

LAB 3 – Use of the Microscope

Introduction

In this laboratory you will be learning how to use one of the most important tools in biology – the **compound light microscope** – to view a variety of specimens. You will also use a slightly different type of light microscope called a **stereoscopic dissecting microscope**.

The first lens used to magnify things was developed in the first century A.D. These were pieces of glass shaped in a convex form – thicker in the middle and tapering off to the sides – and were the first **magnifying glasses** that could increase the image of an object about 10 – 20 X. The creation of glass lenses improved dramatically at the end of the 16th century, vastly improving the magnifying power. By 1609, Galileo Galilei refined the methods of lens making in an effort to view objects in the sky.

About half a century later, the Dutchman Anton van Leeuwenhoek further improved the art of lens making, allowing him to view objects in pond water that had never been viewed by humans – **microorganisms** – life at a tiny level. At the same time, an English physicist named Robert Hooke improved the technology of van Leeuwenhoek and confirmed the existence of tiny organisms in pond water. He also famously examined a piece of cork and observed tiny boxes arranged in such a way that they looked like the “cells” (rooms) in a monastery if you removed the roof and looked in from above.

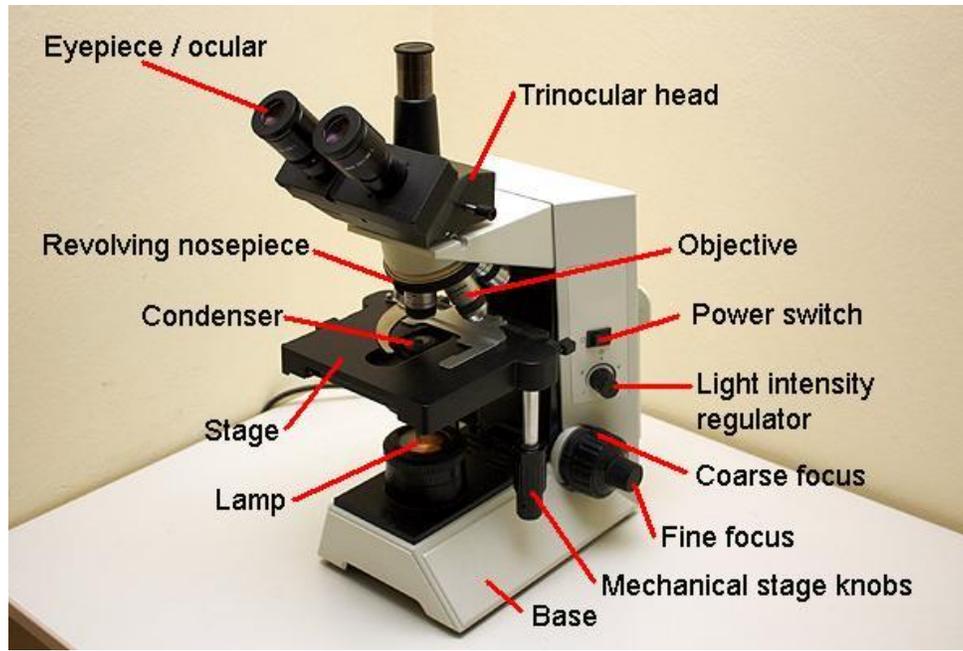
Today the best compound light microscopes are able to magnify objects up to 2,500X without losing their **resolution** – the sharpness of the image itself.

Part 1: THE COMPOUND LIGHT MICROSCOPE

The Parts of the Compound Light Microscope

***Exercise 1A – Getting familiar with the microscope**

You will first get acquainted with the major parts of the compound light microscope before learning the proper way to use it. Get a microscope from the cabinet below your lab bench, being sure to handle it by the arm and base (refer to image on page 2), and place it on the bench in front of you. Remove the cover and place it below, out of the way, and then plug in the microscope. The ocular lens (eyepiece) and stage should be facing you. Read the description of each part of your microscope on the next two pages being sure to follow all instructions, and then complete the matching exercise on your worksheet.



OCULAR LENS (eyepiece) – Your microscope will have either one (monocular) or two (binocular) **ocular lenses**. These are the lenses you will look through when examining a specimen with the microscope. Take a look at the side of your ocular lens and you will notice a label of “10X”. This indicates that each ocular lens magnifies the image by a factor of 10 or 10X.

OBJECTIVE LENSES – Notice the set of **objective lenses** on the **revolving nosepiece**. These lenses allow you to change the degree of magnification. Some of our microscopes have four objective lenses while others have only three. The degree of magnification for each objective lens is indicated on its side. Let’s take a look at each progressing from the shortest to longest objective lenses, being sure to rotate the revolving nosepiece to click each objective lens into position above the stage before examining it:

4X – This objective magnifies the image by a factor of 4. It is referred to as the “**scanning objective**” since it is used to scan the slide to locate the specimen before viewing it at higher magnification. Your microscope may not have this objective lens, in which case you can begin with the 10X objective.

10X – This objective magnifies the image by a factor of 10 and is referred to as the “**low power**” objective.

43X (or 45X) – This objective magnifies the image by a factor of 43 (or 45) and is referred to as the “**high power**” objective.

100X – This objective magnifies the image by a factor of 100. It is referred to as the “**oil immersion objective**” since it requires a drop of immersion oil on the slide to provide good resolution. *You will not be using this objective lens.*

For now, make sure that the low power objective is clicked into position above the stage, and keep in mind that you will only be using the low power and high power objectives. *Also keep in mind that the **total magnification** of any image you see through the ocular lens is the product of the objective and ocular lens magnifications (for example, when using the lower power lens the total magnification is: 10X ocular x 10X low power objective = 100X).*

STAGE and STAGE CLIP – The **stage** is the flat surface upon which you will place each slide you will examine. Notice that there is a moveable **stage clip** that can be used to secure the slide on the stage. Open and close the stage clip to see how it will snugly hold your slide in position.

