11.3:** Nostoc.

### 11.5.3 Gloeocapsa

*Gloeocapsa*<sup>10</sup> (from the Greek gloia (gelatinous) and the Latin caps(a) (case) is a genus of cyanobacteria (Figure 11.4). The cells secrete individual gelatinous sheaths which can often be seen as sheaths around recently divided cells within outer sheaths. Recently divided cell pairs often appear to be only one cell since the new cells cohere temporarily. They are also known as glow caps, a term derived from the yellowish hue given off by the cap.

## 11.6 View Prepared Slides

### 11.6.1 Mixed coccus (Gram stain) (Figure 11.5)

### 11.6.2 Mixed bacillus (Gram stain) (Figure 11.6)

### 11.6.3 Spirillum

*Spirillum*<sup>11</sup> is a genus of Gram-negative bacteria (Figure 11.7). Members of the genus Spirillum are large, elongate, spiral shaped, rigid cells. Some have tufts of amphitrichous flagella at both poles. They are microaerophilic and usually found in stagnant freshwater rich in organic matter.

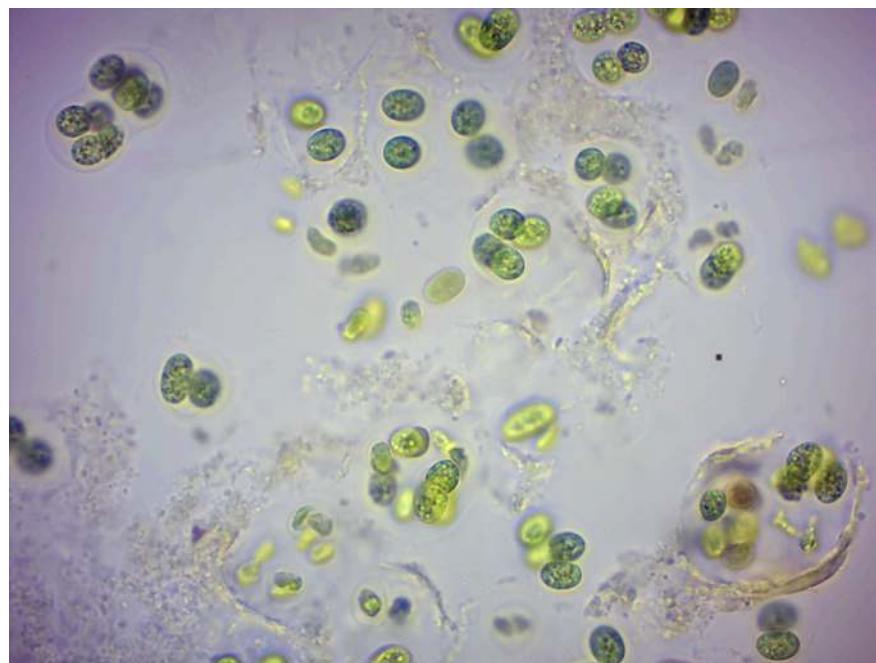
### 11.6.4 Treponema

*Treponema*<sup>12</sup> is a genus of spiral-shaped Gram-negative bacteria (Figure 11.8). The major treponeme species of human pathogens is *Treponema pallidum*, whose subspecies are responsible for diseases such as syphilis,

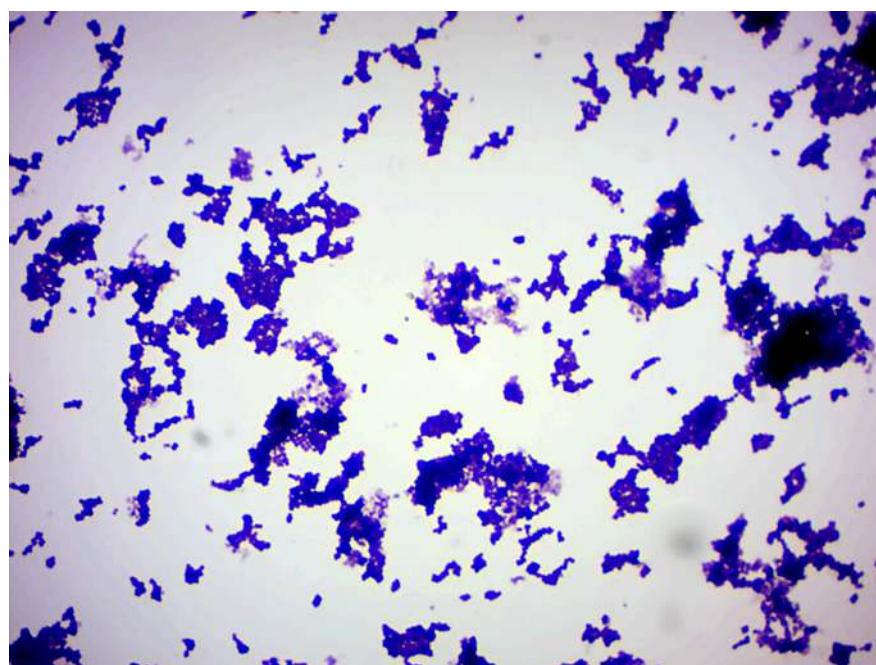
<sup>10</sup><https://en.wikipedia.org/wiki/Gloeocapsa>

<sup>11</sup><https://en.wikipedia.org/wiki/Spirillum>

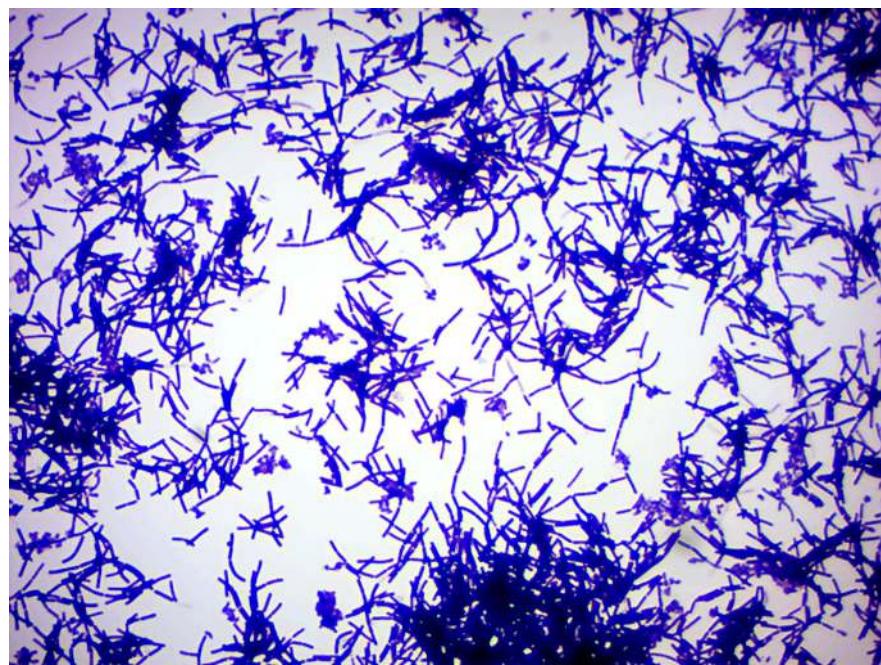
<sup>12</sup><https://en.wikipedia.org/wiki/Treponema>



**Figure 11.4:** Gloeocapsa.



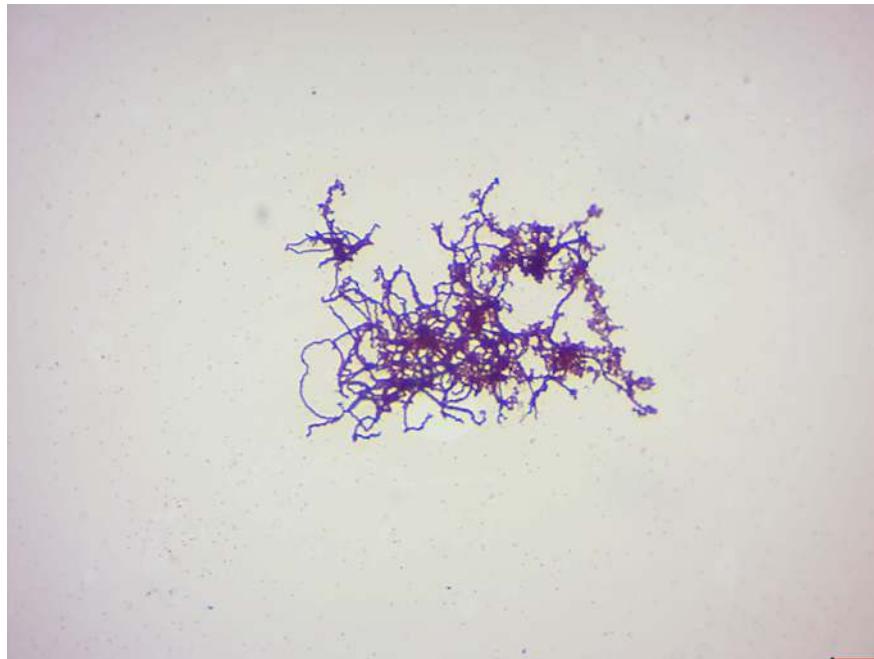
**Figure 11.5:** Mixed cocci.



