

## Biology 1101 Lab

### Lab 3: Microscopes and Cells Lab

Name \_\_\_\_\_

Date \_\_\_\_\_

Lab safety: Full PPE required for this lab - lab coat, long pants, closed-toe shoes, gloves and safety goggles

#### Learning Objectives:

1. Properly clean and carry a compound microscope.
2. Focus a specimen using all objectives of a compound microscope.
3. Identify the parts of the compound microscope and explain the function of each part
4. Define magnification and resolution.
5. Compare and contrast animal and plant cells and be able to distinguish each type under the microscope.
6. Identify the following structures on the slides and explain the functions of each: plasma membrane, cytoplasm, nucleus, nucleolus, cell wall, and plastids (including chloroplasts and amyloplasts).
7. Properly prepare and view wet mount slides under the microscope.

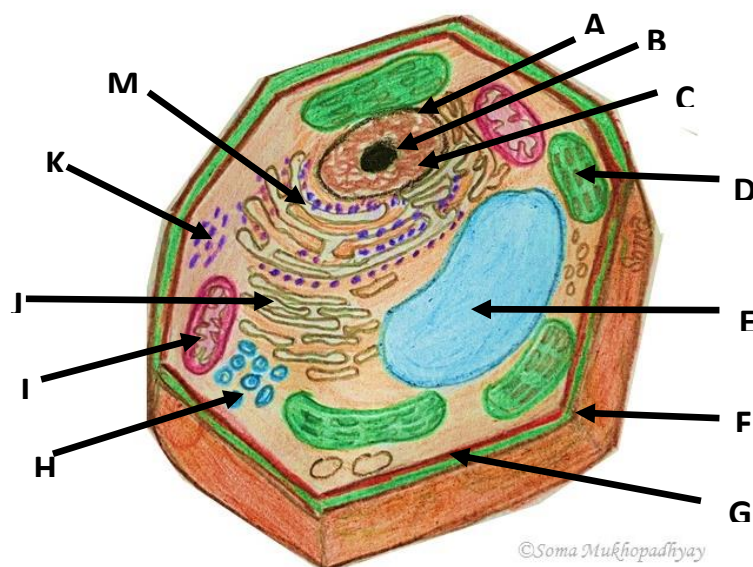


#### Pre-Lab Activity:

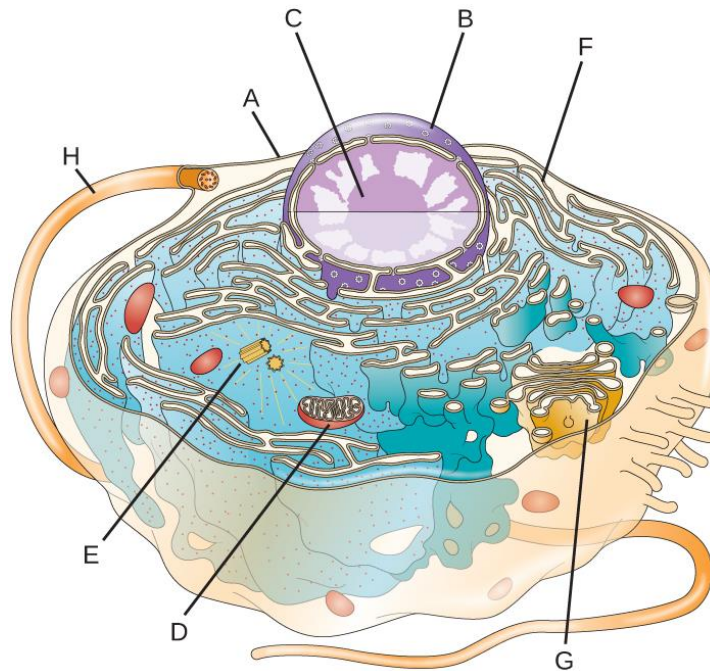
Prior to this lab you should complete the following using your current Biology textbook or refer to the following site: <https://openstax.org/books/biology-2e/pages/4-2-prokaryotic-cells>.