MECHANICAL STAGE KNOBS – To move the slide on the stage when it is secured in the stage clip, you will use the **mechanical stage knobs** on the underside of the stage to move the slide backward/forward and right/left. Adjust each knob to see how one knob controls backward/forward movement and the other knob controls right/left movement.

COARSE FOCUS and FINE FOCUS KNOBS – In order for a specimen on a slide to be in focus, the distance between the specimen and the objective lens must be just right. The **coarse focus knob**, the larger of the two, will move the stage or objective lens (depending on the microscope) up and down quickly and quite visibly, altering the distance between them. *It is very important that the coarse focus knob is only used with the low power or scanning objective lenses, otherwise the microscope or objective lenses could be damaged.* Adjust the coarse focus knob to observe how quickly the focal distance changes. In contrast, the **fine focus knob** will move the stage or objective lens such a small amount that it is hardly noticeable to the naked eye. This is the knob you will use to get the perfect focal distance so the image will be crystal clear.

CONDENSER LENS – Just underneath the stage is the **condenser lens**. This lens serves to capture and focus light from the lamp below onto the slide mounted on the stage. On many microscopes the condenser lens can be adjusted up or down with a knob beneath the stage. Examine the condenser on your microscope to see if it is adjustable. If so, be sure to adjust it as high (close to the stage) as possible since, for our purposes, this is where it should be set.

DIAPHRAGM – The **diaphragm** is located within the condenser and is one of the most important pieces of the microscope, though it is often neglected by many students. The diaphragm allows you to adjust the amount of light passing through the slide by adjusting the **diaphragm lever**. Most of the time the diaphragm will be all the way open to allow the maximum passage of light. However it is important to adjust the diaphragm at times to reduce the amount of light passing through your specimen should the image be too bright or dim, and also to increase the **contrast** to allow you to see the specimen more easily against the background. For now, open the diaphragm all the way, and when using the microscope, *do not forget to use the diaphragm.*

LAMP – The **lamp** emits light to illuminate the specimen so that you can actually see something.

BASE and ARM – The **base** is the bottom of the microscope that sits on the table, and the **arm** is the vertical framework ascending from the base along the back of the microscope. *When handling the microscope always hold the arm while supporting the base with your other hand.*

Proper Use of the Compound Light Microscope

***Exercise 1B** – Steps to follow when using the microscope

If you really want to be able to see a specimen on a slide, you must follow the steps on the next page *every time you look at a new slide*. The microscope will be your friend if you always use the following steps in their proper order. Before you begin, be sure your microscope is plugged in and the power is “on”. Before you start, clean all of the lenses with special **lens paper** which is soft enough to not scratch the lens. *Do not use anything else for this purpose (paper towel, shirt, backpack.....) or you will scratch the lenses.*

- Step 1.** Get a slide of the letter “e” from the tray on the side counter. This an example of a **prepared slide**, a slide that is already made for you and meant to be reused. (*i.e., don’t dispose of it, please return it to the tray when you are finished!*)
- Step 2.** Use a piece of lens paper to clean any smudges (fingerprints, grease, etc.) off the slide. Place the slide on a white piece of paper find the specimen (the letter “e”) on the slide with your naked eye, noticing its location and orientation.
- Step 3.** Lock the low power objective lens into place (it should “snap” into place) if you have not already done so. *You will always (always, always, always.....)* start with either the **low power** or **scanning** objective when you want to view a slide.
- Step 4.** Use the coarse focus knob to move the stage (or objective lens) so that they are as *far apart* from each other as possible. Open the stage clip and place the slide snugly in the corner of the stage clip (make sure the slide is completely flat) before releasing the clip to hold the slide firmly in place. Then use the mechanical stage knobs to position the slide so that the specimen (*i.e., letter “e”*) is centered over the condenser and the light that passes through it.
- Step 5.** Next, using the coarse focus knob once again, move the slide and objective lens as *close* together as the knob will allow.

(NOTE: To this point, you have not yet looked into the oculars. This may be surprising, but this is the proper way to use a microscope so that you will actually see something!)

- Step 6.** Now, look into the ocular lens(es). Using the coarse focus knob, **SLOWLY** increase the distance between the slide and objective until the specimen is in focus.

If the light is too intense, adjust the diaphragm lever (or dial near the lamp if present) until the light level is comfortable before trying to locate the specimen.

If you have difficulty locating and focusing on your specimen (the letter “e”), make sure that it is properly centered and you may need to adjust the course focus more slowly. If you still can’t locate it, ask your instructor for assistance.

- Step 7.** Adjust the diaphragm lever so there is sufficient **contrast** between the specimen and the background, closing it no more than is necessary. *This step is especially important for live specimens since you may not be able to see them otherwise.*

- Step 8.** Now use the fine focus knob to get the specimen in proper focus. *You should now be able to see the object clearly.* Before going to the next step (increasing the magnification), be sure to center your specimen in the field of view as best you can.

- Step 9.** Now that you have centered and focused the object as best you can at low power, rotate the high power objective into place over the slide being sure it “clicks” into position. Use the **fine focus knob (NOT the coarse focus)** to bring the object into perfect focus.

(NOTE AGAIN: You should only use the coarse adjustment knob with the low power objective)

FOLLOW THESE STEPS EVERY TIME YOU WANT TO VIEW A NEW SLIDE
AND YOU WILL BECOME A GOOD MICROSCOPIST!

Part 2: PROPERTIES OF LIGHT MICROSCOPY

In this section we will focus on some of the key properties relating to light microscopy. To help you understand each property you will first read an explanation and then do an exercise to illustrate that particular property. Let us begin with the property of magnification...

Total Magnification

The total magnification of an image is quite simple – it is the product of the ocular lens magnification times the magnification of the objective lens you are using:

$$\text{magnification of ocular} \times \text{magnification of objective} = \text{total magnification}$$

For example, if the ocular lens magnifies the image by a factor of 10 (10X), and the objective lens magnifies the image by a factor of 50 (50X), the total magnification of the image is 500X:

$$10X \times 50X = \underline{500X}$$

Many students make the mistake of adding the two magnifications, so remember that total magnification is the product (multiplication) of the ocular and objective lens magnifications.