**Figure 11.6:** Mixed bacilli.



**Figure 11.7:** Spirilla.



**Figure 11.8:** Treponema.

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#### 11.6.5 Clostridium botulinum

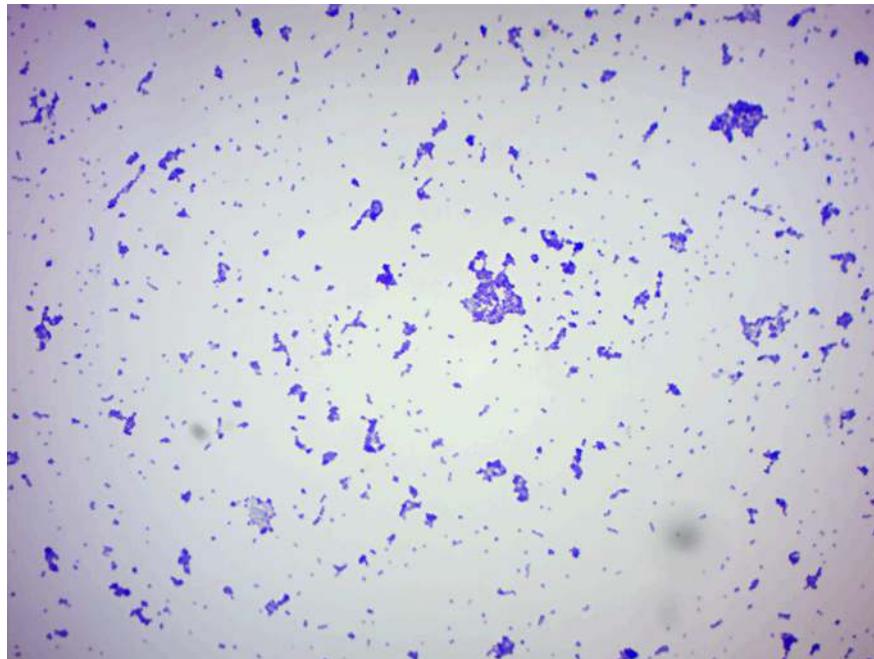
*Clostridium botulinum*<sup>13</sup> is a Gram-positive, rod-shaped, anaerobic, spore-forming, motile bacterium with the ability to produce a neurotoxin known as botulinum toxin. The botulinum toxin can cause a severe flaccid paralytic disease in humans and other animals and is the most potent toxin known to humankind, natural or synthetic, with a lethal dose of 1.3-2.1 ng/kg in humans. *C. botulinum* is an obligate anaerobe, meaning that oxygen is poisonous to the cells. However, it tolerates traces of oxygen due to the enzyme superoxide dismutase, which is an important antioxidant defense in nearly all cells exposed to oxygen. *C. botulinum* is only able to produce the neurotoxin during sporulation, which can only happen in an anaerobic environment. Other bacterial species produce spores in an unfavorable growth environment to preserve the organism's viability and permit survival in a dormant state until the spores are exposed to favorable conditions.

#### 11.6.6 Staphylococcus aureus

*Staphylococcus aureus*<sup>14</sup> (Figure 11.9) is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although *S. aureus* is not always pathogenic (and can commonly be found existing as a commensal), it is a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine.

<sup>13</sup>[https://en.wikipedia.org/wiki/Clostridium\\_botulinum](https://en.wikipedia.org/wiki/Clostridium_botulinum)

<sup>14</sup>[https://en.wikipedia.org/wiki/Staphylococcus\\_aureus](https://en.wikipedia.org/wiki/Staphylococcus_aureus)



**Figure 11.9:** *Staphylococcus aureus*.

#### 11.6.7 Oscillatoria

#### 11.6.8 Nostoc (Figure 11.10)

#### 11.6.9 Anabaena

*Anabaena*<sup>15</sup> is a genus of filamentous cyanobacteria that exist as plankton (Figure 11.11). They are known for nitrogen-fixing abilities, and they form symbiotic relationships with certain plants, such as the mosquito fern. They are one of four genera of cyanobacteria that produce neurotoxins, which are harmful to local wildlife, as well as farm animals and pets. Production of these neurotoxins is assumed to be an input into its symbiotic relationships, protecting the plant from grazing pressure.

## 11.7 Review Questions

1. What are bacteria?
2. What are archaea?
3. What are eukarya?
4. What are cyanobacteria?

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<sup>15</sup><https://en.wikipedia.org/wiki/Anabaena>



**Figure 11.10:** Nostoc.



**Figure 11.11:** Anabaena.

# Chapter 12

## Protista

Protists<sup>1</sup> are any eukaryotic organism that are not an animal, plant or fungus. The protists do not form a natural group, or clade, but are often grouped together for convenience. In the popular five-kingdom scheme proposed by Robert Whittaker in 1969, the protists make up a kingdom called Protista, composed of “organisms which are unicellular or unicellular-colonial and which form no tissues. Some protists are significant parasites of animals (e.g., five species of the parasitic genus *Plasmodium* cause malaria in humans and many others cause similar diseases in other vertebrates), plants (the oomycete *Phytophthora infestans* causes late blight in potatoes) or even of other protists. Protist pathogens share many metabolic pathways with their eukaryotic hosts. This makes therapeutic target development extremely difficult - a drug that harms a protist parasite is also likely to harm its animal/plant host.

The term protista was first used by Ernst Haeckel in 1866. Protists were traditionally subdivided into several groups based on similarities to the “higher” kingdoms such as:

- Protozoa<sup>2</sup>: the unicellular “animal-like” (heterotrophic/parasitic) protozoa which were further subdivided based on motility such as (flagellated) Flagellata, (ciliated) Ciliophora (or Ciliata), (phagocytic) amoeba and spore-forming Sporozoans
- Protophyta: the “plant-like” (autotrophic) protophyta (mostly unicellular algae)
- Molds: the “fungus-like” (saprophytic) slime molds and water molds.

The taxonomy of protists is ever changing. Newer classifications attempt to present monophyletic groups based on morphological (especially ultrastructural), biochemical (chemotaxonomy) and DNA sequence (molecular research) information. However, there are sometimes discordances between molecular and morphological investigations.

### 12.1 View Living Organisms

#### 12.1.1 Amoeba proteus

*Amoeba proteus*<sup>3</sup> (Figure 12.1) is an amoeba closely related to the giant amoebae. This small protozoan uses tentacular protuberances called pseudopodia to move and phagocytose smaller unicellular organisms, (which may be greater in size than of amoeba), which are enveloped inside the cell’s cytoplasm in a food vacuole, where they are slowly broken down by enzymes. It occupies freshwater environments and feeds on other protozoans, algae, rotifers, and even other smaller amoebae. Due to phytochromes, *A. proteus* may appear in a variety of colors (often yellow, green and purple) under a microscope.