1. Label the diagrams below of typical plant, animal, and prokaryotic cells.  
(\*Note: these diagrams are not to scale).

a. Generalized Plant Cell:



## *b. Generalized animal Cell:*



## *c. Generalized Prokaryotic Cell:*

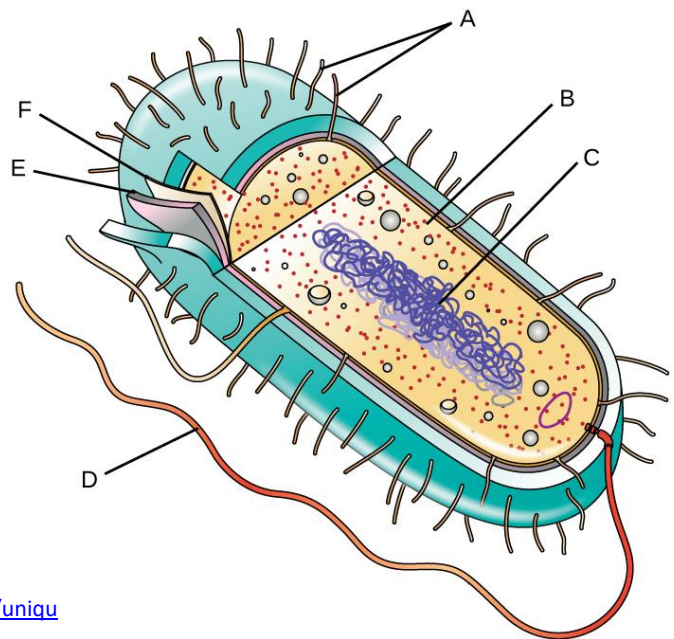


Photo credit (b & c)

<https://ecampusontario.pressbooks.pub/microbio/chapter/unique-characteristics-of-prokaryotic-cells/>

2. What do all three cell types have in common?
3. What is the main difference between the plant cell and the animal cell?

## Introduction Part A: The Compound Microscope

### *Care, Cleaning and proper handling of a Compound Binocular Microscope*

Since all microscopes are delicate precision instruments they must be well cared for. It is important to lift them from the cabinet with the use of two hands. One hand should grab the microscope by its arm and the other hand should be placed under the base of the microscope to ensure the microscope is firmly held. Since the microscope you will be using is also used by several other students you might have to clean the ocular lens and the objective lens for your use. To do this take a piece of lens paper and dampen it with the lens cleaning solution found at each table.

Note: **It is important to only use lens paper and not paper towels, since they will scratch the lens.**

## Activity 1: Using a Compound Microscope

### Materials Needed

For each student, you need:

- Compound Microscope
- Letter “e” slide

### Exercise A - Observation and Identification of the Parts of the Compound Binocular Microscope

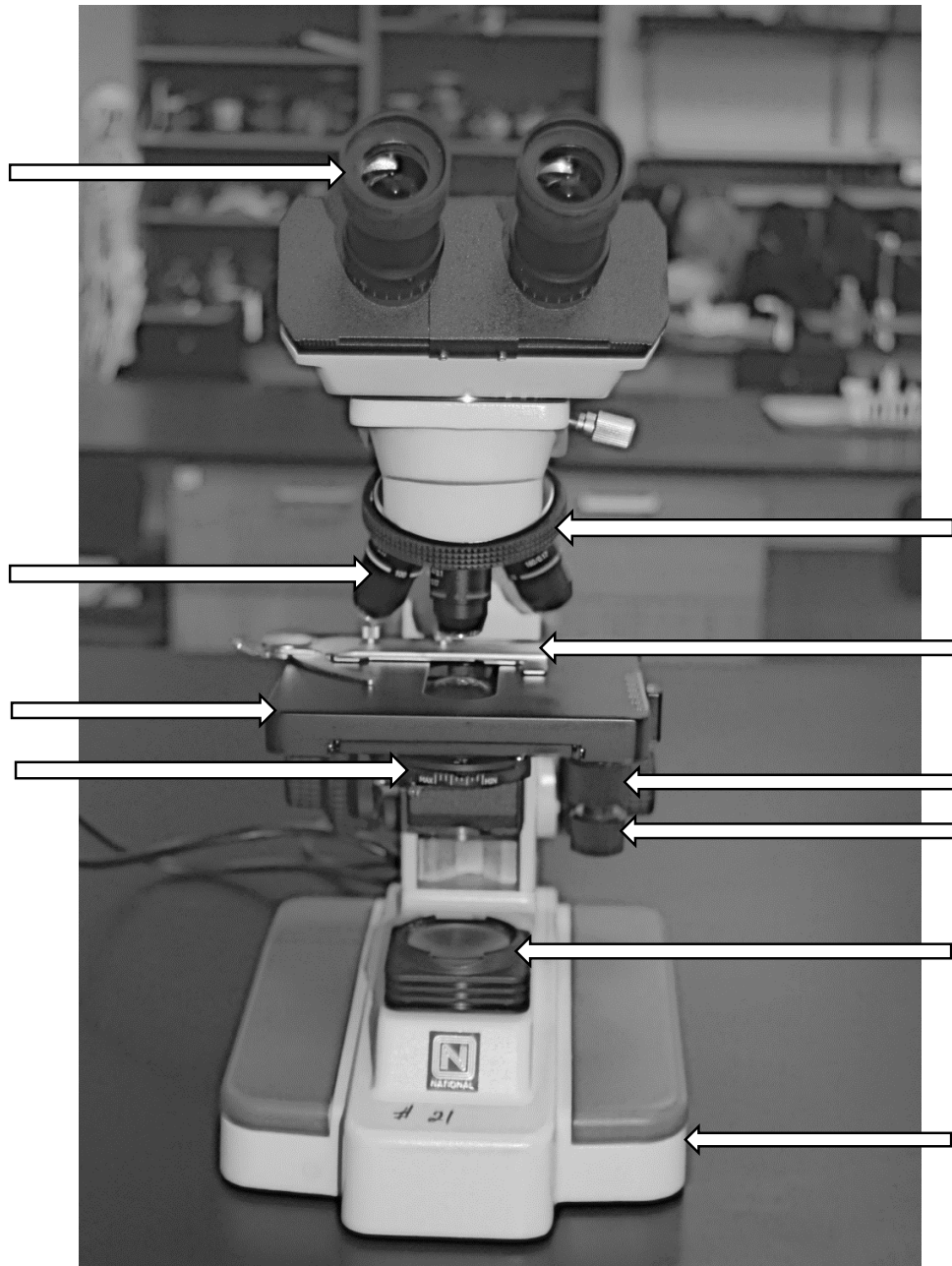
Now that you have the microscope carefully placed on your lab table, it is time to become familiar with the microscope's parts and their functions. To assist you in doing this you will need to find each part described below on the microscope. We will start at the base of the microscope and work our way up to the oculars. Find the parts and label those on the following Figures (1 & 2). Plug the microscope to the electrical outlet.

1. The **light source** is found in the **base** of the microscope (which bears the weight of the microscope). It is activated by turning on the **light switch** at the back of the microscope. The intensity of the light is adjusted by turning the **light intensity control knob** on the base.
2. The **iris diaphragm** is located just above the light source on the bottom side of the stage. Using the lever attached to it you can increase or decrease the amount of light reaching the specimen.
3. Between the stage and the iris diaphragm is the **condenser**. The condenser further aids in the focusing of the light onto the specimen. It can be moved up and down by the black knob called the **condenser knob** that is located on the right side of the stage. For the purpose of this class we do not need to change the position of the condenser.
4. Above the condenser lies the **stage**. It is mounted at a right angle to the arm and positioned just below the rotating nosepiece. The stage is where you will place your specimen. It is through the movement of the stage up and down that you will bring your specimen into focus.
5. Resting on top of the stage is the **mechanical stage**. This contains a spring clip that will hold the slide in place. To the right of the mechanical stage are two **control knobs** that allow you to move the slide left and right and backwards and forwards. This will enable you to look at all areas of the specimen.
6. At the back of the stage is the **arm** of the microscope that supports the head of the microscope. It is connected to the **base** and is a good place for you to grab hold of the microscope when you need to carry it or lift it out of its storage cabinet.
7. Attached to the arm are the coarse and fine adjustment knobs. These knobs move the stage up and down for the purpose of focusing the specimen. The **course adjustment knob** moves the stage a large visible distance with a single turn and as such should be used only with 4X and 10X objectives. It should

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**NEVER** be used with the 40X and 100X objectives. You run the risk of damaging these objectives and breaking the slide if you do not heed this warning.