***Exercise 2A – Determining total magnification**

On your worksheet, calculate the total magnifications for the examples given, then calculate the total magnification when using each of the objective lenses on *your own* microscope.

Field of View (optional)

The **field of view** (FOV) is the actual “circle” you see when looking in the microscope. Although this circular field of view appears to be the same no matter which objective lens you are using, this is not the case. The circular area you are actually viewing will *decrease* as you increase the magnification:

<u>total magnification</u>	<u>field of view</u>
40X	
100X	
450X	
1000X	

A good analogy is to imagine yourself viewing the Earth from space as you gradually move closer and closer to Mission College. Initially your field of view is the entire western hemisphere, but as you approach the Earth's surface your field of view will progressively shrink to encompass the western United States, Southern California, the San Fernando Valley, Sylmar, etc. Although your field of view is shrinking, the image in your field of view is becoming increasingly magnified. This is really no different than looking into your microscope at increasing levels of magnification.

It is also useful to know the **diameter of the field of view** (FOV diameter) at a particular magnification, since you can use this information to estimate the size of the specimen you are viewing. The FOV diameter at low power for your microscope (100X) is ~1.8 mm. Using this FOV diameter, you can calculate the FOV diameter at other magnifications. This is done by multiplying by the ratio of the magnifications:

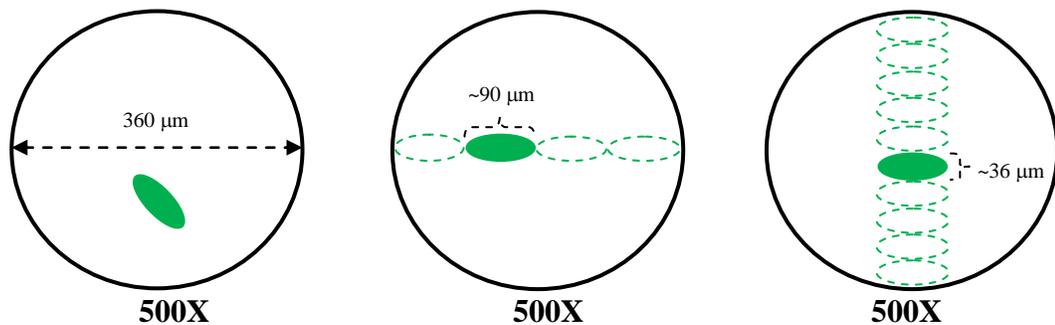
$$\text{known FOV diameter} \times \frac{\text{total mag. (known FOV)}}{\text{total mag. (unknown FOV)}} = \text{unknown FOV diameter}$$

If you want to know the FOV diameter at 500X, you could calculate it as follows:

$$1.8 \text{ mm} \times 100\text{X}/500\text{X} = 1.8 \text{ mm} \times 1/5 = 0.36 \text{ mm} = \underline{360 \mu\text{m}}$$

Once you know the FOV diameter, you can estimate the dimensions of your specimen. For example, assume you are viewing the specimen below at 500X total magnification and, based on your calculation above, you know FOV diameter to be 360 μm . It appears that ~4 of your specimens would fit across the FOV end to end (i.e., length = 1/4 of FOV), and ~10 side to side (i.e., width = 1/10 of FOV). Thus you would estimate the dimensions of your specimen to be:

$$\begin{aligned} \text{LENGTH} &= 1/4 \times 360 \mu\text{m} = \underline{90 \mu\text{m}} \\ \text{WIDTH} &= 1/10 \times 360 \mu\text{m} = \underline{36 \mu\text{m}} \end{aligned}$$



***Exercise 2B – Field of view and estimating size (optional)**

Before you can estimate the size of a microscopic specimen, you must first determine the diameter of the field of view at the magnification you are using. Once you have that information you are prepared to estimate the size of any specimen you observe at that magnification:

- 1) Calculate the FOV diameter for each possible total magnification on your microscope given the FOV diameter at low power (100X) is 1.8 mm.
- 2) Examine a prepared slide of *Paramecium* at low power and estimate the length and width of a single *Paramecium*.
- 3) Examine a prepared slide of *Euglena* at high power and estimate the length of a single *Euglena*.

Depth of Focus (optional)

Once you have a specimen in focus under the microscope, if you adjust the fine focus knob up and down the specimen will come in and out of focus. Thus, there is a range in the vertical dimension in which the specimen on your slide will appear in focus. The “thickness” of the vertical range in which the specimen remains in focus is referred to as the **depth of focus**. As it turns out, the *depth of focus decreases* as the *magnification increases* as illustrated below:

<u>total magnification</u>	<u>depth of focus</u>
40X	
100X	
450X	
1000X	

To make sure this concept is clear, imagine the range in which you can adjust the distance between the objective lens and the slide (via the focus knobs) to be a loaf of bread standing on end. The image produced in your microscope will only be in focus if the objective lens is positioned within a particular slice of that loaf of bread. This slice of bread is the depth of focus, and it will get thinner as you increase the magnification.

This property of microscopy becomes very noticeable if the specimen you are examining is actually thicker than the depth of focus at the magnification you are currently using. For example, if the depth of focus is only 1 μm thick and the specimen you are observing is 2 μm thick, there will always be a portion of the specimen outside the depth of focus. This portion will thus be out of focus and cause the image to appear blurry no matter how carefully you adjust the fine focus knob.

<u>observed specimen</u>	<u>magnification</u>	<u>depth of focus</u>	<u>image</u>
	high power		blurry
	low power		focused

In this example, the image will look blurry when viewed at high power magnification no matter what you do. To get a focused image in this case you will have to increase the depth of focus and thus lower the magnification.

