

### Objectives

1. Become familiar with the basic design and use of the compound microscope and stereomicroscope.
2. Be able to measure objects using an ocular micrometer.
3. Become aware of other major types of microscopes and their uses.

### Introduction

More than any other invention, the lens has expanded our knowledge of the world around us and within us. Whether turned toward the heavens, in telescopes, or turned toward a drop of pond water, the lens projects our meager sensory abilities over vast distances, from microns to light years.

A lens is basically a device for refracting (bending) light in a uniform fashion. As light passes through the curved surfaces of a magnifying lens, its path is changed so that

the rays converge. The light from an object, passing through the lens, takes a path through the eye like of a larger object, and projects a larger image on the retina (the light-sensing surface). Therefore, the object appears to be larger than it actually is. This apparent image is called a “virtual” image. Microscope optics are more complex—several lenses are involved—but the basic principle of magnification is the same.

In today’s lab you will have an opportunity to learn the basics of microscope use. These skills will come in handy in subsequent classes, and could even lead you into a fascinating hobby. There are numerous amateur microscopy clubs worldwide, and the pursuit has become even more attractive now that digital cameras and video are readily applied to light microscopes. Visit some of the society and club web pages linked to the 121 page.

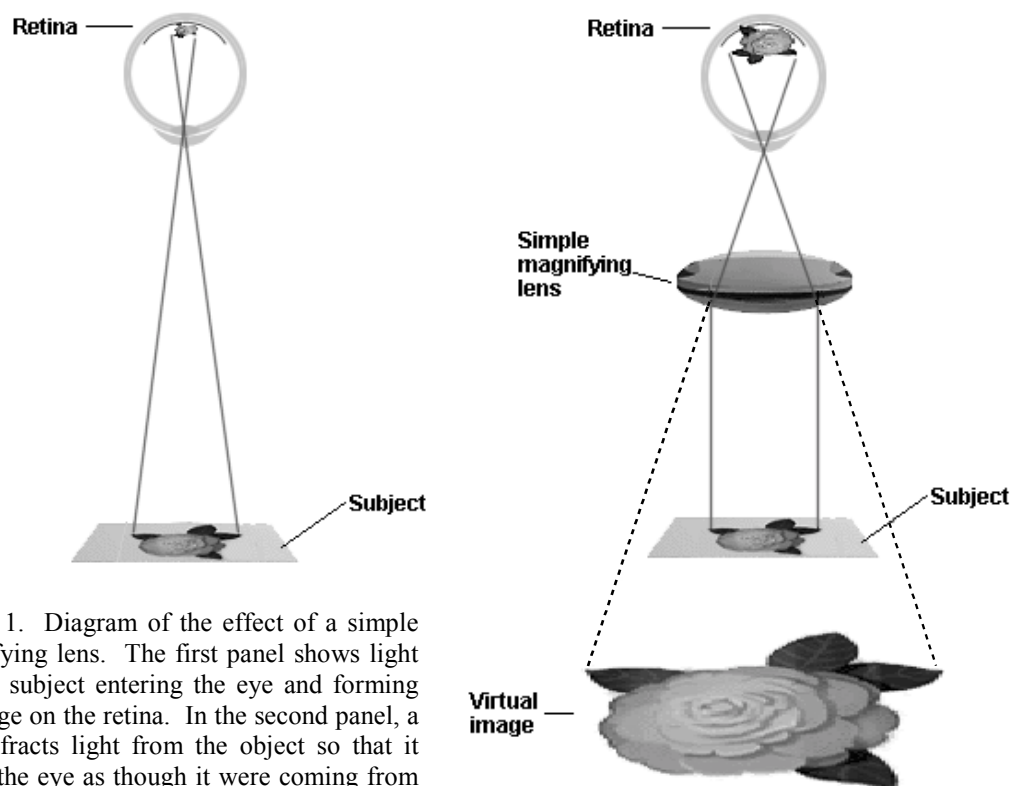


Figure 1. Diagram of the effect of a simple magnifying lens. The first panel shows light from a subject entering the eye and forming an image on the retina. In the second panel, a lens refracts light from the object so that it enters the eye as though it were coming from a much larger object (the virtual image).

**Care and handling– a summary of do's and don'ts**

Microscopes are delicate and expensive. Please handle them carefully. Also, use them properly so that you can get the most out of the experience. Follow these guidelines each time that you use the scopes:

1. Always carry the microscope with TWO HANDS, one hand on the arm and one supporting the base.
2. Never hold the microscope sideways or upside down. The ocular lens or other parts may fall off!
3. Check the ocular lenses for fingerprints or other smudges. If necessary, use LENS PAPER to clean lenses. Don't use Kimwipes or paper towels– they are abrasive and will scratch the lens coatings. Breath on the lens to fog it, and then wipe with clean lens paper.
4. Don't touch the end of the objective, and don't allow it to touch a specimen. If you suspect that the objective lens has been soiled, let the instructor clean it.
5. Begin with the low power objective in place and the STAGE ALL THE WAY UP. Then focus by lowering the stage. Turn the right hand focus knob clockwise.
6. Start with the low power objective, find the object, focus, and only then rotate the higher power objectives into place. An object that is in focus on low power should be nearly in focus when you switch to high power (if so, the scope is *parfocal*).
7. In high power, use the FINE ADJUSTMENT KNOB ONLY. It is possible to ram the objective into the slide if you are not careful. So be careful!
8. Adjust the interocular width for your eyes.
9. Adjust the diopter for your eyes as follows: Relax your eyes. Look into the right eyepiece with your right eye, and focus with the focus controls. Keep your eyes relaxed– focus the scope, not your eyes. Then close your right eye and look through the left ocular with your left eye. If the image is not sharp, focus by turning the diopter ring. Now both eyes should be happy.
10. When you are finished:
  - A. Remove any slide from the stage and make sure that the stage is clean.
  - B. Turn the light off.
  - C. Put the low power objective in place.
  - D. Turn the stage all the way up.
  - E. Replace the dust cover.

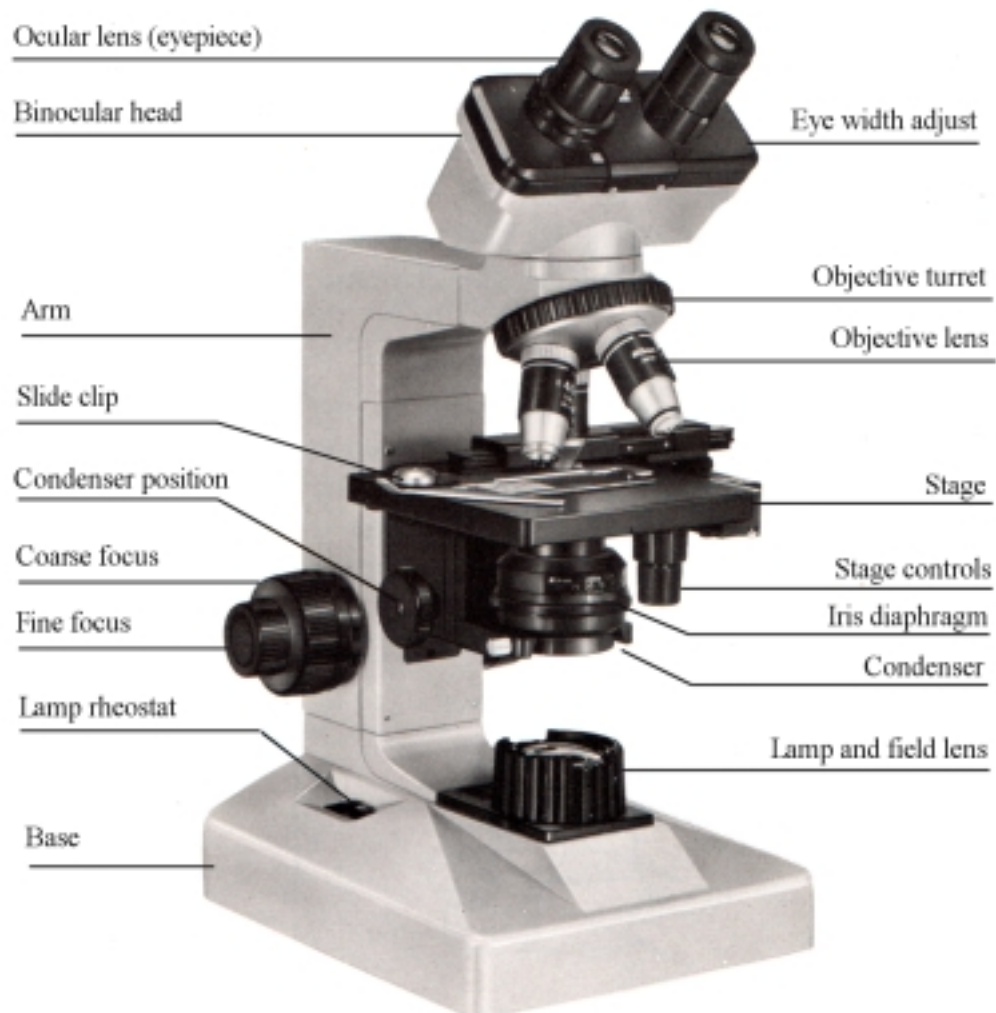


Figure 2. Parts of the Nikon Alphaphot binocular compound microscope. A *compound* microscope has two sets of magnifying lenses– the ocular and the objective lenses. The total magnification is the product of the ocular (usually 10X) and the objective (usually 4-100X) magnifications. A *binocular* microscope has two ocular lenses– one for each eye. The width between these and the difference in focus can be adjusted to fit the user’s eyes. These microscopes are designed to use transmitted light. A lamp in the base of the scope shines light through a condenser, which focuses the light upward to an object on the stage. Light transmitted through the object enters the objective lens. The virtual image from the objective lens is further magnified by the ocular lens. *You should be able to identify and explain the basic function of each labeled part.*

### 1– Learn the parts and their functions

Work in pairs. One or two of the compound microscopes will be assigned to you and your lab partner. Read the CARE AND HANDLING instructions carefully before you start.