8. The **fine adjustment knob** is used to move the stage up or down only very slightly. Since these scopes are **parfocal** (all the objective lens focus the image in the same plane), once you have focused your specimen at the 4X or 10X then when you progress to the next objective you will only need to use the fine focus to make the minor adjustment needed for the specimen to be in focus.
9. Above the stage and attached to the **rotating nose piece** are the four objective lenses. They are called objective lenses because they are closest to the object or specimen you are looking at. The magnification of the lenses are **4X** marked by a red ring, **10X** marked by a yellow ring, **40X** marked by a blue ring and **100X** marked by a white ring. 4X lens is referred as the scanning lens, 10X as the low





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power lens and 40X as the high power lens. The 100X is the oil immersion lens and must be used by placing a drop of immersion oil on the slide before clicking it into place. The 100X lens will **NOT** be used in this course. Please note that the length of the lens increases as their power of magnification increases.

Figure 1: Label different parts in the diagram

10. The image magnified by the objective lens in use is passed up through the body tube into the oculars. Each **ocular** contains two lenses for a total magnification of 10X. The total magnification of the microscope is the product of the ocular lens and the magnification of the objective lens in use. This means that if one has the 10X objective lens in place the total magnification that you will see is the product of  $10 \times 10$  or 100X. It is this combined magnifying power that makes this microscope a compound microscope.