To help you understand and appreciate the concept of depth of focus, complete the exercises that follow:

***Exercise 2C – Depth of focus in the vertical dimension (optional)**

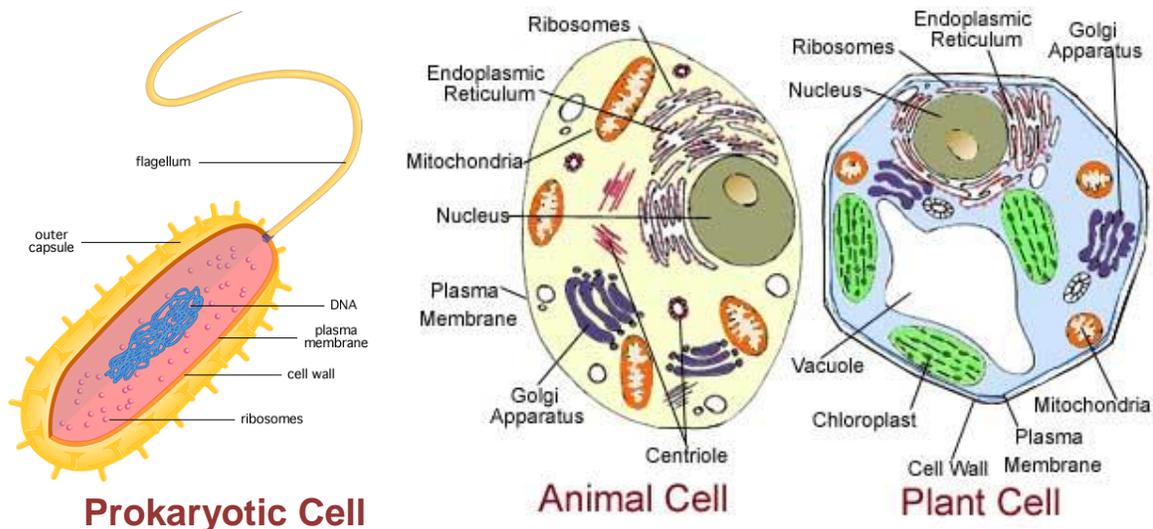
Obtain prepared slides of *Paramecium* and “colored threads” and observe them as follows:

- 1) Observe a single *Paramecium* at low power (100X) and then at high power (430X or 450X), and answer the corresponding questions on your worksheet.
- 2) Examine the colored thread slide at low power (100X), and determine the vertical order (top to bottom) of the three colored threads as you *slowly* adjust the focus up and down through the threads.

Part 3: A MICROSCOPIC VIEW OF CELLS

Review of Cell Structure

All living organisms consist of one or more cells and come in a tremendous variety. There are single-celled prokaryotic organisms such as the bacteria, single-celled eukaryotic organisms such as the protozoa (e.g, *Paramecium*) and yeasts (a type of fungus), and multicellular eukaryotes such as most fungi (e.g., molds, mushrooms) and all members of the plant and animal kingdoms. Before you examine cells from some of these organisms, let’s review some of the general features of our three basic cell types:



Notice that a prokaryotic cell does not have any distinct internal compartments. This does not mean that prokaryotic cells have no internal organization, they simply do not have any structures we refer to as **organelles**. In contrast, animal and plant cells contain a variety of organelles. Take a moment to review the functions of the various cell structures and organelles shown and then complete the next exercise on your worksheet:

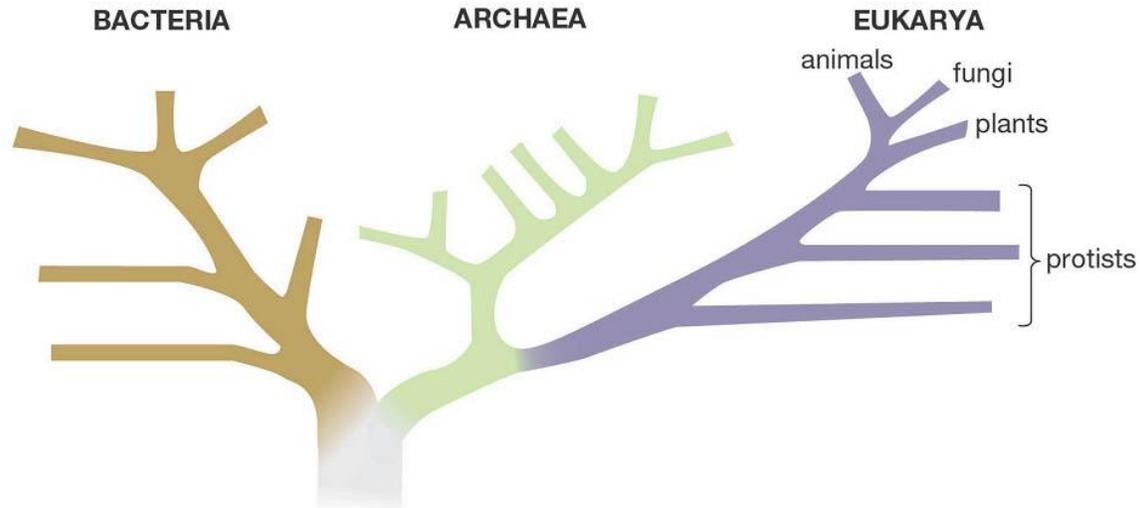
<u>STRUCTURE/ORGANELLE</u>	<u>FUNCTION</u>
plasma membrane	barrier between inside/outside of cell
cell wall	extracellular structure that protects and supports cell
capsule	protective gelatinous outer layer of some prokaryotes
flagellum	large cellular extension used for motility
nucleus	organelle containing the genetic material (DNA)
endoplasmic reticulum	synthesis of lipids, proteins in “secretory pathway”
Golgi apparatus	modification, sorting of “secretory pathway” proteins
mitochondria	organelle in which cellular respiration occurs
chloroplast	organelle in which photosynthesis occurs
central vacuole	storage of water and other materials in plant cells
centrioles	structures involved in animal cell division
ribosomes	small structures that carry out protein synthesis

***Exercise 3A – Review of cellular structures and organelles**

- 1) Fill in the correct labels for each cell diagram on your worksheet.
- 2) Complete the matching exercise on your worksheet relating each cell structure and organelle with its function in cells.
- 3) Fill in the chart on your worksheet indicating which structures and organelles are found in which cell types.