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<sup>1</sup><https://en.wikipedia.org/wiki/Protist>

<sup>2</sup><https://en.wikipedia.org/wiki/Protozoa>

<sup>3</sup>[https://en.wikipedia.org/wiki/Amoeba\\_proteus](https://en.wikipedia.org/wiki/Amoeba_proteus)



**Figure 12.1:** Amoeba proteus.

### 12.1.2 Paramecium caudatum

*Paramecium caudatum*<sup>4</sup> (Figure 12.2) is a unicellular, ciliate eukaryote. They can reach 0.25mm in length and are covered with minute hair-like organelles called cilia. The cilia are used in locomotion and feeding. *P. caudatum* feed on bacteria and small eukaryotic cells, such as yeast and flagellate algae. In hypotonic conditions (freshwater), the cell absorbs water by osmosis. It regulates osmotic pressure with the help of bladder-like contractile vacuoles, gathering internal water through its star-shaped radial canals and expelling the excess through the plasma membrane. When moving through the water, they follow a spiral path while rotating on the long axis. Paramecia have two nuclei (a large macronucleus and a single compact micronucleus). They cannot survive without the macronucleus and cannot reproduce without the micro-nucleus. Like all ciliates, Paramecia reproduce asexually, by binary fission. During reproduction, the macronucleus splits by a type of amitosis, and the micronuclei undergo mitosis. The cell then divides transversally, and each new cell obtains a copy of the micronucleus and the macronucleus. Fission may occur as part of the normal vegetative cell cycle. Under certain conditions, it may be preceded by self-fertilization (autogamy), or it may follow conjugation, a sexual phenomenon in which Paramecia of compatible mating types fuse temporarily and exchange genetic material. During conjugation, the micronuclei of each conjugant divide by meiosis and the haploid gametes pass from one cell to the other. The gametes of each organism then fuse to form diploid micronuclei. The old macronuclei are destroyed, and new ones are developed from the new micronuclei. Without the rejuvenating effects of autogamy or conjugation a Paramecium ages and dies. Only opposite mating types, or genetically compatible organisms, can unite in conjugation.

### 12.1.3 Euglena

*Euglena*<sup>5</sup> (Figure 12.3) is a genus of single-celled flagellate eukaryotes. It is the best known and most widely studied member of the class Euglenoidea, a diverse group containing some 54 genera and at least 800 species. Species of *Euglena* are found in fresh and salt waters. They are often abundant in quiet inland waters where they may bloom in numbers sufficient to color the surface of ponds and ditches green (*E. viridis*) or red

<sup>4</sup>[https://en.wikipedia.org/wiki/Paramecium\\_caudatum](https://en.wikipedia.org/wiki/Paramecium_caudatum)

<sup>5</sup><https://en.wikipedia.org/wiki/Euglena>



**Figure 12.2:** *Paramecium caudatum*.

(*E. sanguinea*). When feeding as a heterotroph, Euglena takes in nutrients by osmotrophy, and can survive without light on a diet of organic matter, such as beef extract, peptone, acetate, ethanol or carbohydrates. When there is sufficient sunlight for it to feed by phototrophy, it uses chloroplasts containing the pigments chlorophyll a and chlorophyll b to produce sugars by photosynthesis. Euglena's chloroplasts are surrounded by three membranes, while those of plants and the green algae (among which earlier taxonomists often placed Euglena) have only two membranes. This fact has been taken as morphological evidence that Euglena's chloroplasts evolved from a eukaryotic green alga. Thus, the intriguing similarities between Euglena and the plants would have arisen not because of kinship but because of a secondary endosymbiosis. Molecular phylogenetic analysis has lent support to this hypothesis, and it is now generally accepted.

#### 12.1.4 Peranema

*Peranema*<sup>6</sup> (Figure 12.4) is a genus of free-living flagellate, with more than 20 accepted species, varying in size between 8 and 200 micrometers. They are found in freshwater lakes, ponds and ditches, and are often abundant at the bottom of stagnant pools rich in decaying organic material. Although they belong to the class Euglenoidea, and are morphologically similar to the green Euglena, *Peranema* have no chloroplasts, and cannot feed by autotrophy. Instead, they capture live prey, such as yeast, bacteria and other flagellates, consuming them with the help of a rigid feeding apparatus called a “rod-organ.” Unlike the green Euglenids, they lack both an eyespot (stigma), and the paraflagellar body (photoreceptor) that is normally coupled with that organelle. However, while *Peranema* lack a localized photoreceptor, they do possess the light-sensitive protein rhodopsin, and respond to changes in light with a characteristic “curling behavior.”

#### 12.1.5 Chlamydomonas

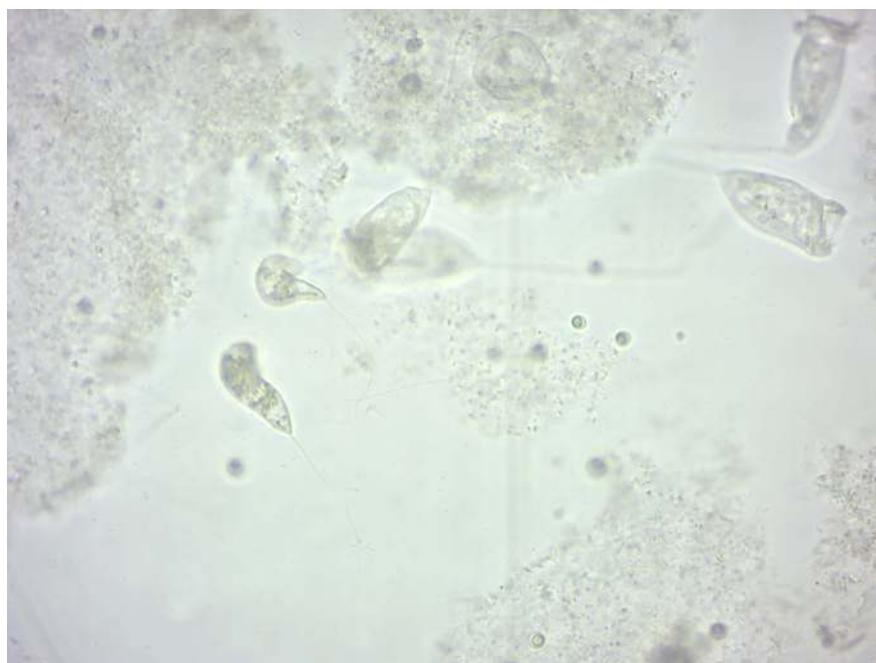
*Chlamydomonas*<sup>7</sup> (Figure 12.5) is a genus of green algae consisting of unicellular flagellates, found in stagnant water and on damp soil, in freshwater, seawater, and even in snow as “snow algae”. *Chlamydomonas* is used

<sup>6</sup><https://en.wikipedia.org/wiki/Peranema>

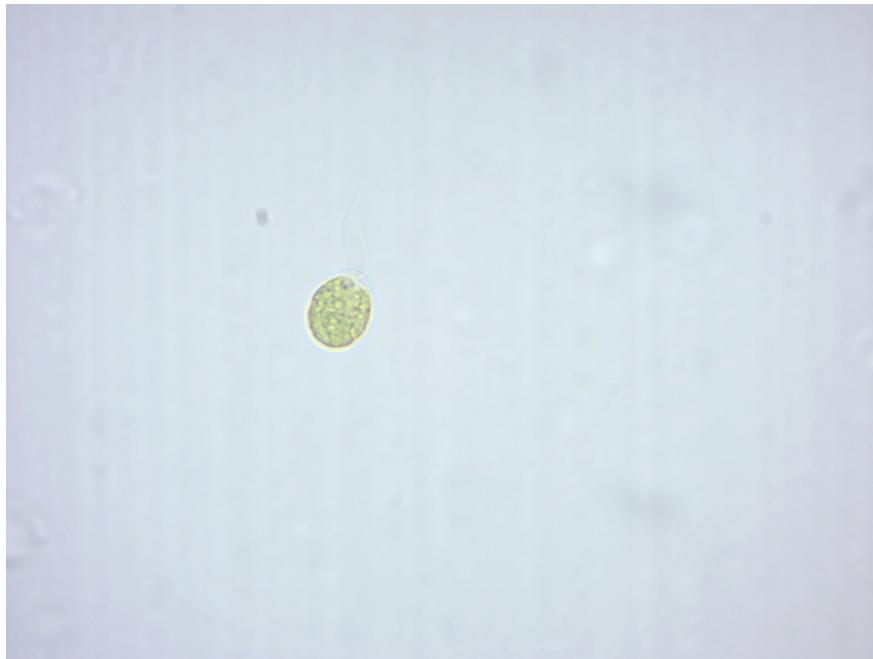
<sup>7</sup><https://en.wikipedia.org/wiki/Chlamydomonas>



**Figure 12.3:** Euglena.



**Figure 12.4:** Peranema.



**Figure 12.5:** Chlamydomonas. Note the flagella.

as a model organism for molecular biology, especially studies of flagellar motility and chloroplast dynamics, biogeneses, and genetics. One of the many striking features of Chlamydomonas is that it contains ion channels, (channelrhodopsins), that are directly activated by light. These proteins are used in optogenetics.