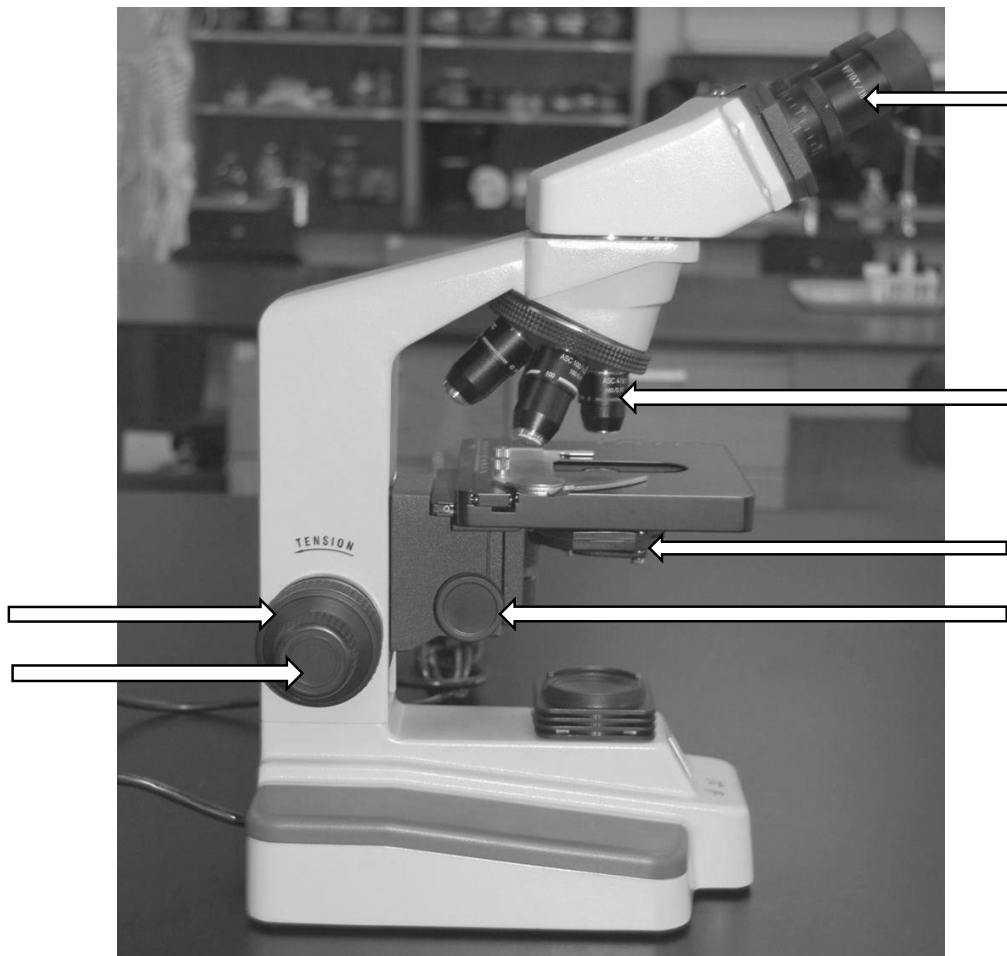


Figure 2: Label different parts in the diagram

### Exercise B - Calculating Total Magnification

Complete the table below to show total magnification for each objective lens.

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$$\text{Ocular Lens X Objective Lens} = \text{Total Magnification}$$

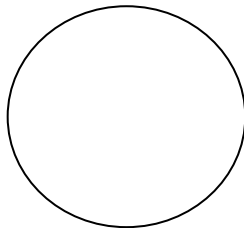
Calculate the total magnification for the remaining three objectives on the microscope below:

Ocular Lens Magnification	Objective Lens Magnification	Total Magnification
10X	4X (scanning)	40X
	10X (low power)	
	40X (high power)	
	100X (oil, will not use)	

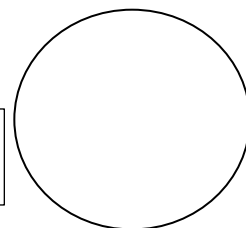
### Exercise C - Viewing a Specimen

- Using a piece of lens paper clean the **ocular lens**, each of the four **objectives**, the **condenser** (through the opening in the stage) and **the light source**.
- If the **4X objective** is not currently in line with the body tube rotate the 4X objective until it clicks into place.
- Plug in the microscope and turn on the light source.
- Either on your lab bench or on the table are some letter "e" slides. Open the **stage clip** and place one of these slides on the **stage** so that you can read the "e" as you look at the stage. Using the **stage control knobs** (hanging in a bar from the right side of the stage) move the slide until the "e" is positioned over the opening above the condenser lens and is illuminated by the light.
- Raise the stage** up as close as it will go to the objective using the **coarse adjustment knob**. The microscope has an automatic stop built in to prevent the slide from hitting the 4X (please note this stop will not prevent the slide from hitting the 40X or 100X objectives). **As you look through your ocular lens use the coarse adjustment knob to move the stage away from the objective until the object is in focus.** If nothing comes into view after several turns of the coarse adjustment knob you will need to check for the following errors: 1) the letter "e" was not positioned over the opening in the stage, 2) you lowered the stage too quickly and missed the letter "e", or 3) you have not lowered the stage far enough to see the letter "e". It may be necessary to repeat steps 4-5 to avoid any of the above three errors.
- Once you have the letter "e" in focus **use the mechanical stage control knobs to move it into the center of your field of view**. If the "e" still needs a minor focusing adjustment use the fine focus knob to complete the focusing. If the field of view is too bright you can decrease the light by closing the iris diaphragm.
- Draw the letter "e" in the following circles as it appears in your field of view (through the microscope oculars) and as appears when you look at it on the stage (without the microscope) in the appropriate circle below.

a) Letter "e" through microscope



b) Letter "e" without scope



Answer the following questions based on your observations.

- a. Does the letter "e" appear different when viewed through the microscope? \_\_\_\_\_
  - b. If so how? \_\_\_\_\_
  - c. Is the letter larger or smaller when viewed through the microscope? \_\_\_\_\_
  - d. While looking through the oculars move the slide away from you. Which way did the letter move in your field of view? \_\_\_\_\_
  - e. While looking through the oculars move the slide to the right. Which way did the letter appear to move in the field of view? \_\_\_\_\_ Is it possible to bring the entire letter "e" into clear focus with the fine adjustment? or is the outer edge slightly out of focus when the center is clear? \_\_\_\_\_
8. Now move the 10X objective into line with the body tube. Since this microscope is parfocal you should only need to make minor adjustments to the focus using the fine focus adjustment knob. You may find that you now need to open the **iris diaphragm** to let more light in.