Find the labeled parts indicated in Figure 2. The scope should be turned so that the stage and the ocular lenses are facing you. Begin with the *ocular lens*. The magnifying factor or “power” of the lens is inscribed on the ocular, followed by an “X” to indicate that it is a factor. Note the power of the oculars.

The two ocular lenses are mounted on the *eye width adjust* bracket which can be spread to match the distance between your eyes. Grasp this bracket with one hand on either side and pull it apart or push it together to change the *interocular distance*. Try it.

Now find the *objective lenses* mounted on the *turret*. The magnification power of each objective is inscribed on the side of each objective, along with information about the numerical aperture of the lens. Note the power of each objective. The total magnification is the product of the magnifying factors of the ocular and objective lenses. Calculate the power of each of the possible lens combinations.

Only the objective that is pointed directly at the stage is part of the optical path. The shortest (lowest power objective should be in that position. Carefully rotate the turret to bring the next largest lens into position. It will click into place. Then rotate back to the short one. Remember that you should start and end with the shortest (lowest power) objective in place.

Now turn your attention to the base of the microscope. Make sure that the power cord is plugged in. Find the *lamp rheostat*, turn on the lamp, and try adjusting the bright-

ness. You should turn the lamp intensity down when you are not using it, but you don’t need to turn it off. The lamp shines through the *field lens*, which focuses the light on the condenser.

The purpose of the *condenser* is to control the amount and the focus of the light reaching the object on the stage. The condenser has two important parts. The *iris diaphragm* (Figure 3) is similar to that inside a camera lens, or the iris of your eye. It acts to widen or narrow a hole that admits light to the condenser. The size of the opening is adjusted by turning a ring or lever on the side of the condenser. Find that control.



Figure 3. A very large iris diaphragm (from a telescope).

The second part of the condenser is a lens system that focuses the light on the object on the stage. The *condenser position* control raises and lowers the whole assembly. Generally speaking, the condenser should be raised as high as possible (i.e. as close to the object on the stage as possible). However, sometimes lowering the condenser may be useful.

The *stage controls* are rack and pinion mechanisms that move the slide clip from front to back and left to right. Keep your left hand on fine focus, and your right hand on the stage controls. The *slide clip* is a spring that holds the glass slides on the mechanical part of the stage.

## 2– Use of the microscope

Obtain a “letter e” slide. Place the slide with the “e” right side up in the clip on the stage and focus on low power. Follow the guidelines on page 23 and make the distance and diopter adjustments for your eyes.

Open and close the iris diaphragm while looking through the oculars. Now turn the lamp rheostat. Note that both affect light intensity. Generally, you should adjust the diaphragm to improve contrast and depth of field, and then adjust the rheostat to get a comfortable light intensity.

Now rotate the next objective (10X) into place and look again. Is the light dimmer or brighter than it was with the 4X objective? Why? Adjust the diaphragm and rheostat to get appropriate lighting at this magnification.

Compare the orientation of the letter “e” looking directly at the slide and looking at it through the microscope. Sketch the image as it appears in the microscope. Now use the stage controls to move the slide from right to left, as you view it directly. Compare this with what you see through the ocular. What two differences do you notice about the orientation of the image through the microscope?

Obtain a slide with two crossed threads and examine it on low and medium power. Focus up and down on the point where the threads cross. Notice that the plane of focus is very shallow. Compare the view with the iris diaphragm closed and open.

## 3– Measuring with the microscope

The *field of view* is the circular area that appears to your eye through the ocular lens. This field of view is smaller at higher magnification. That is, a smaller area is visible at higher magnification.

You may have noticed that a scale line appears when you view through the right ocular. This is an *ocular scale*—inscribed on a round piece of glass that sits in the ocular tube. The image of the ocular scale is magnified only by the ocular lens, so it doesn't change size as you switch objectives. Of course, the image of the field of view does!

In order to use the ocular scale to measure objects, it is necessary to determine what distance the scale units represent at different magnifications. Obtain a *stage scale* from your lab instructor. The stage scale is a special glass slide with a 1-mm scale inscribed on it and divided into tenths and hundredths of a mm.

Use the stage scale to calibrate the ocular scale for each of the different objectives (magnifications). That is, determine the metric length of an ocular unit at each magnification. Place the stage scale in the slide clip. Use the stage controls and rotate the ocular lens to align the ocular micrometer with the stage micrometer. Measure the length of the whole ocular scale if possible, and then divide that measure by 100 to determine the length of an ocular unit in mm. Record this factor for each objective, and compare the results with the magnifications.

### *The iris diaphragm and depth of field.*

If you have used a camera you may know that closing the aperture of the iris diaphragm (increasing the F-stop) lets less light through the lens, and increases the *depth of field*. Greater depth of field means that more of the field of view, from closer to further away, is in sharp focus. Contrast is also increased (contrast is the difference between dark and light parts of the image). Sometimes you want more light, sometimes you want more depth of field and contrast. Remember that light intensity is best controlled with the rheostat.