Now that you are well acquainted with the structures and organelles found in cells, you will use your microscope to observe cells of organisms in most of the major groupings. The most comprehensive groupings are the **domains**, of which there are three: the **Bacteria**, **Archaea** and **Eukarya**.

All species in the domains Bacteria and Archaea are single-celled prokaryotes. Until recently, the bacteria and archaea were grouped into a single kingdom called Monera. However, research in the last few decades has revealed that these organisms, though similar in microscopic appearance, are vastly different in DNA sequence and physiology. As a result they are now placed into entirely different domains. The domain Eukarya consists of all eukaryotes – i.e., organisms made of cells with a nucleus and other organelles. Within the domain Eukarya are the four traditional kingdoms still in use: **Protista**, **Fungi**, **Plantae** and **Animalia**.



Let us now take a look at cells from organisms in these major groups.

Bacteria

By far the most abundant organisms on our planet are the single-celled prokaryotes known as the **bacteria** and **archaea**. Since archaea are very difficult to culture in a laboratory setting, the only prokaryotes you will observe are bacteria. You will observe two species of bacteria, one having a round or **coccus** shape and the other having a rod or **bacillus** shape. Since bacteria are much smaller than eukaryotic cells, you will need to observe them at 1000X under the oil immersion lens in order to see them clearly.

***Exercise 3B – Examining bacteria**

Slides showing two distinct types of bacteria have been set up on the side counters. The bacteria are not alive and have been stained different colors to add contrast and help you distinguish between the two types.

- 1) There should be two distinct types of bacteria on your slide. Draw a sample of each type of bacteria on your worksheet and answer the corresponding questions.

Protozoa

The kingdom **Protista** consists of two general types of eukaryotic organisms: the **protozoa** and the **algae**. The protozoa (literally “before animals”) are single-celled eukaryotes that have “animal-like” qualities, whereas the algae are photosynthetic organisms that do not have the features of true plants. In the next exercise, you will observe a variety of live protozoa as you learn how to prepare your own **wet mounts** of these organisms:

***Exercise 3C – Examining protozoa**

You will prepare three different **wet mounts** of live protozoa as outlined below: *Paramecium* (view at low power), *Euglena* (view at high power) and a sample of **pond water or hay infusion** (low or high power). The protozoa you will see can move quite fast under the microscope, so prepare each wet mount as follows to ensure that they are slowed enough for you to view them:

- 1) Place one drop of “protoslo” on a clean glass slide (*this will help slow the critters down!*)
- 2) Using a transfer pipet, add one drop of sample (from the bottom of the container) to the protoslo and slowly add a cover slip over the sample, *laying it down gently at an angle.*
- 3) Mount the slide on your microscope and prepare to view the slide at low power.
- 4) To help you find the level of focus for the protozoa you want to examine, focus on either a bubble or the edge of the cover slip.
- 5) Close the diaphragm lever *almost* all the way to increase the contrast, and locate a specimen. Unless you do this, there will not be enough contrast to see any specimens.

Yeast

The kingdom **Fungi** includes multicellular fungi such as molds and mushrooms, as well as single-celled fungi which are collectively known as the **yeasts**. Yeasts are immensely important to humanity. They are essential for producing certain foods and beverages (e.g., bread, beer, wine, chocolate), and have allowed scientists to effectively study the nature of eukaryotic cells and to produce commercial medicines such as insulin for diabetics. In the next exercise you will look at the species of yeast commonly referred to as “baker’s yeast” or “brewer’s yeast”: *Saccharomyces cerevisiae*.

***Exercise 3D – Examining yeast**

Your instructor will set up a wet mount of live yeast to be viewed at 1000X at your table. The dye methylene blue will be added to provide contrast between the yeast and the background.

- 1) Examine the slide for live yeast, which should look like “golden eggs” on a bluish background. You may also see some dead yeast cells which will be dark blue. Draw a few of the live yeast cells on your worksheet and identify the nucleus in each.

Plant Cells

The kingdom **Plantae** includes organisms such as mosses and ferns as well as the familiar cone-bearing plants (*Gymnosperms*) and flowering plants (*Angiosperms*). All plants are multicellular and sustain themselves by the process of photosynthesis. Most plants have distinct organs and tissues consisting of different cell types. Despite their differences, most plant cells have the same basic structures as illustrated on page 9. For the next exercise, you will observe live plant cells in a leaf from the aquatic plant *Elodea*:

***Exercise 3E – Examining plant cells**

Prepare and observe your own wet mount of a leaf from the aquatic plant *Elodea* as instructed below, and answering the corresponding questions on your worksheet:

- 1) At the front of the lab, obtain a clean glass slide and place a drop of water on it.
- 2) Using a pair of tweezers, break off a single *Elodea* leaf and place it in the drop of water on the slide, making sure it is as flattened out as possible.
- 3) Obtain a single glass cover slip and placing it at angle next to the leaf, *gently* and *slowly* lay it down over the leaf at an angle until it lies flat on top of it.
- 4) Observe the leaf at low power and draw a sample of what you see on your worksheet.
- 5) Observe the leaf at high power and draw several cells including whatever organelles you can see. Be sure the label the nucleus, chloroplasts, central vacuole, and cell wall.

Animal Cells

All species in the kingdom **Animalia** are multicellular, consisting of a wide variety of organs, tissues and cell types. Like the protists, fungi and plants, animals belong to the domain Eukarya since their cells have a nucleus and other organelles as shown in the diagram on page 9. The animal cells you will observe today will actually be your own (*Yes, you are a member of the kingdom Animalia!*).