### 12.1.6 Gymnodinium

*Gymnodinium*<sup>8</sup> is a genus of dinoflagellates<sup>9</sup> It is one of the few naked dinoflagellates, or species lacking armor (cellulosic plates). The dinoflagellates (Greek dinos “whirling” and Latin flagellum “whip, scourge”) are a large group of flagellate eukaryotes that constitute the phylum Dinoflagellata. Most are marine plankton, but they are common in freshwater habitats, as well. Their populations are distributed depending on temperature, salinity, or depth. Many dinoflagellates are known to be photosynthetic, but a large fraction of these are in fact mixotrophic, combining photosynthesis with ingestion of prey (phagotrophy). In terms of number of species, dinoflagellates form one of the largest groups of marine eukaryotes, although this group is substantially smaller than the diatoms. Some species are endosymbionts of marine animals and play an important part in the biology of coral reefs. Other dinoflagellates are unpigmented predators on other protozoa, and a few forms are parasitic.

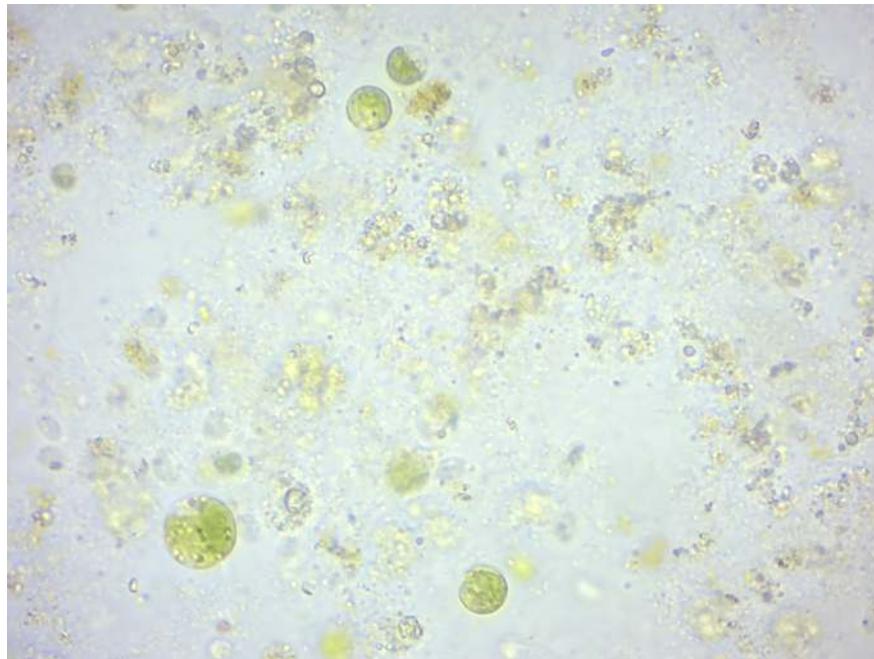
### 12.1.7 Pandorina

*Pandorina*<sup>10</sup> (Figure 12.6) is a genus of green algae composed of 8, 16, or sometimes 32 cells, held together at their bases to form a sack globular colony surrounded by mucilage. The cells are ovoid or slightly narrowed at one end to appear keystone- or pear-shaped. Each cell has two flagella with two contractile vacuoles at their base, an eyespot, and a large cup-shaped chloroplast with at least one pyrenoid. The colonies coordinate their flagellar movement to create a rolling, swimming motion. Pandorina shows the beginnings of the colony polarity and differentiation seen in Volvox since the anterior cells have larger eyespots. Asexual

<sup>8</sup><https://en.wikipedia.org/wiki/Gymnodinium>

<sup>9</sup><https://en.wikipedia.org/wiki/Dinoflagellate>

<sup>10</sup><https://en.wikipedia.org/wiki/Pandorina>



**Figure 12.6:** Pandorina.

reproduction is by simultaneous division of all cells of the colony to form autocolonies that are liberated by a gelatinization of the colonial envelope. Sexual reproduction occurs by division of each cell of the colony into 16-32 zoogametes. Zoogametes show indications of heterogamy, a slight difference in the size and motility of the pairs that fuse to form the smooth walled zygote.

### 12.1.8 Volvox

*Volvox*<sup>11</sup> (Figure 12.7) is a genus of freshwater algae found in ponds and ditches, even in shallow puddles. It forms spherical colonies of up to 50,000 cells that were first reported by Antonie van Leeuwenhoek in 1700. *Volvox* diverged from unicellular ancestors approximately 200 million years ago. Each mature *Volvox* colony is composed of up to thousands of cells from two differentiated cell types: numerous flagellate somatic cells and a smaller number of germ cells lacking in soma that are embedded in the surface of a hollow sphere or coenobium containing an extracellular matrix made of glycoproteins. Adult somatic cells comprise a single layer with the flagella facing outward. The cells swim in a coordinated fashion, with distinct anterior and posterior poles. The cells have anterior eyespots that enable the colony to swim towards light. An asexual colony includes both somatic (vegetative) cells, which do not reproduce, and large, non-motile gonidia in the interior, which produce new colonies through repeated division. In sexual reproduction two types of gametes are produced. *Volvox* species can be monoecious or dioecious. Male colonies release numerous sperm packets, while in female colonies single cells enlarge to become oogametes, or eggs. *Volvox* is facultatively sexual and can reproduce both sexually and asexually. The switch from asexual to sexual reproduction can be triggered by environmental conditions and by the production of a sex-inducing pheromone. Desiccation-resistant diploid zygotes are produced following successful fertilization.

<sup>11</sup><https://en.wikipedia.org/wiki/Volvox>



**Figure 12.7:** Volvox.

### 12.1.9 Oedogonium

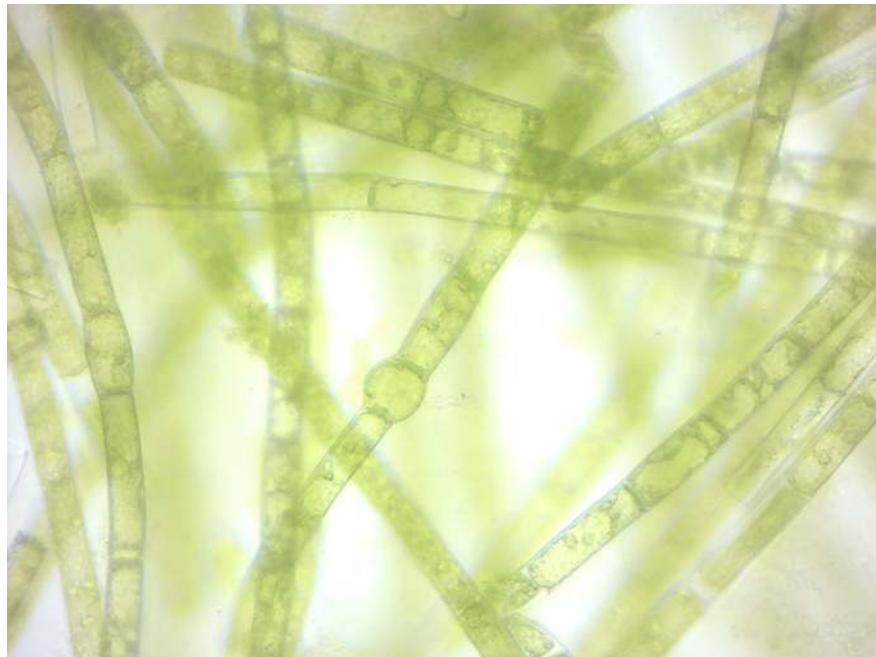
*Oedogonium*<sup>12</sup> (Figure 12.8) is a genus of filamentous green algae, with unbranched filaments that are one cell thick. *Oedogonium* can be free-floating, though it is usually attached to aquatic plants by a holdfast. It appears greenish and inhabits calm, fresh water. *Oedogonium* can reproduce asexually by fragmentation of the filaments, through some other types of non-motile spores, and also through zoospores, which have many flagella. These develop in a zoosporangium cell, one zoospore per zoosporangium. After settling and losing its flagella, a zoospore grows into a filament. *Oedogonium* can also reproduce sexually. Its sexual life cycle is haplontic, i.e., the zygote undergoes meiosis. Antheridia produce and release sperm, and oogonia produce and release an egg,. The egg and sperm then fuse and form a zygote which is diploid (2n). The zygote then undergoes meiosis to produce the filamentous green alga which is haploid (1n).