*Resolution* of an image is an important concept. Resolution is defined as the minimum distance between two points at which they can still be distinguished as separate points. The resolution of an optical image limits the detail or information content in the same way that pixel number limits a digital image. More magnification doesn't help because it doesn't add any more pixels— it just makes the existing ones bigger.

The resolution of light microscopy is about 1 micrometer. Resolution is limited by the wavelength of the radiation used to form the image, and the wavelengths of visible light are about 0.4-0.8 micrometers. Other kinds of illumination, such as X-rays, or electron beams, can give better resolution because they have shorter wavelengths. Of course, these images can't be viewed directly!

#### 4– Prepare a wet mount.

Obtain a slide and a coverslip. Handle both by the edges to avoid adding fingerprints. Be sure that you have just one coverslip—they tend to stick together. If there are two stuck together, a close look will show a rainbow pattern—the light is diffracted as it passes the layers of glass and the thin layer of air that separates them.

Your instructor will provide water containing protists and algal cells. Go to the bottom, where the goodies are. Place a drop of this suspension on the center of a clean glass slide and add a coverslip. Lower one edge of the coverslip first and contact the drop from the side, then lower the other edge. This approach will help to avoid trapping bubbles. Bubbles can be confusing at first—they are easy to see, but not very interesting to look at!

Focus on the edge of the coverslip or any visible object under the coverslip. Then use the stage controls to scan the wet mount on 100X. There should be a variety of interesting organisms present. Practice your tech-

nique by following these and by adjusting the microscope to get the best possible views. Bring these to the attention of the instructor so that they can be projected for the rest of the class to see.

#### 5– Use of darkfield illumination

Lighting is a key issue in improving the contrast and therefore the detail visible in an image. Two lighting techniques that can dramatically enhance the image are **dark field** and **phase contrast**. Both of these methods use a “hollow” cone of light to illuminate the subject. This hollow cone is produced basically by blocking the center of the projected light from the condenser with an opaque disc (called a “stop”). Thus, there is no light shining directly at the objective lens from below, and the field of view is dark. Only oblique light rays reach the object, and if it were perfectly transparent, none of this light would reach the objective. However, the object refracts and reflects some of the light, so that it enters the objective lens and forms the image. Because the field is dark, the object appears bright on a dark background.

In **darkfield** illumination, the stop on the condenser is the only modification. In

#### *Keep it moving*

It's easy to spot someone who is experienced with microscopes. When observing, they always have their left hand on the fine focus and their right on the stage controls. They focus up and down nearly continuously as they scan back and forth with the stage controls. They also adjust the condenser and rheostat occasionally to get good lighting. If you don't do these things, you see only one plane of one part of the object, and you never find out if the light could have been better adjusted. Don't be afraid to work with the microscope. You'll see a lot more if you do.

**phase contrast** microscopes, the objective lens is also modified with a dark ring, matched in size to the stop, that blocks any direct illumination from the condenser and thus improves the effect.

Another variation of darkfield is called **Rheinberg illumination**. The stop that blocks the direct light is colored and translucent, rather than opaque, so that direct light of one color illuminates the field of view. The hollow cone of white light striking the object has other colors, which are refracted to the objective. You can see examples of this effect on the Cells Alive website (linked to the lab web pages).

Let's use darkfield illumination to view an object. Your instructor will provide a suitable slide for viewing. The method is particularly useful when viewing objects that are very transparent (i.e. have low contrast in brightfield illumination). It will work best with the lower power objectives (4x to 10x).

View the slide in bright field and make adjustments to get the best image possible. Next, place a stop (opaque disc) on the field lens (below the condenser) and open the iris diaphragm. View the object again, and adjust the position of the stop, the light intensity, and the iris diaphragm to get the best image possible. Can you see more detail

than before? If translucent colored stops are available, also try to achieve Rheinberg illumination.

### **6- Stereomicroscope**

The stereomicroscope or "dissecting microscope" is designed for low magnifications (2-40X) and is used to view relatively large objects. Illumination is usually *reflected* light, not transmitted. However, some stereoscopes also have a light source below the stage. There is usually no condenser. Stereoscopes are always binocular. They have paired internal objectives and light paths to provide a 3-dimensional view. The longer distance between the lens and the subject, and the use of reflected light, make the stereoscope useful for large or opaque objects and those which must be manipulated. Your lab instructor will provide specimens to examine with the dissecting microscope.

### **7- Clean-up**

Make sure that the glass slides that you used are clean, dry, and returned to their box. Coverslips (but not slides) can be discarded in the waste container for glass. Set up your scope for storage according to the directions on page 23.

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**7. Assignment**

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A. Answer the following questions and be prepared to turn in your typewritten answers next time. Any of these are possible quiz questions. The text of these questions is available on the Bio 121 lab web page— you can copy it into your document and thereby avoid having to retype the questions. Use your own words to answer the questions— do not transcribe statements in the lab manual.