Answer the following questions based on your observations.

- a. Is the letter larger or smaller when viewed through the microscope? \_\_\_\_\_
  - b. While looking through the oculars move the slide away from you. Which way did the letter move in your field of view? \_\_\_\_\_
  - c. While looking through the oculars move the slide to the right. Which way did the letter appear to move in the field of view? \_\_\_\_\_
  - d. Is it possible to bring the entire letter "e" into clear focus with the fine adjustment? or is the outer edge slightly out of focus when the center is clear? \_\_\_\_\_
9. Now move the 40X objective into line with the body tube. Since this microscope is **parfocal** you should only need to make minor adjustments to the focus using the fine focus adjustment knob. You may find that you now need to open the iris diaphragm to let more light in.

Answer the following questions based on your observations.

- a. Is it possible to bring the entire letter "e" into clear focus with the fine adjustment? or is the outer edge slightly out of focus when the center is clear? \_\_\_\_\_

## Introduction Part 2: Observing the Differences between Plant and Animal Cells

In today's lab you will be examining a variety of different cell types using the compound microscope. All cells have certain common features, including a fluid-filled **cytoplasm** surrounded by a **plasma membrane**, DNA (genetic material) and ribosomes (for protein synthesis). Some cells, including the prokaryotes, fungi, plants, and certain protists, also have a **cell wall** that lies outside the plasma membrane and functions in protection and structural support.

Biologists recognize two major categories of cell types – the **prokaryotes** and the **eukaryotes**. Prokaryotes lack a membrane-bound nucleus, have few or no organelles and are smaller than eukaryotes. Prokaryotes include organisms in the Domains Bacteria and Archaea. Organisms in Domain Eukarya (protists, plants, fungi and animals) have eukaryotic cells. These cells have a membrane-bound nucleus that houses their DNA and contain extensive internal organelles ("little organs") that perform specific functions. As you

complete this lab, note the size and structural differences between the prokaryotic and eukaryotic cells you observe.

A tremendous amount of diversity exists within each category of cells. Differences occur in size, shape, and presence and number of various organelles and other structures. Each cell's structure correlates with its specific function. You will be examining several different plant and animal cell types to explore eukaryotic cell diversity. Plant cells have a cell wall composed of cellulose and a large **central vacuole** that stores water, pigments and wastes. Various **plastids** are also present, which produce or store various products. **Chloroplasts** perform photosynthesis, using light energy to produce carbohydrates. Other plastids include the **amyloplasts**, which store starch. Animal cells lack cell walls, plastids, and a central vacuole, but share many other common organelles with plants, including mitochondria, the endoplasmic reticulum, Golgi apparatus and cytoskeleton. Most of these shared structures will not be visible in the slides we examine today.

### Activity 2: Observing Cells Using a Microscope

#### Material Needed

For each student or group of 2, you need:

- Compound microscope
- Fixed Bacterial slide
- 2 Glass slides
- 2 glass coverslips
- Onion skin sample
- Toothpick for cheek cell sample
- Iodine Stain
- Methylene blue stain

#### Exercise A - Observation of preserved/prepared slide for Prokaryotic (Bacteria) cells

As mentioned in the lab introduction, prokaryotic cells are much smaller than eukaryotic cells, and thus can be more difficult to view under the microscope. Like plants, they have cell walls, but usually lack internal organelles.

#### Procedure

1. Either on your lab bench or on the front table are some bacterial shapes/types slides. Open the **stage clip** and place one of these slides on the **stage**. Follow the steps you used previously for viewing a specimen to examine this prepared slide of bacteria.
2. Once you think that you have located a cell, switch to high power and refocus. \*You will need to view the cells on high power. REMEMBER: do NOT use the coarse adjustment knob at this point.