### 12.1.10 Spirogyra

*Spirogyra*<sup>13</sup> (Figure 12.9; common names include water silk, mermaid's tresses, and blanket weed) is a genus of filamentous chlorophyte green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in freshwater areas, and there are more than 400 species of *Spirogyra* in the world. *Spirogyra* measures approximately 10 to 100 m in width and may grow to several centimeters in length. *Spirogyra* can reproduce both sexually and asexually. In vegetative reproduction, fragmentation takes place, and *Spirogyra* simply undergoes the intercalary mitosis to form new filaments. Sexual Reproduction is of two types: 1. Scalariform conjugation requires association of two different filaments lined side by side either partially or throughout their length. One cell each from opposite lined filaments emits tubular protuberances known as conjugation tubes, which elongate and fuse, to make a passage called the conjugation canal. The cytoplasm of the cell acting as the male travels through this tube and fuses with the female cytoplasm, and the gametes fuse to form a zygospore. 2. In lateral conjugation, gametes are formed in a single filament. Two adjoining cells near the common transverse wall give out protuberances known as conjugation tubes, which further form the conjugation canal upon

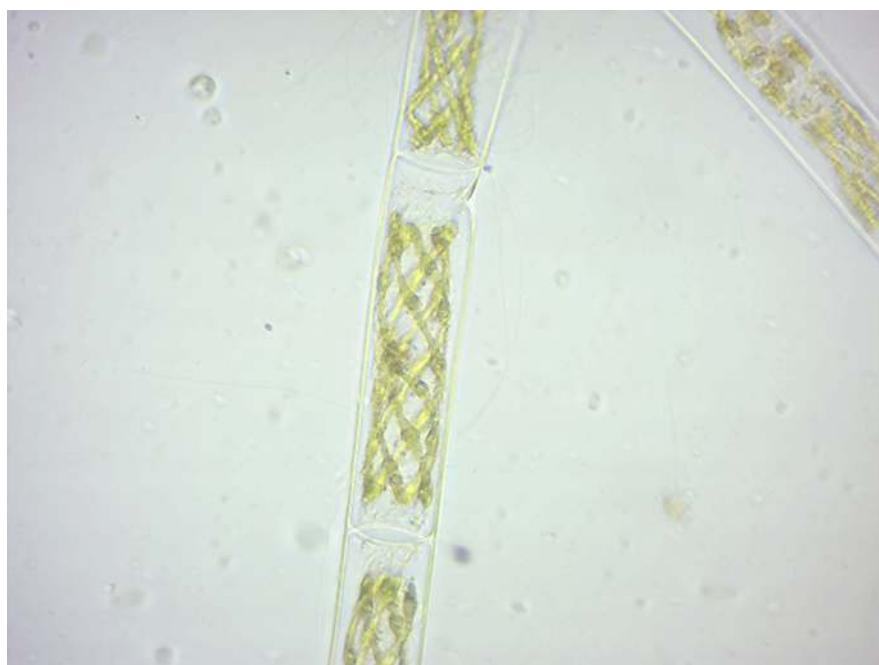
<sup>12</sup><https://en.wikipedia.org/wiki/Oedogonium>

<sup>13</sup><https://en.wikipedia.org/wiki/Spirogyra>

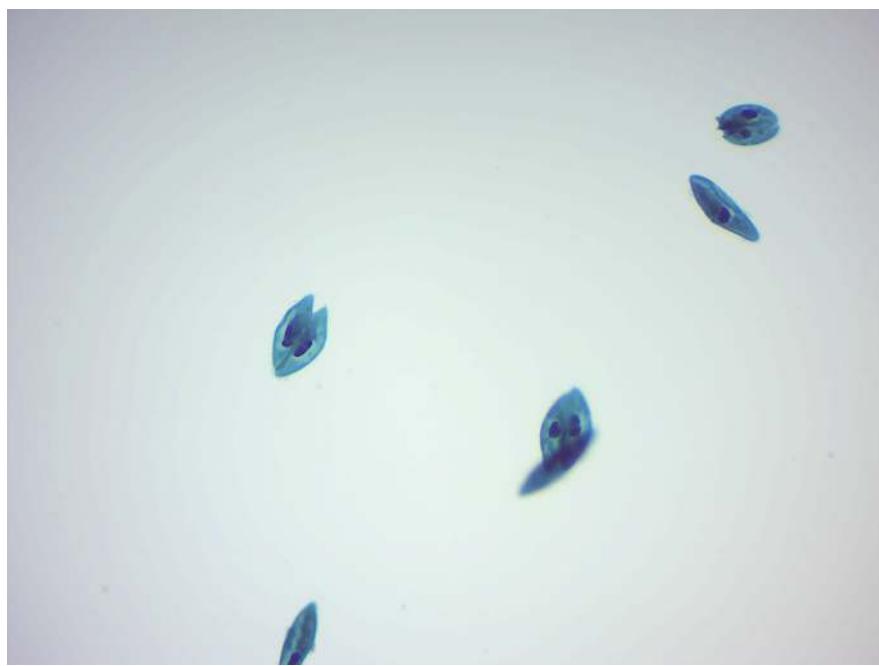


**Figure 12.8:** *Oedogonium*.

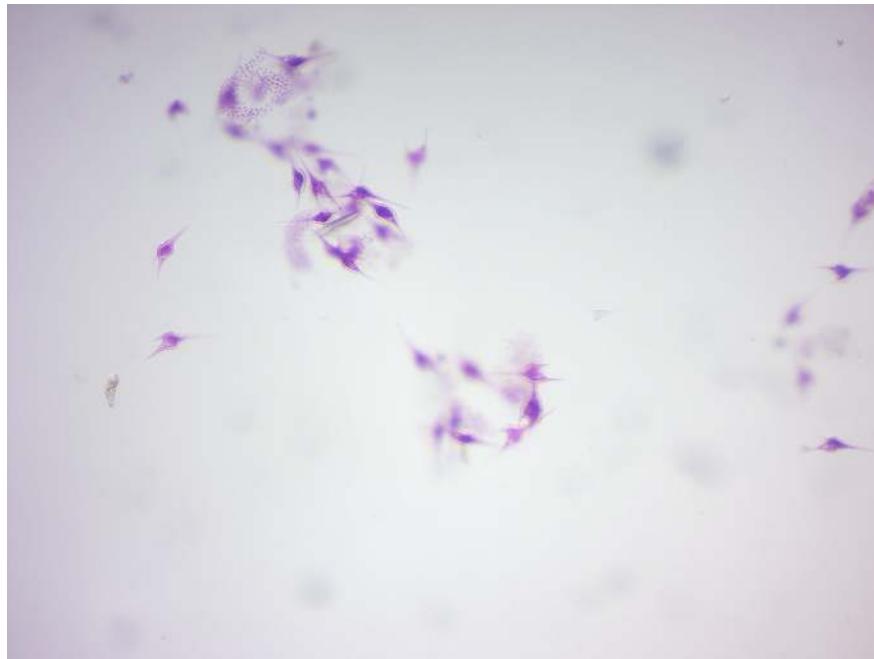
contact. The male cytoplasm migrates through the conjugation canal, fusing with the female. The rest of the process proceeds as in scalariform conjugation. The essential difference is that scalariform conjugation occurs between two filaments and lateral conjugation occurs between two adjacent cells on the same filament.



