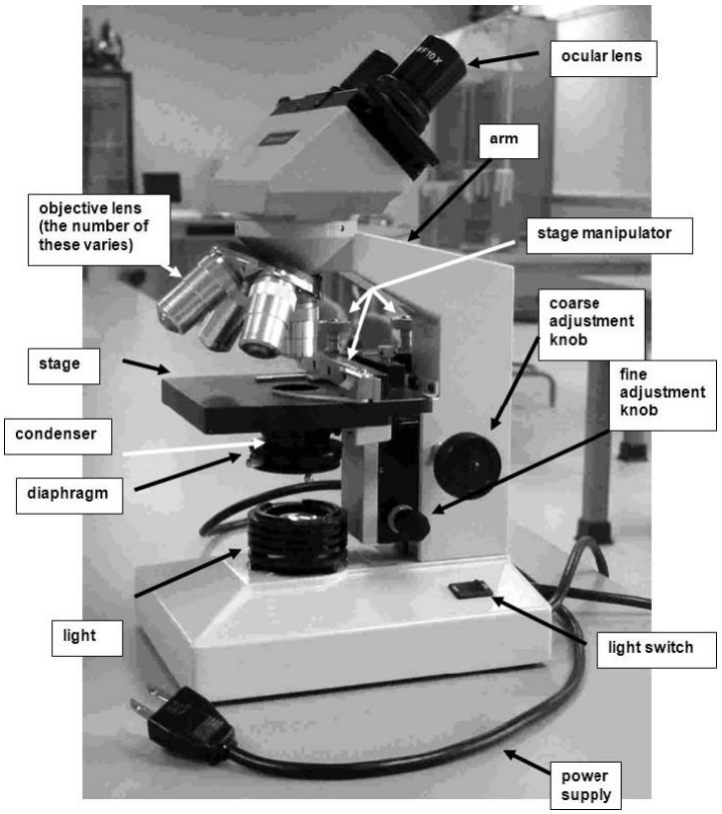
LABORATORY #3 -- BIOL 111
Microscope Use & Intro to Cells

Today you will need a microscope to observe cells. A good experience with a microscope requires good magnification in combination with sharp focus of what you observe. Focus and clarity in microscopy is called resolution. Specifically, resolution is the ability to distinguish two objects clearly. To resolve 2 objects, they must be separated by some minimum distance, i.e., the limit of resolution. If they are any closer they will appear as one object. The best human eyes have a limit of resolution of about 0.1 mm.

To observe objects that are too small to be seen (or resolved) with the naked eye, we must use a microscope. The simple purpose of the microscope is to observe phenomena that are too small to be seen clearly. All microscopes are designed to both magnify and to increase resolution. The limit of resolution of the light microscope is 0.2 μm (2/1000 of a millimeter) which is 500 times better than your eye. This means that many objects of biological importance, from cells and some subcellular structures to small tissues and organs are visible with the aid of a light microscope. You will see for yourself how the tissue of an onion, and a leaf of a plant are organized at the cellular level, as well as organelles of the individual cells. You will see individual cells making up the inner lining of your cheeks. Hopefully you will appreciate more generally, the common make up of all living things!

The compound light microscope
This microscope is used for magnifying objects, typically 100X (or "100 times larger than actual") to 1000X. There are several additional features of this microscope. First, there are two adjustment knobs: coarse and fine. **The coarse adjustment knob is only used when using the lowest power objective lens.** When the higher power objective lenses are used, focus using only the fine adjustment knob.



A magnifying glass is a simple microscope because it consists of only one lens. This microscope is called compound because it consists of two or more lenses. The two lenses on the microscope are called the ocular lens (the eyepiece, or lens closest to your eye) and the objective lens (the lens that is variable and can be changed). The ocular lens typically magnifies objects 10 times, whereas the objective lenses (used one at a time and mounted on a nosepiece) vary from 4 to 100 times.

As two or more lenses are used together, their effects are multiplicative; this means that a lens that magnifies objects 10 times used with a lens that magnifies objects 4 times will together magnify an object 40 times (4x X 10x = 40x). **Total magnification** refers to this magnification produced together by the ocular lens and the objective lens in use. **When discussing magnification, the user should be clear about whether "objective lens**

magnification" or "total magnification" is being used.