1. Define *refraction*.
2. How does the *lamp rheostat* control light intensity?
3. How does the *iris diaphragm* control light intensity?
4. Define *depth of field*.
5. Define *contrast*.
6. How does closing the iris diaphragm affect contrast and depth of field?
7. Why is the image dimmer when you switch from a low power to a high power objective?
8. Is the field of view (the area visible) smaller or larger at higher magnification? Why?
9. Why doesn't the image of the ocular micrometer change size when you switch objectives?
10. Why is the ocular micrometer visible through only one ocular and not both?
11. Define *resolution*.
12. Would a light microscope be useful for viewing objects less than 1 micron in size? Why or why not?
13. If you could shine a light sideways at the object being viewed, rather than from underneath, would it appear dark field or bright field? Explain your answer.

B. The second part of your assignment is to research and write a one-page essay describing the function and use of one type of microscope that we did not use in class. For example, you might report on the confocal laser microscope, or the scanning electron microscope. There are several other types of scopes. Use your textbook and the Internet to find information. You can get started with the links available on the Biology 121 lab page.



**Objectives**

1. Observe a variety of cell types and contrast the structures of prokaryote and eukaryote cells.
2. Observe and learn the functions of major cellular organelles.
3. Learn the general features of cells in the eukaryote Kingdoms Protista, Fungi, Plantae, and Animalia.
4. Measure the dimensions of several cell types to gain understanding of cell size.

Materials: The following items should already be at your station: Neutral red, methylene blue, iodine, toothpicks, slides, coverslips, pipettes, kimwipes, and lens paper.

Live material: *Oscillatoria*, yogurt, *Elodea*, *Paramecium*, yeast, biology students

Prepared slides: Root tip, mammalian ileum X-section

**Introduction**

The exploration of the structure and function of cells is a venture that is comparable in scope to any of the other great intellectual enterprises of mankind— it is a voyage to worlds within. Cells are tremendously complicated structures. Despite great strides in research, there is still far more unknown than known.

Microscopists made observations of cells in plant tissues as early as 1665, but the idea that plants and animals are built of cells was first stated clearly in 1839, by Theodor Schwann. Schwann called his idea the “cell theory”, and it was the first great generalization in biology. Biologists realized that, despite the tremendous variety of life, the study of cells could reveal features shared by all organisms. Soon it was realized that all cells arise from pre-existing cells by division. Louis Pasteur and others disproved earlier hypotheses about the spontaneous generation of life. In the following dec-

ades, biologists armed with little more than light microscopes and careful logic made great progress in deducing the structure and function of cells and their organelles.

In this lab exercise, you will observe several examples of cells, including some of your own, and the organelles within them. You should review the introductory discussions of cell structure and function in your textbook before you come to lab. You may be quizzed on these topics at the beginning of lab.

**Prokaryote cells**

Prokaryote cells are smaller and structurally simpler than eukaryotes. They lack a cell nucleus and certain other organelles. Prokaryote organisms are nearly always single-celled or simple strings or mats of similar cells, not complex multicellular organisms. The cells are enclosed by a *cell wall*, a rigid box that encloses the cell. Many species also produce a mucilaginous *capsule* or sheath outside the cell wall.

Although structurally simple, prokaryote organisms are biochemically and metabolically *more diverse* than eukaryotes. In particular, different groups use different enzyme systems and chemical pathways for obtaining energy. There are two major prokaryote groups, the *Archaea* and the *Eubacteria*. Archaea often live in extreme levels of temperature, salinity and/or pH. The Eubacteria include organisms commonly known as *bacteria* and *blue-green algae*. They are widely distributed and very diverse, including species that are eaten as food, others that cause deadly disease, or still others that live in and on our bodies as essential symbiotes.

*I. Oscillatoria.* This alga is a good choice for a first look at a prokaryote because it is easy to find. Pipette a drop of water from the culture onto a slide and cover with a coverslip. Observe first on low power and

then move up to higher magnification. Each cell is a flattened cylinder that is only about half as long as it is wide. Many cells

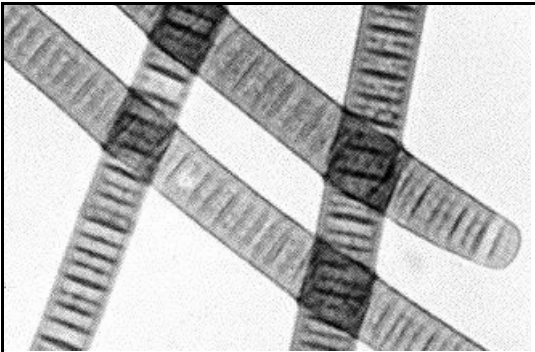


Figure 1. *Oscillatoria*

link together to form the filament. The photosynthetic pigments cause the blue-green color. Measure the diameter and length of the cells, and record your answers in the table on page 36.

You may have noticed that the filaments can move—slowly! Observe and briefly describe this movement.

Secretion of the capsule is thought to be involved in this movement, but the mechanism is not well understood.