**Figure 12.9:** Spirogyra.



**Figure 12.10:** Paramecium in conjugation.



**Figure 12.11:** Ceratium, a dinoflagellate.

## 12.2 View Prepared Slides

### 12.2.1 Amoeba proteus

### 12.2.2 Paramecium 4 types of protista

### 12.2.3 Paramecium caudatum

### 12.2.4 Paramecium in conjugation (Figure 12.10)

### 12.2.5 Euglena

### 12.2.6 Dinoflagellates

### 12.2.7 Ceratium (Figure 12.11)

### 12.2.8 Peridinium (dinoflagellate)

### 12.2.9 Foraminifera

*Foraminifera*<sup>14</sup> (Figure 12.12; Latin meaning hole bearers; informally called “forams”) are members of a phylum or class of amoeboid protists characterized by: streaming granular ectoplasm for catching food and other uses; and commonly an external shell (called a “test”) of diverse forms and materials. Most foraminifera are marine, the majority of which live on or within the seafloor sediment (i.e., are benthic), while a smaller variety float in the water column at various depths (i.e., are planktonic). These shells are commonly made

<sup>14</sup><https://en.wikipedia.org/wiki/Foraminifera>



**Figure 12.12:** Foraminifera.

of calcium carbonate ( $\text{CaCO}_3$ ) or agglutinated sediment particles. Over 50,000 species are recognized, both living (10,000) and fossil (40,000).

### 12.2.10 Radiolaria

*Radiolaria*<sup>15</sup> (Figure 12.13), also called Radiozoa, are protozoa of diameter 0.1-0.2 mm that produce intricate mineral skeletons, typically with a central capsule dividing the cell into the inner and outer portions of endoplasm and ectoplasm. The elaborate mineral skeleton is usually made of silica. They are found as zooplankton throughout the ocean, and their skeletal remains make up a large part of the cover of the ocean floor as siliceous ooze.

### 12.2.11 Diatoms

*Diatoms*<sup>16</sup> (Figure 12.14) are a major group of microalgae and are among the most common types of phytoplankton. Diatoms are producers within the food chain. A unique feature of diatom cells is that they are enclosed within a cell wall made of silica (hydrated silicon dioxide) called a frustule. These frustules show a wide diversity in form, but are usually almost bilaterally symmetrical, hence the group name. These shells are used by humans as diatomaceous earth, also known as diatomite. Fossil evidence suggests that they originated during, or before, the early Jurassic period. Only male gametes of centric diatoms are capable of movement by means of flagella. Diatom communities are a popular tool for monitoring environmental conditions, past and present, and are commonly used in studies of water quality.

<sup>15</sup><https://en.wikipedia.org/wiki/Radiolaria>

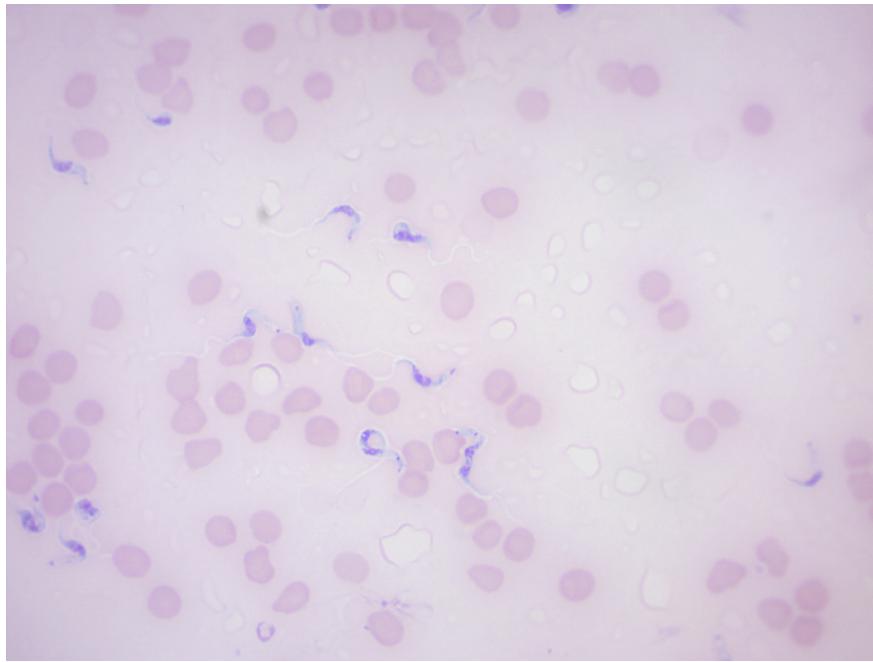
<sup>16</sup><https://en.wikipedia.org/wiki/Diatom>



**Figure 12.13:** Radiolaria.



**Figure 12.14:** Diatoms.



**Figure 12.15:** *Trypanosoma brucei gambiense* among red blood cells.

### 12.2.12 *Trypanosoma cruzi* and *Trypanosoma brucei gambiense*

*Trypanosoma cruzi*<sup>17</sup> is a species of parasitic euglenoids. Amongst the protozoa, the trypanosomes characteristically bore tissue in another organism and feed on blood (primarily) and also lymph. This behaviour causes disease or the likelihood of disease that varies with the organism: for example, trypanosomiasis in humans (Chagas disease in South America). Parasites need a host body and the haematophagous insect triatomine (descriptions “assassin bug”, “cone-nose bug”, and “kissing bug”) is the major vector in accord with a mechanism of infection. The triatomine likes the nests of vertebrate animals for shelter, where it bites and sucks blood for food. Individual triatomines infected with protozoa from other contact with animals transmit trypanosomes when the triatomine deposits its faeces on the host’s skin surface and then bites. Penetration of the infected faeces is further facilitated by the scratching of the bite area by the human or animal host.

*Trypanosoma brucei*<sup>18</sup> (Figure 12.15) is a species of parasitic kinetoplastid belonging to the genus *Trypanosoma*. The parasite is the cause of a vector-borne disease of vertebrate animals, including humans, carried by genera of tsetse fly in sub-Saharan Africa. In humans *T. brucei* causes African trypanosomiasis, or sleeping sickness. In animals it causes animal trypanosomiasis, also called nagana in cattle and horses. *T. brucei* has traditionally been grouped into three subspecies: *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*. The first is a parasite of non-human vertebrates, while the latter two are the known parasites of humans.

### 12.2.13 *Plasmodium vivax*

*Plasmodium vivax*<sup>19</sup> is a protozoal parasite and a human pathogen. This parasite is the most frequent and widely distributed cause of recurring (benign tertian) malaria, *P. vivax* is one of the five species of malaria parasites that commonly infect humans. Although it is less virulent than *Plasmodium falciparum*,

<sup>17</sup>[https://en.wikipedia.org/wiki/Trypanosoma\\_cruzi](https://en.wikipedia.org/wiki/Trypanosoma_cruzi)

<sup>18</sup>[https://en.wikipedia.org/wiki/Trypanosoma\\_brucei](https://en.wikipedia.org/wiki/Trypanosoma_brucei)

<sup>19</sup>[https://en.wikipedia.org/wiki/Plasmodium\\_vivax](https://en.wikipedia.org/wiki/Plasmodium_vivax)



**Figure 12.16:** Chlamydomonas. Note the flagella.

the deadliest of the five human malaria parasites, *P. vivax* malaria infections can lead to severe disease and death, often due to a pathologically enlarged spleen. *P. vivax* is carried by the female Anopheles mosquito, since it is only the female of the species that bites.

