

Background

You are used to seeing the world at a certain scale of observation. You are very familiar with the large, macroscopic world where your eyes cannot distinguish (resolve) objects less than half a millimeter or so. But there is an unseen world around you. This invisible world is teeming with a great diversity of life forms that become apparent when viewed through the lens system of a microscope. Today you will explore this unseen world, learn the fundamentals of microscopy, and then conduct an investigation with the microscope. You will learn how to use a compound microscope, which uses a system of two lenses to magnify a specimen. One lens, the eyepiece or ocular lens, can provide a modest amount of magnification (10x), but when this lens is used in combination with one of several objective lenses, the total magnification is the product of each individual lens (*i.e.* 10x ocular \times 40x objective = 400x total magnification).

A compound microscope also has another lens called the condenser. The condenser is found below the stage and is not involved with magnification. Instead, this lens focuses light on the specimen. The condenser may have an attached iris diaphragm, which can alter the amount of light on the specimen and thus plays a role creating contrast.

☞ Some History:
One of the first microscopes was used by a Dutch naturalist named Leeuwenhoek in the seventeenth century. His single lens scope could magnify an image 266 times.

THE LAB

1. Anatomy of a Microscope

Carefully remove a microscope from the cabinet and bring it to your desk (use two hands). Then make a rough sketch of the microscope in your lab notebook. As your instructor presents a mini-lecture on the parts of a microscope, you should label each part on your drawing and make a note on its function. Be sure that you can identify the following parts: base, stage, arm, ocular lens, objective lenses, coarse focus, fine focus, condenser, diaphragm, and light source control. A poster of a microscope, with the parts labeled, is available if you need more information.

2. Making Wet Mounts

The preparation of fresh, live material for view under a microscope is a basic skill in a biology laboratory. One technique to do this is called a wet mount. You will learn this skill by making a wet mount of your cheek cells (which are constantly renewed since they are frequently scraped off when eating). Use a clean toothpick and gently scrape the inside of your cheek to remove the cells and some saliva, and then smear the wet end of the toothpick across the center of a clean microscope slide. Since your cells are mostly water, and therefore transparent, a stain must be used to provide some contrast. The appropriate stain for these cells is called methylene blue. It could be added now, but there's another way (see below).

A prepared slide is like an Oreo®; there's the glass slide, the specimen and then a small cover slip. If you try to lay the cover slip flat over the specimen, you will likely trap air bubbles inside. First add water (or 0.9% NaCl) to make a penny-sized pool of liquid. Place an edge of the cover slip to one side of the pool, pull it closer until it contacts the liquid and then lower the other side from a 45° angle until it lies flat. The coverslip will be floating on the pool.

View your cheek cells at several different magnifications. Tinker with the diaphragm, and other settings, until you find the best view. Then draw your cheek cells and label any parts you can identify.

You can add a stain (or solution of salt or nicotine or alcohol) after the coverslip is already in place by placing a tiny drop of stain touching the liquid at one side of the coverslip. If diffusion isn't fast enough, touch a paper towel to the opposite side to draw liquid out of and dye into the space between slide and coverslip.

3. Resolution vs. Magnification

This exercise is to help you learn the difference between magnification and resolution—two elements of microscopy that can be at odds with each other. **Resolution** is the ability to see fine detail, or more formally, the ability to distinguish two discreet points (your instructor may demonstrate this). Every microscope has a limit for resolution. **Magnification**, on the other hand, is simply the enlargement of the object's image. Use the hole punch and take a sample from a colored newspaper picture and another from the magazine. Examine the pictures and look for ink dots. Then make a wet mount of both with a drop of water. Examine each slide with the low power objective (which is both magnified and resolved well), and then do the same but with medium power objective. Can you still see the individual dots that make up the image? Now try the same but with the high power objective. You progressively increased the magnification but what happened to the resolution? Is a highly magnified image always better?

4. From invisible to paper

Make wet mounts of the following on unlined paper:

Your cheek cells at 400x.

Human Red Blood Cells at 400x

Frog Red Blood Cells at 400x

Euglena at 100x or 400x.

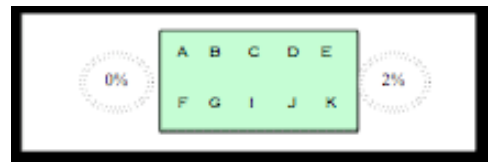
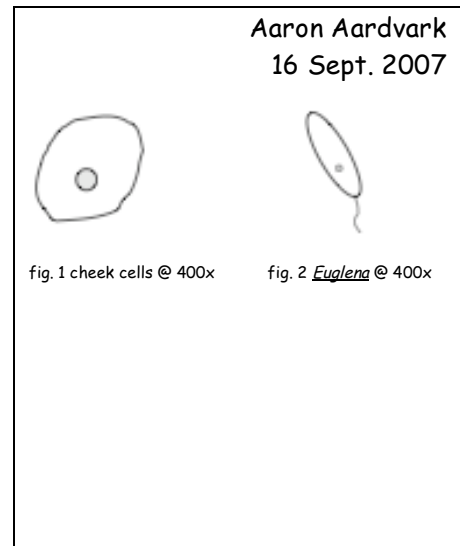
Use a pencil; a hard one is best (2H). Draw large enough to show all the detail you can see. Use the layout shown at right. Title each figure (sketch) as shown. Label any parts you can identify (nucleus, membrane, flagellum).

The freshwater protist *Euglena* faces osmotic challenges in seasonal streams and shallow cultures. Heavy rainfall can dilute the solute concentration in the water and consequently decrease the salinity, or conversely, sun exposure can increase the concentration of salts through evaporation. Multiple microhabitats, which are small areas with unique physical conditions, can exist within the same culture and pose additional problems. *Euglena*, a single eukaryotic cell, meets all of these challenges within the confines of one plasma membrane. Like many protists, *Euglena* can try to regulate its internal conditions in response to external ones.

Like plants, *Euglena* is photosynthetic, but capable of rapid movement over short distances and, if conditions change beyond its tolerance, *Euglena* can move to another microhabitat. **Question:** Is *Euglena* more tolerant to an increase or a decrease in salinity?

To do:

- Use a microscope, slides with wide coverslips, dropper bottles of distilled water and 2% salt solution, and *Euglena* culture to answer the above question.
- Read all of the instructions before you begin.
- Place enough *Euglena* culture on the center of a slide to float a wide coverslip. It is good that there be a little fluid peeking out around the edges of the coverslip.
- Decide which solutions are hypertonic and hypotonic to the culture water and add them by drops on either side of the cover slip to produce a concentration gradient that increases from left to right. The added drops must be in contact with the fluid under the coverslip.
- Remember that when you put the slide under the microscope that left and right will appear reversed.
- You can sample different areas under the coverslip perhaps using a grid like the one implied by the letters A through K in the diagram above.
- Thoroughly discuss the setup and possible modifications of it with your group. Formulate a hypothesis and decide what and how you will measure or count (your dependent variable). Develop a prediction about your hypothesis: what specific condition(s) must exist for you to accept the hypothesis? Finally, design an experiment to evaluate your hypothesis. Your experiment must include: enough data to make a confident conclusion
- Record all of the details of your experimental design in your lab notebook.



Discussion Questions

In the *Euglena* experiment, which treatment served as a control? Why is this so important in evaluating your results? Is *Euglena* more tolerant to an increase or a decrease in salinity? Support your conclusion with numbers and a graph. Describe the osmotic challenges faced by the *Euglena* in the hypertonic and hypotonic environments. Can you identify any sources of error in the experiment? Explain.