Several species of *Oscillatoria* are common in freshwater. Some species produce a compound called 2-methylisoborneol (MIB), which has a rather strong musty odor, and can cause problems by lending this odor to drinking water. MIB can even cause pond-reared catfish to taste poorly.

2. *Lactobacillus*. Next, prepare a slide of natural yogurt, which consists largely of a bacterium called *Lactobacillus*. When you make your slide, use a toothpick to mix a very small amount of yogurt into a drop of water on the slide. Add a coverslip and examine carefully. The cells are very small. Closing the condenser diaphragm will help to improve contrast. Determine the length

and diameter of a typical individual cell and record on page 34.

These bacteria secrete enzymes that digest milk sugar (lactose) and produce lactic acid, which lowers the pH and denatures the milk proteins. Other bacteria are discouraged by the low pH. Although good in yogurt, *Lactobacillus* can rapidly turn wine into vinegar, so it is not popular among wine-makers.

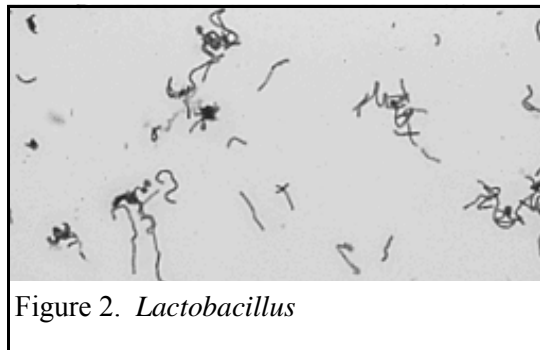


Figure 2. *Lactobacillus*

Heterotrophic bacteria such as *Lactobacillus* are generally much smaller than photosynthetic bacteria. This size difference is related to function. Heterotrophic bacteria feed by absorbing food molecules directly from their environment (**saprophy**). This process is facilitated by small size and the consequently high ratio of surface to volume.

### Eukaryote Cells

Eukaryote (“true nucleus”) cells are distinguished from prokaryotes by larger size and more complex internal organization, including elaborate internal membrane systems that partition the cell into functional compartments. In addition to a membrane-bound *nucleus*, eukaryote cells also possess particularly complex organelles called *mitochondria*. Mitochondria carry out the production of ATP. They are similar to prokaryote cells in several ways, and it is very likely that they were originally independent organisms. Photosynthetic eukaryotes also

possess similar organelles called *chloroplasts*, which carry out photosynthesis. Like mitochondria, chloroplasts are cell-like, contain their own DNA and reproduce by division. The eukaryote cell is therefore seen as a symbiosis that achieved its structural complexity, in part, by a combination of ancestral cells.

3. *Elodea*. Obtain a leaf of the aquatic plant *Elodea* and use a forceps to tear off a piece. Pull this piece back as you remove it. The leaf is just two cell layers thick, and with luck you can tear it so that the layers separate, affording a better view of the cells.

Place the bit of leaf in a drop of water on a slide and add a coverslip. As you observe this specimen, look for the areas, perhaps along the torn edge, where a single layer of cells is exposed. Focus up and down so that you can visualize the cell walls and the distribution of the numerous green chloro-

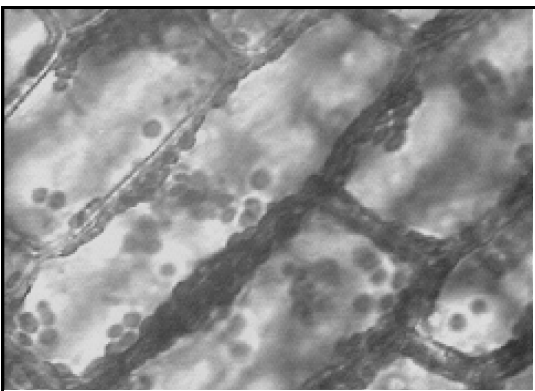


Figure 3. *Elodea*— an aquatic plant with very transparent cells

plasts. Are they uniformly distributed throughout the cell? What other organelle is present that occupies much of the internal volume of the cell? Do the chloroplasts move? Careful observation may also reveal the cell nucleus— however, it is very transparent. Adding a drop of methylene blue dye will improve the view of the nucleus.

Measure the length and width of the cells and the diameter of the chloroplasts and record.

4. *Amyloplasts in potato* **Amyloplasts** are organelles in plant cells that store sugar in the form of **starch**, an important polysaccharide. These organelles are abundant in the cells of organs that are modified for storage, such as the modified stems that we call potatoes. The amyloplasts also provide a rather dramatic view of the way that **staining** with selective dyes can increase the visibility of organelles

Use a razor blade to cut a *very* thin slice of potato. I'm talking paper-thin (you have to be able to see through it). Place this slice in a drop of water on a slide, add a coverslip, and observe on low and medium power. Next, add a drop of iodine solution at the edge of the coverslip. Touch a Kimwipe or other absorbent paper to the opposite side of the coverslip, in order to wick the solution up and draw the iodine under the coverslip. Observe again. Iodine binds selectively to starch and yields a blue color that enhances the visibility of the amyloplasts.