#### 12.2.14 Mixed green algae

#### 12.2.15 Chlamydomonas (Figure 12.16)

#### 12.2.16 Pandorina (Figure 12.17)

#### 12.2.17 Volvox (Figure 12.18)

#### 12.2.18 Volvox sexual stages (Figure 12.19)

#### 12.2.19 Spirogyra

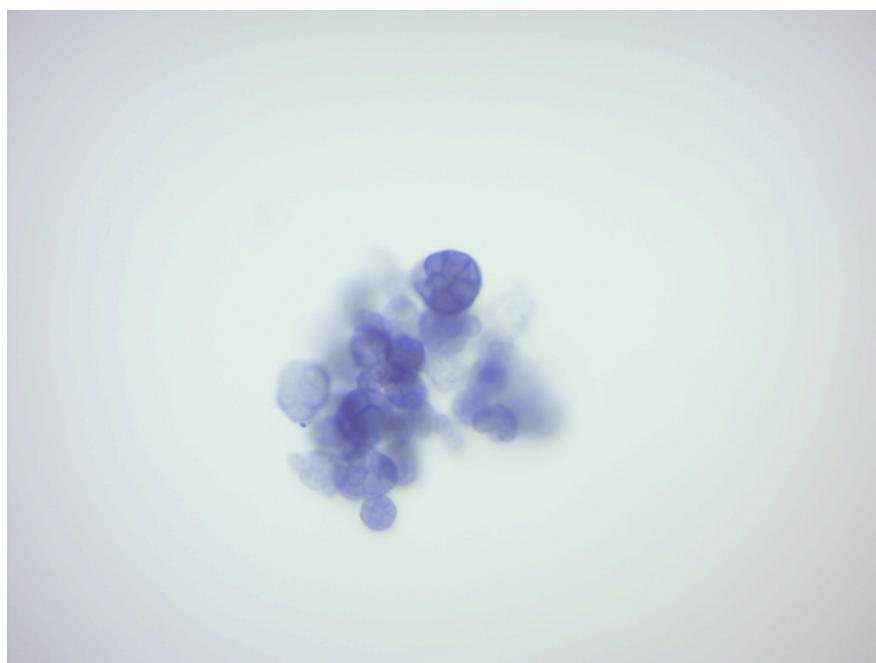
#### 12.2.20 Oedogonium zygospores

#### 12.2.21 Oedogonium macrandous

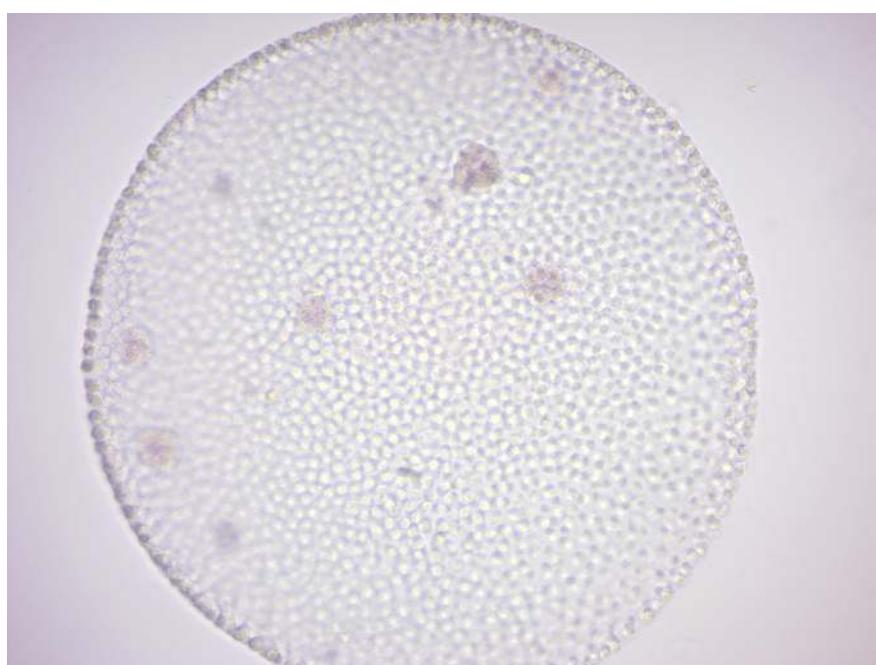
#### 12.2.22 Fucus male and female conceptacle

*Fucus*<sup>20</sup> is a genus of brown algae found in the intertidal zones of rocky seashores almost throughout the world. It has a relatively simple life cycle and produce only one type of thallus which grows to a maximum size of 2 m. The thallus is perennial with an irregular or disc-shaped holdfast or with haptera. The erect

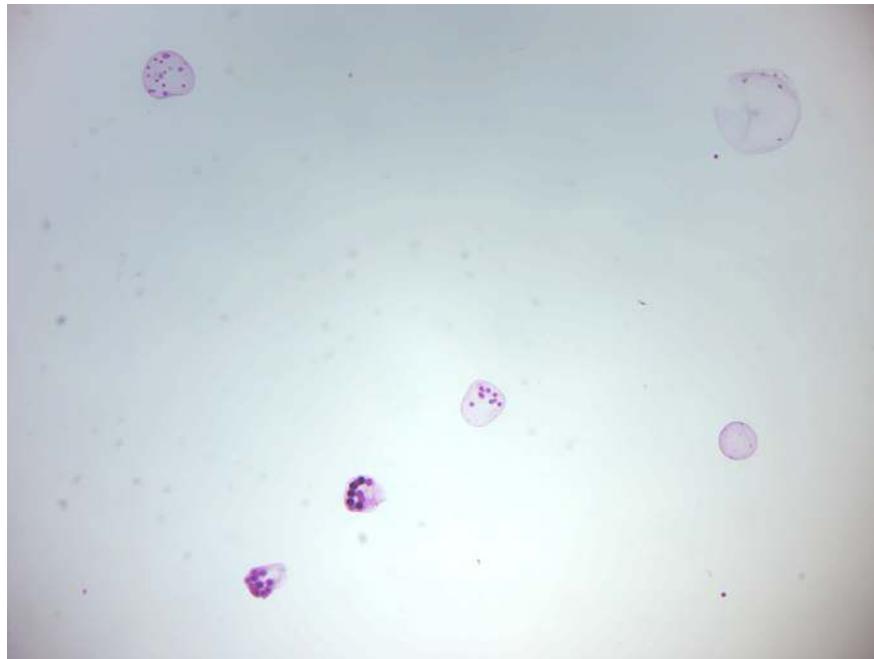
<sup>20</sup><https://en.wikipedia.org/wiki/Fucus>



**Figure 12.17:** Pandorina.



**Figure 12.18:** Volvox.



**Figure 12.19:** Volvox sexual stages.

portion of the thallus is dichotomous or subpinnately branched, flattened and with a distinct midrib. Gas-filled pneumatocysts (air-vesicles) are present in pairs in some species, one on either side of the midrib. The gametangia develop in conceptacles embedded in receptacles in the apices of the final branches. They may be monoecious or dioecious. Fertile cavities, the conceptacles, containing the reproductive cells are immersed in the receptacles near the ends of the branches. After meiosis oogonia and antheridia are produced and released, fertilisation follows and the zygote develops directly into the diploid plant. It may be considered to be analogous to the life cycle of the flowering plant, but in algae the oogonia are released and fertilised in the sea while in flowering plants the ovules are fertilised while attached to the parent plant and then released as a seed.

### 12.2.23 Fucus male conceptacle (Figure 12.20)

### 12.2.24 Fucus female conceptacle (Figure 12.21)

### 12.2.25 *Polysiphonia*

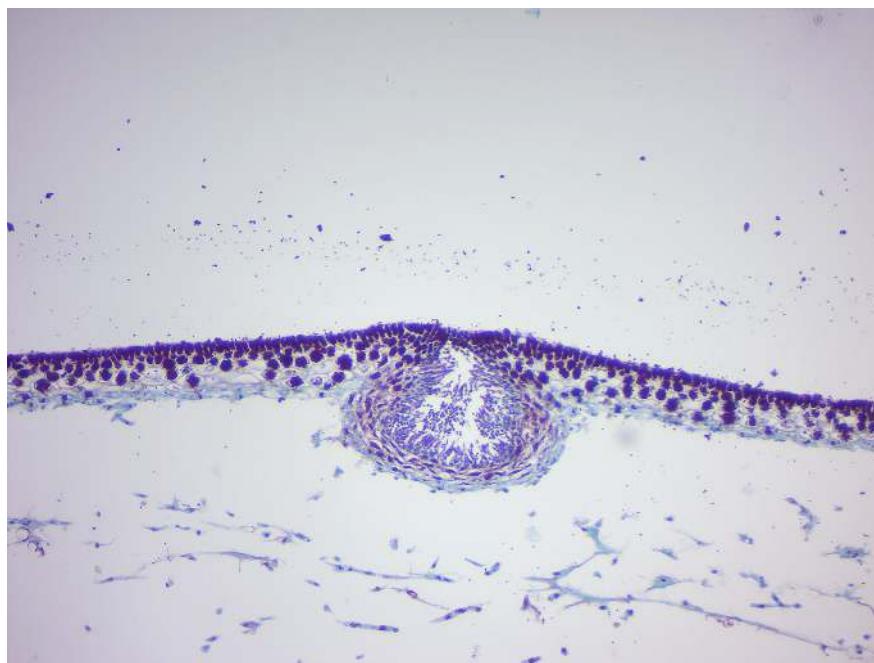
*Polysiphonia*<sup>21</sup> is a genus of filamentous red algae with about 19 species on the coasts of the British Isles and about 200 species worldwide.