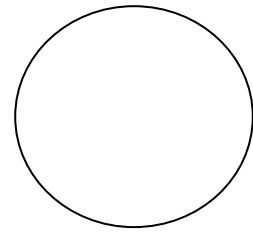
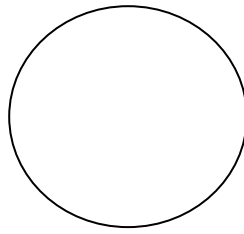
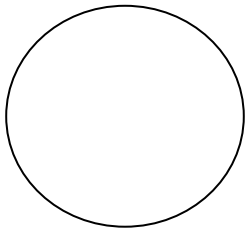
Bacterial cells typically come in one of three shapes:

- spheres (coccus)
- rods (bacillus)
- spirals (spirillum)

You will see all three shapes on your slide.



3. For each shape, sketch the cells at high power, being sure to draw the cells to scale.



a) Shape: \_\_\_\_\_

b) Shape: \_\_\_\_\_

c) Shape: \_\_\_\_\_

## Exercise B: Preparing Wet Mount Slides

Wet mounts are one of the most common types of slide preparations, and you will make several of these slides today. In a wet mount, a drop of liquid (water, stain, etc.) is suspended between a slide and a glass coverslip. Follow the instructions below to correctly prepare a wet mount.

### General Procedure

Before beginning your first wet mount slide (procedure 1), read through the general procedure below for how all wet mount slides are prepared.

1. Obtain a clean glass slide and coverslip. **\*\*Caution: coverslips are very thin and can break easily. Handle carefully!\*\***
2. If you will be viewing a specimen not already suspended in liquid (cheek cell, onion skin, etc.) place this specimen on the slide first.
3. Using a pipet, draw up a small portion of the liquid (water **or stain**) and place a single drop onto the slide. (On top of your specimen from step #2, if applicable).
4. Holding the coverslip by the edges, place one edge of the coverslip on the slide so that it touches the edge of the liquid. Slowly lower the coverslip over the sample, as shown in the image below.

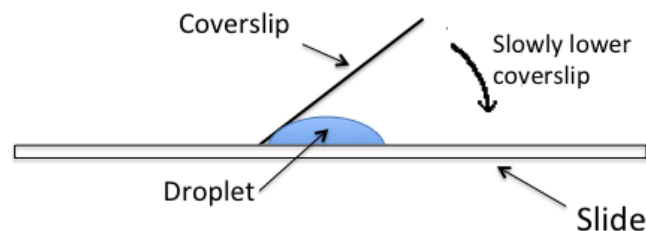


Figure 3: Preparing a wet mount slide

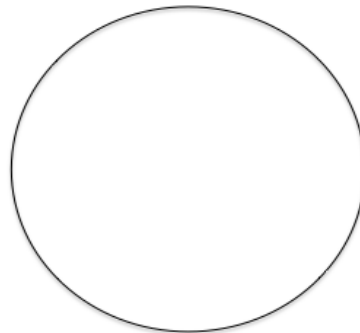
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5. If you have prepared the slide correctly, there should be minimal air bubbles between the slide and coverslip. However, if you have any large bubbles, you can attempt to remove them by gently pushing on the coverslip with the erasure end of a pencil.
6. There should be sufficient liquid to fill the space between the slide and coverslip, and to securely hold the coverslip in place. If there is too much liquid, the coverslip will slide around. If this is the case, hold a piece of paper towel close to the edge of the coverslip to draw out excess fluid. **\*\*Make sure the bottom and top of the slide/coverslip are dry before placing the slide under the microscope!\*\***

### Procedure 1: Wet Mount Slides of Plant Cells

1. Prepare a slide of an onion by carefully peeling the outer layer off of a piece of onion (take thin slice).
2. Place the onion skin onto the middle of a clean slide and add a coverslip according to the wet mount preparation instructions.
3. Place a drop of **iodine** on the edge of the cover slip and once it has spread under the entire cover slip, dry the excess liquid from the slide.
4. View the slide at low and high magnifications, noting the shape of the cells, and the **cell walls**.
  - a. Draw what you see under the highest power you can focus under in the circle below.
  - b. Label the nucleus, cell wall, cytoplasm and any other organelles that you can see.