***Exercise 3F – Examining animal cells**

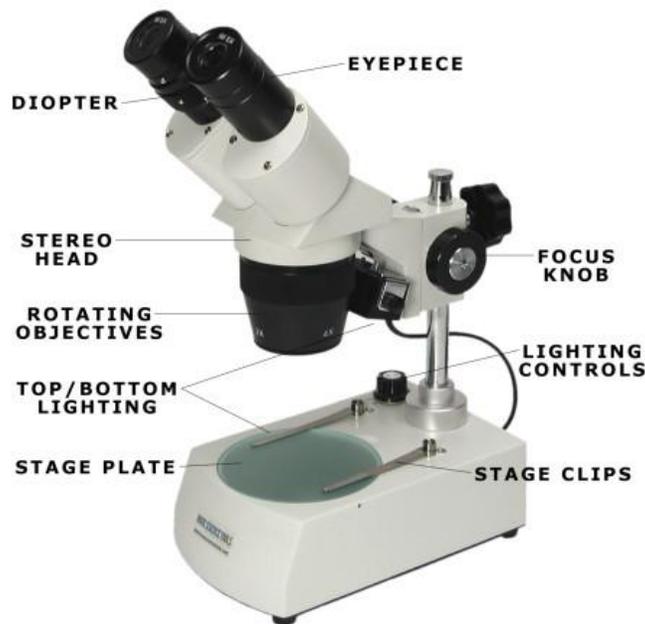
Prepare a wet mount of your own cheek cells as described below, and answer the corresponding questions on your worksheet. Since your cheek cells are transparent, you will add the dye methylene blue to provide contrast between your cells and the background.

- 1) At the front of the lab, obtain a clean glass slide and place one drop of water and one drop of the dye methylene blue on the slide.
- 2) Obtain a toothpick and *gently* rub one end of it on its side along the inside of your cheek.
- 3) Place the end of the toothpick that made contact with your cheek in the mixture of methylene blue and water on the slide, and move it around to transfer some cheek cells.
- 4) Discard the toothpick in the biohazard bag, and gently place a cover slip over the slide as you did before.
- 5) Observe your cheek cells at low and high power and draw samples of cheek cells you see at each magnification, being sure to identify the cell nucleus.

Part 4: THE STEREOSCOPIC DISSECTING MICROSCOPE

Up until now you have been exclusively using a compound light microscope. While it is ideal for viewing tiny microbes that can be mounted on a slide, there are biological specimens that are too large and/or thick to be mounted on a slide and viewed with the compound microscope (yet too small for the naked eye). In this case you will want to use the **stereoscopic dissecting microscope** or “dissecting microscope” for short. Two advantages of this microscope are 1) you can manipulate your specimen (turn, flip, dissect) using your hands or tools while viewing it under magnification (hence term “dissecting”), and 2) by looking through both oculars you can see the image in three dimensions (“stereoscopic”).

The dissecting microscope is a *simple* light microscope since the image you see is magnified through a single magnification lens. Your microscope has two such lenses that you can switch between, allowing you to view your specimen at 15X or 30X. While the total magnifications possible on this microscope are low, they provide the advantages of a very large field of view and a very thick depth of focus. This will allow you to see most, if not all, of your specimen clearly and in three dimensions.



Your dissecting microscope contains a single focus knob and two different light sources controlled by knobs on either side of the arm of your microscope. Turn them on and you will notice that one light source is below the stage and the other is above the stage. The light below the stage produces light that will pass through a transparent specimen, what we call **transmitted light**. The light above the stage produces light that will bounce or reflect off the specimen. We refer to this as **reflected light**, which is used to illuminate a non-transparent specimen from above.

You will first examine some 3-dimensional biological objects to get used to using the dissecting microscope, and then you will examine some samples of the fruit fly *Drosophila*. *Drosophila* has been an incredibly valuable organism for over a century in the study of genetic inheritance and embryological development.

***Exercise 4 – Using the stereoscopic dissecting microscope**

Examine the samples indicated below, and as you do so, adjust the lighting to give you the best image, and answer the corresponding questions on your worksheet.

- 1) Examine the letter “e” slide at 30X, noting its orientation viewed with the microscope relative to your naked eye.
- 2) To become more familiar with viewing specimens under the dissecting microscope, examine the objects provided on your bench at both 15X and 30X with transmitted and/or reflected light.
- 3) Using the tweezers provided, carefully and very gently place one *Drosophila* fruit fly from each container onto the stage, and note the eye color of each fly (*optional*).

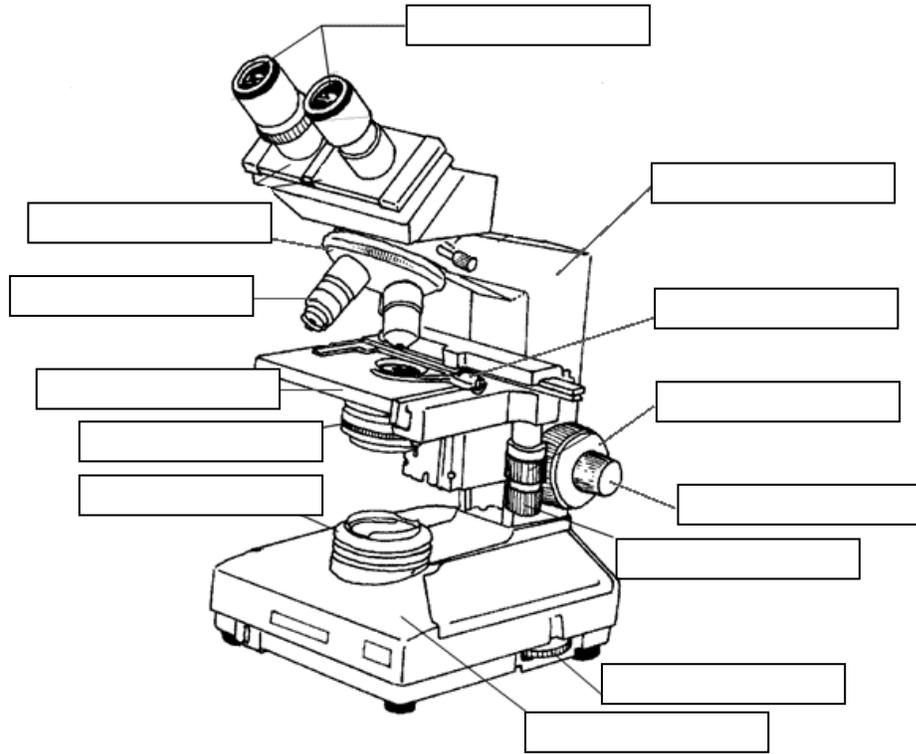
LABORATORY 3 WORKSHEET

Name _____

Section _____

Exercise 1A – Parts of the compound microscope

Write the correct label for each part of the microscope shown below:



Exercise 1B – Using the compound microscope

Match each part of the compound microscope on the left with its function on the right:

- | | |
|-------------------------------|---|
| ___ base and arm | A. eyepiece, what you look in to see an image |
| ___ coarse focus knob | B. adjusts position of slide left/right, front/back |
| ___ condenser lens | C. used to bring the image into sharp focus |
| ___ diaphragm | D. flat surface on which slide is placed |
| ___ fine focus knob | E. secures slide in place before viewing |
| ___ high power objective lens | F. focuses light from the lamp on the slide |
| ___ lamp | G. used only with the low power objective |
| ___ low power objective lens | H. used to handle the microscope properly |
| ___ mechanical stage knobs | I. adjusts the amount of light passing through slide |
| ___ ocular lens | J. used when you first examine a slide |
| ___ stage | K. light source used to illuminate specimen |
| ___ stage clip | L. used to produce a more magnified image |

Exercise 1B – Using the microscope

Answer the following questions as you work through this exercise:

How is the letter “e” on the slide oriented when you see it with the naked eye as you mount it on the stage (i.e., is it right side up or upside down)?

How is the letter “e” on the slide oriented when you see it under low or high power magnification?

What effect, if any, does the compound light microscope have on the *orientation* of the image?

Exercise 2A – Total Magnification

Fill in the charts below. For your actual microscope, you will find the magnifications of the ocular and objective lenses printed on the side of each lens. If your microscope does not have a scanning objective lens, leave that line blank.

<i>SAMPLES</i>			<i>YOUR MICROSCOPE</i>			
ocular lens magnification	objective lens magnification	total magnification	ocular lens magnification	objective lens name	objective lens magnification	total magnification
5X	50X			scanning		
5X	100X			low power		
10X	50X			high power		
20X	20X			oil immersion		

Exercise 2B (optional) – Field of View and Estimation of Size

Calculate the diameter of the field of view for each total magnification on your microscope in millimeters (mm) and then convert this value to micrometers (µm):

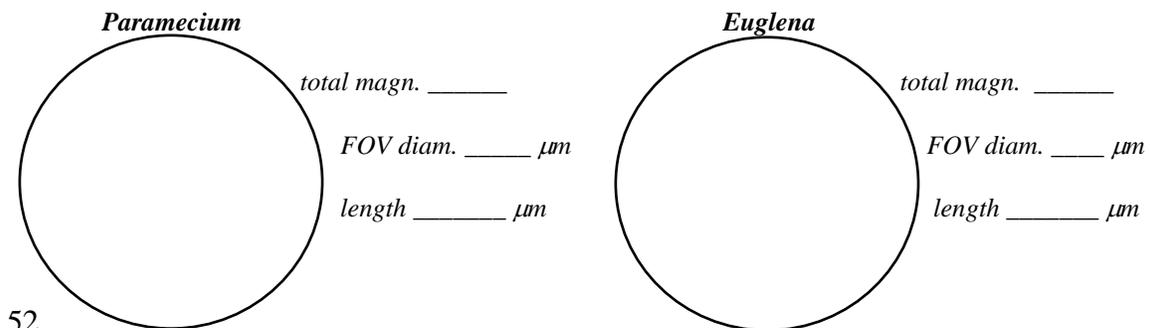
Scanning (40X): $1.8 \text{ mm} \times 100\text{X}/40\text{X} = \text{_____ mm} = \text{_____ } \mu\text{m}$

Low power (100X): $\text{FOV diameter} = 1.8 \text{ mm} = \text{_____ } \mu\text{m}$

High power (____X): $1.8 \text{ mm} \times 100\text{X}/\text{____X} = \text{_____ mm} = \text{_____ } \mu\text{m}$

Oil immersion (1000X): $1.8 \text{ mm} \times 100\text{X}/1000\text{X} = \text{_____ mm} = \text{_____ } \mu\text{m}$

Draw and estimate the length of a single Euglena (high power) and Paramecium (low power):



Exercise 2C (optional) – Depth of Focus

Answer the following questions as you complete this exercise:

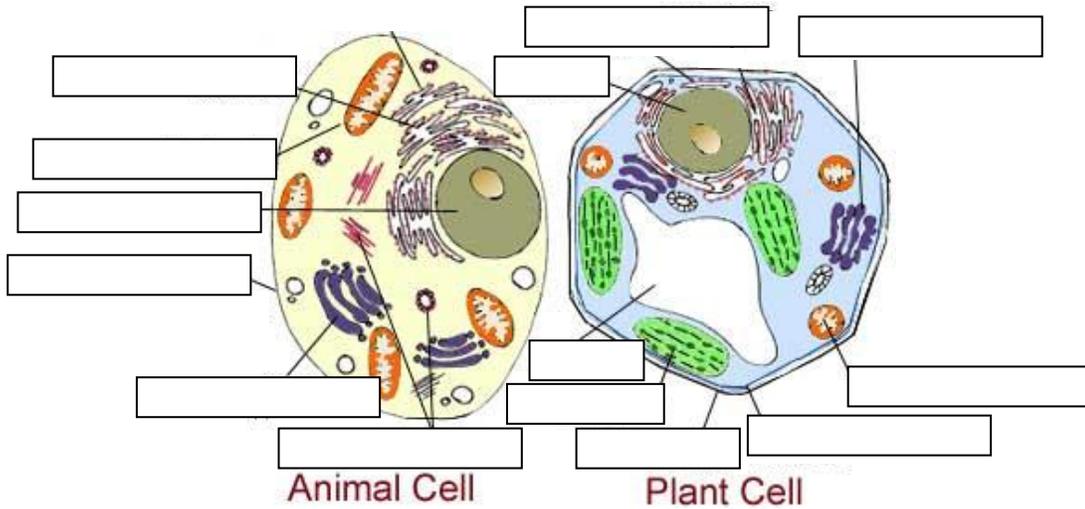
Describe the clarity of images of *Paramecium* at low power vs high power.