### 12.2.26 *Stemonitis*

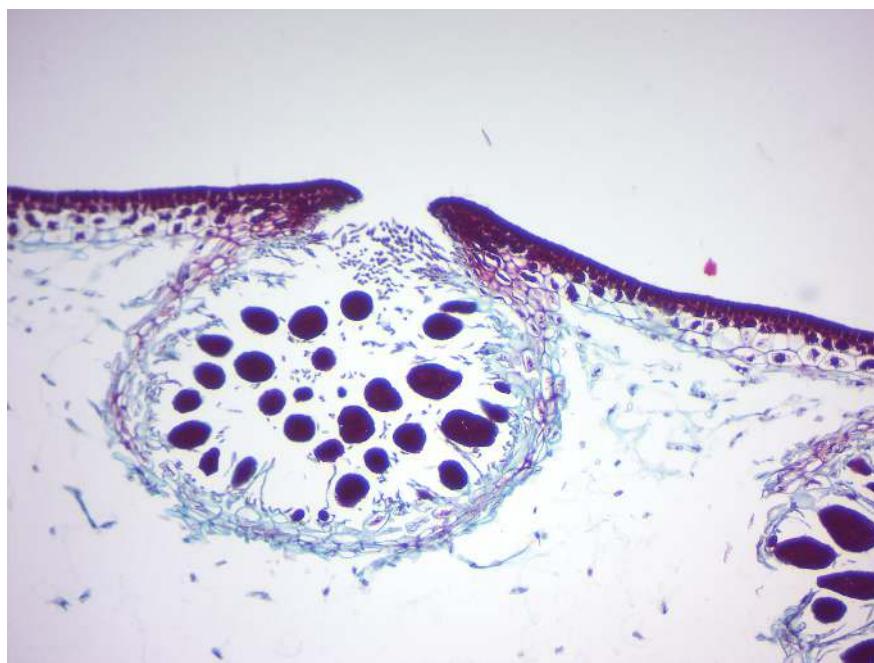
*Stemonitis*<sup>22</sup> (Figure 12.22) is a distinctive genus of slime moulds found throughout the world (except Antarctica). They are characterized by the tall brown sporangia, supported on slender stalks, which grow in clusters on rotting wood.

<sup>21</sup><https://en.wikipedia.org/wiki/Polysiphonia>

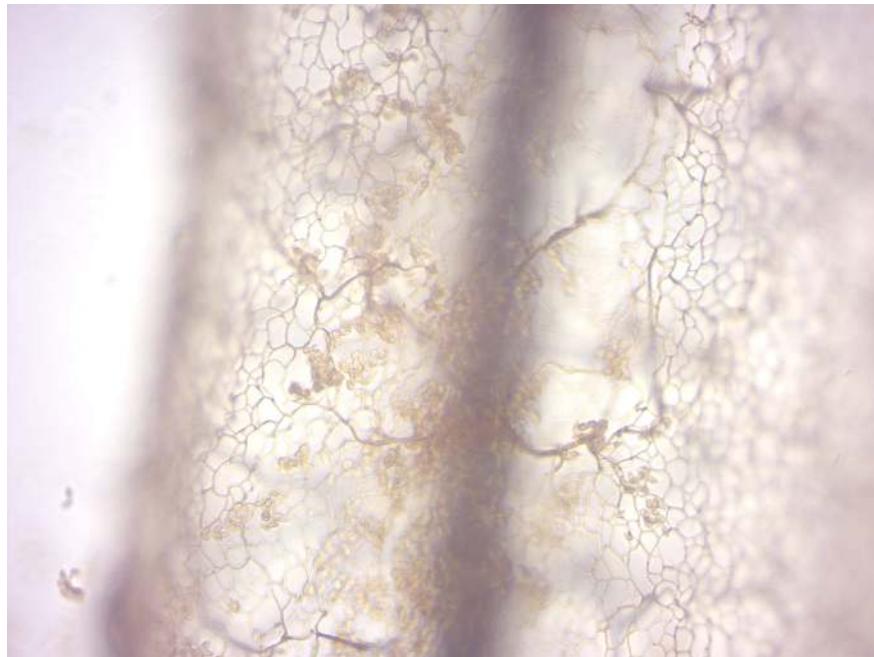
<sup>22</sup><https://en.wikipedia.org/wiki/Stemonitis>



**Figure 12.20:** Fucus male conceptacle



**Figure 12.21:** Fucus female conceptacle



**Figure 12.22:** Stemonitis.

#### 12.2.27 *Saprolegnia*

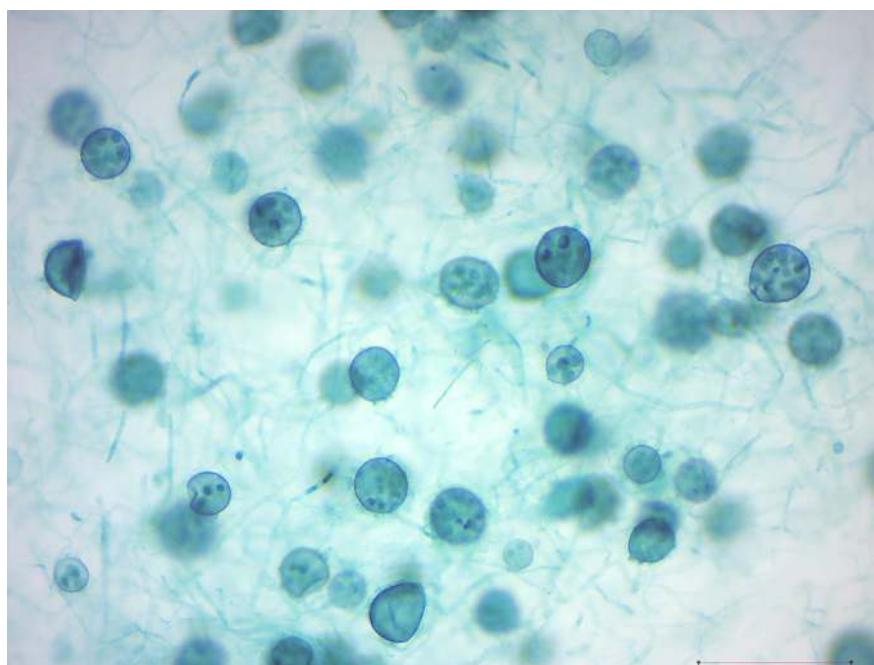
*Saprolegnia*<sup>23</sup> (Figure 12.23) is both a saprotroph and necrotroph. Typically feeding on waste from fish or other dead cells, they will also take advantage of creatures that have been injured. An infection is known as oomycosis. *Saprolegnia* is tolerant to a wide range of temperature, 3 °C to 33 °C, but is more prevalent in lower temperatures. While it is found most frequently in freshwater, it will also tolerate brackish water and even moist soil. *Saprolegnia* filaments (hyphae) are long with rounded ends, containing the zoospores. *Saprolegnia* generally travels in colonies consisting of one or more species. They first form a mass of individual hyphae. When the mass of hyphae grows large enough in size to be seen without use of a microscope, it can be called a mycelium.

It has a diploid life cycle which includes both sexual and asexual reproduction. In the asexual phase, a spore of *Saprolegnia* releases zoospores. Within a few minutes, this zoospore will encyst, germinate and release another zoospore. This second zoospore has a longer cycle during which most dispersal happens; it will continue to encyst and release a new spore in a process called polyplanetism until it finds a suitable substrate. When a suitable medium is located, the hairs surrounding the spore will lock onto the substrate so that the sexual reproduction phase can start. It is also during this stage of polyplanetism that the *Saprolegnia* are capable of causing infection; the most pathogenic species have tiny hooks at the end of their hairs to enhance their infectious ability. Once firmly attached, sexual reproduction begins with the production of male and female gametangium, antheridia and oogonium respectively. These unite and fuse together via fertilization tubes. The zygote produced is named an oospore.

### 12.3 Review Questions

1. What are protists?
2. What are ciliata?
3. What are flagellata?

<sup>23</sup><https://en.wikipedia.org/wiki/Saprolegnia>



**Figure 12.23:** *Saprolegnia*.

4. How do amoeba move?
5. What are slime molds?