5. *Epithelial cells*. Use the broad end of a toothpick to gently scrape some epithelial cells from the inside of your cheek. Stir these into a drop of methylene blue dye on a slide and add a coverslip. Observe on low power and locate a few cells that are spread out (the clumps are confusing). These cells are flat and they fold very easily. Switch to high power, and locate the cell nucleus. Numerous bacteria will also be visible, on and around the cheek cells, as tiny dark specks only a few microns in diameter. Note the vast difference in size between the bacteria and the cheek cells. Measure the approximate diameters of the cheek cells and their nuclei and record.

Epithelial cells are constantly being shed from the surfaces of the skin and the mucus

membranes. One study reported that most of the dust in homes is actually skin cells!

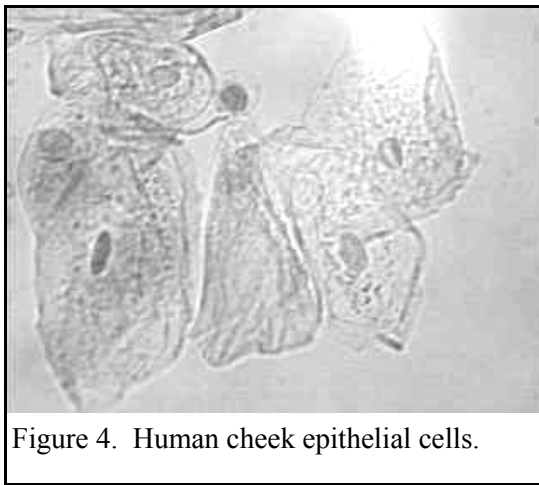


Figure 4. Human cheek epithelial cells.

**6. *Paramecium*.** The Kingdom Protista includes remarkably complex single-celled eukaryotic organisms. It is amazing to see single cells that are able to locomote, capture prey, and respond to stimuli. Some of these unicellular organisms cells live alongside multicellular animals that are of similar size and habits.

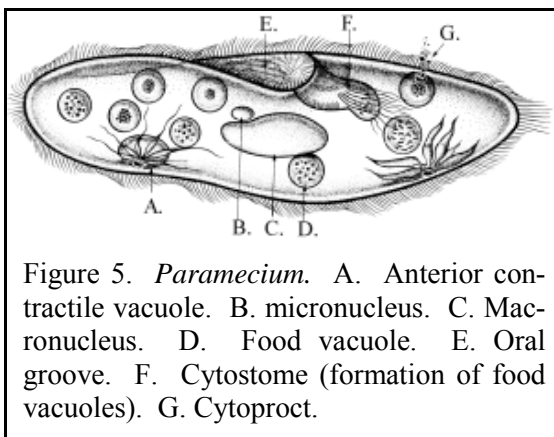


Figure 5. *Paramecium*. A. Anterior contractile vacuole. B. micronucleus. C. Macronucleus. D. Food vacuole. E. Oral groove. F. Cytostome (formation of food vacuoles). G. Cytoproct.

Place a drop of *Paramecium* culture on a slide. Mix in a small drop of bread yeast culture. The yeast cells will be ingested by the *Paramecium*, making it possible to identify food vacuoles. Let the mixture set for 5 minutes, then add a drop of methylcellulose, a viscous substance that will slow down the

movements of the *Paramecium*. It may take some searching to locate *Paramecium*. You might try looking at the slide directly- the cells are large enough to be seen by eye.

Measure the length and width of *Paramecium* and record. Then observe carefully on high power to see the organelles in Figure 5. Rows of hair-like **cilia** beat in a coordinated fashion to drive the cell through the water. Besides propelling the cell, the cilia also sweep food particles into the oral groove where they are collected into **food vacuoles** that form at the cytostome (“cell mouth”). These vacuoles will be clearly visible because they will contain yeast cells (measure the yeast cells too, for the table on page 36). The ingestion of particles by a cell is called **phagocytosis**. The food vacuoles contain enzymes to digest the food. They circulate throughout the cell (similar to the movements of the chloroplasts of *Elodea*) and then empty to the outside through the cytoproct (“cell anus”).

The **contractile vacuoles** of *Paramecium* regulate cell volume by repeatedly filling and emptying water to the outside. This process can be seen if you watch one cell carefully for a few minutes. Why does *Paramecium* need to bail out so much water? Hint- what is osmosis?

In most eukaryote cells, there is only a single nucleus with two copies of the chromosomes (diploid). However, there are exceptions. For example, these protists have two kinds of nucleus. The **micronucleus** is comparable to the diploid nuclei of other eukaryotes. The **macronucleus** consists of many extra copies of the chromosomes and carries out most of the **transcription** (RNA synthesis) for the cell.

### Cell division and growth

Examine a prepared slide of *Tilia* or *Allium* root tip. This slide is a thin section (slice)

of a region where rapid growth is occurring. The section is *longitudinal*, meaning that it cuts along the length of the root, and not across the diameter. The section has been stained with vital dyes to make the cell walls and the cell nuclei more visible.