Explain why the image at high power was less clear than at low power (assuming that’s the case).

Indicate the order of colored threads on your slide from top to bottom.

Exercise 3A – Cell Structure

Label each structure for the plant and animal cell diagrams below:



Match each cell structure/organelle on the left with its function on the right:

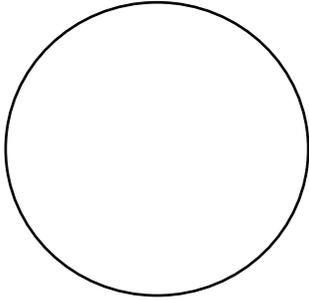
- | | |
|---------------------------|--|
| ___ nucleus | A. modification, sorting of proteins |
| ___ endoplasmic reticulum | B. where cellular respiration occurs |
| ___ Golgi apparatus | C. gelatinous outer layer of prokaryotic cells |
| ___ mitochondrion | D. small structure that carries out protein synthesis |
| ___ chloroplast | E. projection used for motility |
| ___ plasma membrane | F. synthesis of lipids, secretory pathway proteins |
| ___ cell wall | G. stores water and other materials in plant cells |
| ___ capsule | H. contains the genetic material (DNA) |
| ___ flagellum | I. where photosynthesis occurs |
| ___ ribosome | J. provides external support/structure in some cells |
| ___ centriole | K. barrier between inside/outside of cell |
| ___ central vacuole | L. plays an important role in cell division |

Place a check mark or “X” indicating a structure/organelle is present in the indicated cell type:

CELL TYPE	nucleus	endoplasmic reticulum	Golgi apparatus	mitochondrion	chloroplast	central vacuole	plasma membrane	cell wall	capsule
bacteria									
plant									
animal									

Exercise 3B – Bacteria

Draw samples of each of the two types of bacterial cells seen in the microscope, and be sure your drawing represents the arrangement of cells as well as cell shape.



Describe the arrangement of the round bacteria (**cocci**) relative to each other.

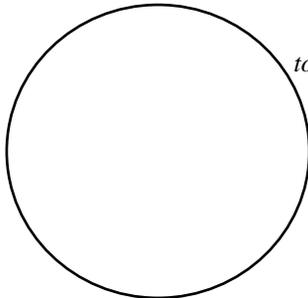
Describe the arrangement of the rod-shaped bacteria (**bacilli**) relative to each other.

total magnification **1000X**

Exercise 3C – Protozoa

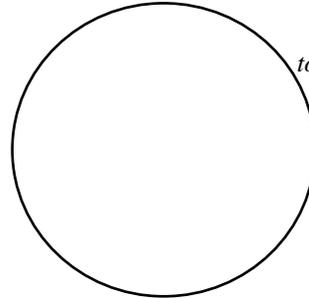
Draw samples of live *Paramecium* and *Euglena* as seen in the microscope

Euglena



total magn. _____

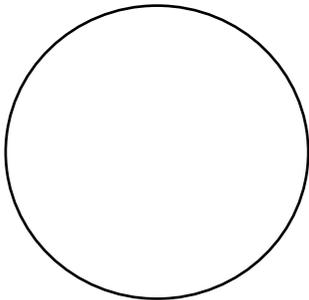
Paramecium



total magn. _____

Exercise 3D – Yeast

Draw samples of yeast cells seen in the microscope, and be sure to identify and label the cell nucleus.

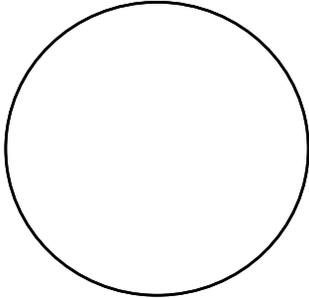


total magnification **1000X**

Exercise 3E – Plant Cells

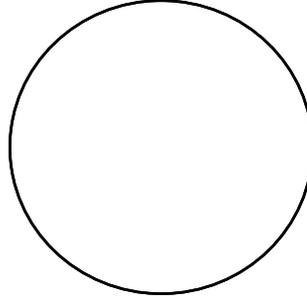
Draw Elodea cells at low and high power and estimate the length and width of a typical Elodea cell at high power:

Elodea (low power)



total magnification _____

Elodea (high power)

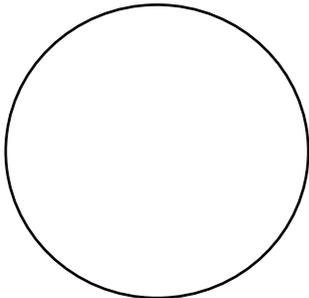


total magnification _____

Exercise 3F – Animal Cells

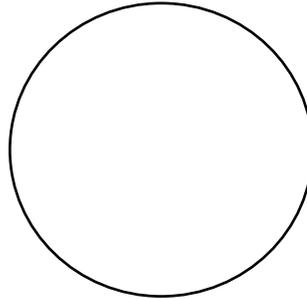
Draw several cheek cells at low and high power and estimate the length of a typical cheek cell at high power:

Cheek cells (low power)



total magnification _____

Cheek cells (high power)



total magnification _____

Exercise 4 – Dissecting Microscope

Answer the following questions:

How is the letter “e” oriented when viewed in the dissecting microscope relative to your naked eye?

How does this compare to the compound microscope?

OPTIONAL: Draw a sample of an object as seen under the dissecting microscope.