Measuring

It is easy to calculate the size of a microscopic object. All you need is the diameter of the circle of light and the number of objects which fit across this diameter. If, for example, the field diameter is 1mm and 10 objects fit across the diameter, then the size of the object is 1mm/10 = 0.1mm.

Today, we will use a total magnification of 400x, that is, the ocular lens is 10x and the objective lens is 40x (10x X 40x = 400x). At 400x, the field diameter is 0.45mm.

Note: there is almost no reason to use the 100X objective (or 1000X total magnification) in this course. This means that it would be really great if you didn't bother with the 100X objective lens!

Cells

The basic unit of life is the cell. Some organisms are composed of a single cell (e.g., a bacterium like *E. coli*) whereas others are multicellular (e.g., YOU). Regardless of what type of organism one considers, all are composed of cells and these cells are remarkably similar, from algae to anteaters. This means that examining a single cell can give you a picture of the cell anatomy of almost any living thing.

There are two classes of cells: prokaryotic ("before nucleus") and eukaryotic ("true nucleus"). Recall members of the domains Bacteria and Archaea are single celled and are of the prokaryotic cell type—and are thus are called "prokaryotes". All members of the domain Eukarya are composed of eukaryotic cells and are called "eukaryotes."

We will be examining eukaryotic cells (from plants and animals). Eukaryotic cells differ from prokaryotic cells in that eukaryotic cells have specialized compartments (like the nucleus) as well other membrane-bound structures with specific functions. The "little organs" of a cell are called organelles. In contrast, activities performed by prokaryotes occur in the single compartment of fluid called cytoplasm, surrounded by one cell membrane. Organelles allow eukaryotic cells to have a higher level or organization. In multicellular organisms, this increased level of organization allows further specialization of cells of different tissues. (Recall chapter 1---showing living things at different levels of organization.

We will examine plant (from onions, and an aquatic plant called *Elodea*) and animal cells (your own inner cheek cells of your mouth). When you examine the cells you will be able to see some cellular structures and organelles fairly easily, whereas some organelles are too small to see even with the highest magnification of your microscope. (But you will learn about them in lecture—and by studying chapter 4.

We examine both plants and animal cells and look for differences in structures and organelles. In contrast to animals, plant stay in one place, and their cells make up rather rigid structures and thus have cell walls which restrict changes in shape of the cells. Plants also perform photosynthesis in some tissues, which requires special organelles called chloroplasts, which contain green chlorophyll. As you will notice, plant cells almost look "empty", having a large central organelle for storage of polysaccharides. These compartments are called vacuoles.

Procedure
Using the directions provided to you by your instructor, prepare three slides:
One with onion skin (epidermis), one with an *Elodea* leaf, and one with human cheek cells.

Slide 1. Using a drop of IKI (iodine in potassium iodide) prepare a wet mount slide of onion skin from the inner side of a bulb leaf. **The sample must be extremely thin!!** Observe the cells at low, medium and high magnification. Address question 1.

Slide 2. Using a drop of water, prepare a wet mount of an *Elodea* leaf. Observe the cells at low, medium and high magnification. Address question 2.

Slide 3. Prepare a slide of cheek cells stained with methylene blue. Observe the cells at low, medium and high magnification. Address question 3.

Name_____

Requirements Lab 2
READ ALL QUESTIONS COMPLETELY. Use complete sentences to answer questions. Don't forget size units in your numerical answers.

1. *(5 pts)* On the back of this page, prepare a diagram of **one** onion skin cell. (label the magnification you are sketching from)
   a. Why did we stain the onion cells?
   b. Determine the length & width of a cell. Show your math!
   c. Determine the diameter of the nucleus. Show your math!
      (Include scale bars indicating your with and length measurements.)
   d. Label the cell wall, plasma membrane, and nucleus.

2. What organelles are present in the elodea leaf cells that were not in the onion skin cells? Why did we not have to stain to see these organelles? What do you think the main purpose of the elodea leaf cells is? Is this the main purpose of the onion skin cells? Explain. *(3 pts)*

3. After observing your cheek cells, how are they visibly different than the onion cells? How are they the same? Determine the diameter of your animal cells. Which cells are larger: cheek cells or onion cells? Show your math! *(3 pts)*

4.
On the diagram of the plant leaf cell, label the cell wall, plasma membrane, nucleus, chloroplasts and mitochondria. What is the function of the nucleus? (This is a cell…not an atom!). What is the purpose of the vacuole?