Work up to high power and focus several ranks of cells *above* the tip of the root. Note the variety of appearance of the cell nuclei. Most nuclei will have a stippled appearance. In a few cells, the boundaries of the nucleus will be indistinct or absent, and bundles of dark-stained linear or V-shaped objects will be evident. These objects are the condensed **chromosomes** of cells that are undergoing cell division for growth (**mitosis**). The chromosomes have been copied and the two bundles are being separated prior to growth of a cell wall to divide the two new cells.

In what direction are the pairs of new cells separated— along the length of the root tip or across its width?

Where is cell division occurring? Are the dividing cells scattered uniformly throughout the root, or are they concentrated in a certain area? Is this area (**meristem**) at the very tip? Choose an average cell in this region and measure for the table on page 36.

Scan the slide from the root tip moving upward and note the length of the cells. How does cell length change with position in the root? Do you suppose that the cells further away from the meristem are older or younger than those close to it? Does diameter of the cells also change?

Given your observations, how do you think that roots are able to penetrate the soil? What do you suppose must happen to cells at the tip as it is pushed through the soil? Examine the cells at the tip— what do you notice about their condition? Would it be advantageous for the dividing cells to be at the very tip?

Now focus on the nuclei of the older, more elongated cells well above the root tip. In these nuclei, look for 1 or two darker areas— these are **nucleoli (singular nucleolus)** which are regions where **ribosomes** are being manufactured. Most eukaryote cells possess nucleoli, but they are not always visible.

### Tissues and organs

Examine a prepared slide of mammal intestine (ileum) in cross-section. This preparation is useful for illustrating cellular organization in a complex multicellular organism. The condition of “multicellularity” is more than multiple cells— it involves differentiation of cell types. There are over a hundred recognizable cell types in a mammal, each specialized for particular functions and dependent on the functions of the others. Specialization and cooperation among cells permits the development of complex body plans and larger body size, unlike a single-celled organism where one cell must carry out all processes.

Cells with similar functions are grouped into **tissues**, which in turn are combined to make **organs**. In this case, the organ is the small intestine, which is a hollow tube that takes up food molecules and transports them into the blood and lymphatic systems.

Working from the outside of the tube, the first layer of tissue is **longitudinal muscle**, followed by a layer of **circular muscle**. Note the orientation of the elongate muscle cells, which are called **muscle fibers**. The fibers are stained pink, while their nuclei are purple. Muscle fibers contain specialized **contractile proteins** which use ATP to forcefully shorten. Which layer would contract to shorten the intestine? Which would act to narrow the diameter?

Moving still further toward the lumen, you will encounter the **submucosa**, a thin layer

of connective tissue with scattered muscle fibers and blood vessels. On most slides, some parts of the submucosa will contain **lymph nodules**. This **lymphatic tissue** consists of phagocytic cells that appear dark purple because of the closely packed cell nuclei. The lymph nodules act as filters for the extracellular fluid and are important in preventing bacteria from entering the body.

The next tissue is the **mucosa**. The mucosa has projections called **villi (singular = villus)** that increase the surface area available for absorption of food molecules. Examine the surface of the villi– they are covered

with a glandular **epithelium**– a layer of similar cells that lines a surface in the body. Some of these cells are **goblet cells** that contain a large oval secretory vacuole and produce mucus. Measure a typical epithelial cell for the table on page 36.

Examine the villi at several points and try to visualize how the varied shapes could result from cutting these finger-like projections at various angles. Also look for smooth muscle fibers within each villus– they are capable of changing shape. Why is the intestine such a muscular organ?

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**Assignment– Cell size and surface area**


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**Prepare to hand in next time:**

- A. Record the length (L) and the width or diameter (W) of each of the following cell types examined in lab. Use units of micrometers.

		Ocular scale units		Micrometers		
		Power	L	W	L	W
1	<i>Oscillatoria</i>					
2	<i>Lactobacillus</i>					
3	<i>Elodea</i> cell					
4	<i>Elodea</i> chloroplast					
5	cheek epithelium					
6	<i>Paramecium</i>					
7	yeast					
8	root meristem					
9	gut epithelium					

- B. Make a sketch showing specimens #1-7 in outline, drawn to the same scale. These do not have to be fancy drawings– just outlines that indicate the size and shape of each cell type. You may superimpose the outlines to save space– be creative. Include a scale line showing what distance on your sketch is equivalent to 10 microns (0.1 mm).
- C. Using the dimensions that you determined (above) and assuming cylindrical shape, calculate the surface area, volume, and surface/volume ratio of a cell of 1) *Lactobacillus* and 2) *Paramecium* (there is a geometry link on the Biology home page, under “useful links”, in case you don’t remember the formulas for surface area and volume of a cylinder). Report the results of your calculations. Knowing how these two organisms feed (saprophy and phagocytosis, respectively) explain how their size is appropriate for their feeding habits.
- D. How does *Paramecium* increase the surface area available for bringing food molecules into the cytoplasm (hint– where were those yeast cells) ? How does a mammal increase the surface area available for bringing food molecules into the body?