Plant Cell



Total Magnification: \_\_\_\_\_x

5. Disposal and Cleaning: **\*\*When you are finished with the plant cell slide, dispose the cover slip in the broken glass container. Clean the slide with soapy water, dry them and put those back in the slide box at instructor's bench. \*\***

### Procedure 2: Wet Mount Slides of Animal Cells

The tissue that lines your cheeks contains multiple layers of flattened cells that are constantly sloughing off as you eat and drink. The layered nature of these cells serves to protect the underlying tissue against this abrasion. New cells are constantly being produced in the lower layers to replace those that are lost. It is easy to collect some cells from places like this.

**\*\*Make sure you are wearing gloves and goggles\*\***

1. Obtain a clean slide and a cover slip.
2. Add a drop of diluted **methylene blue** stain to the slide.
3. Gently rub the inside of your cheek with a toothpick (roll it) and smear the collected fluid onto the slide. Discard the toothpick in the trash.

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4. Apply the cover slip.
5. Use the SCANNING objective to focus. You probably will not see the cells at this power. Cells will only look like blue/black dots.
6. Switch to low power. Cells should be visible, but they will be small and look like nearly clear purplish blobs. If you are looking at something very dark purple, it is probably not a cell.
  - a. Once you think you have located a cell, switch to high power and refocus. (Remember, do NOT use the coarse adjustment knob at this point). Note the cell shape, plasma membrane, cytoplasm, and nucleus.
  - b. Draw what you see under the highest power you can focus under in the circle below. Label the nucleus, cytoplasm, and any other organelles that you can see.
  - c. Compare your observations with the picture shown in Figure 4.
  - d. Record your observations in the circle provided.

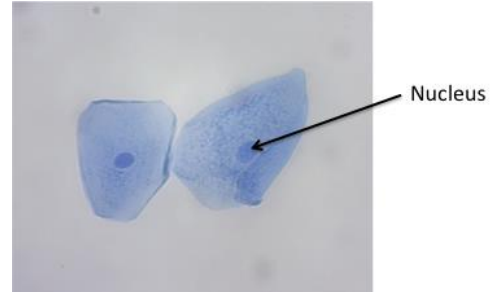
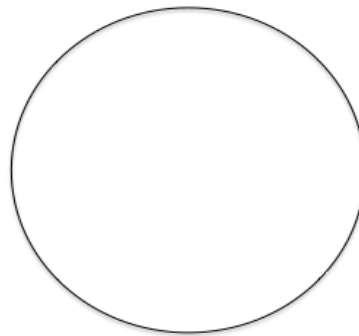


Figure 4: Cheek cells under microscope

Animal Cell



Total Magnification: \_\_\_\_\_x

7. Disposal and Cleaning: **\*\*When you are finished with the cheek cell slide, place the slide with the cover slip in the marked container on the instructor bench.\*\*** Those will be washed with bleach later and disposed off.

### Storing the Microscope After Use

- Turn off the light and then unplug.
- Return the objective lens to 4X and lower the stage.
- Remove the slide from the stage.
- DO NOT wrap cord around the base of your scope. Coil the cord neatly next to the microscope.
- Carefully return to cupboard holding with both of your hands and lock the door.

### Reference

Tugmon C., (2007) Care and Use of a Compound Microscope, Department of Biological Sciences, Augusta University

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### Post-Lab Activity:

Name: \_\_\_\_\_

After completion of the lab, answer the following questions. Your instructor may require you to turn this in.

1. In the chart below, list the function of each of the listed parts of the microscope. On the microscope image below, label the parts that have an arrow.

<b>Oculars</b>	
<b>Stage</b>	
<b>Condenser</b>	
<b>Iris Diaphragm Lever</b>	
<b>Objectives</b>	
<b>Fine Adjustment Knob</b>	
<b>Coarse Adjustment Knob</b>	
<b>Rotating Nose Piece</b>	



2. Since both animal cells and plant cells have a nucleus, what type of cell are they?
3. What would these cells be called if they didn't have a nucleus?
4. List the ways that eukaryotic cells are different from prokaryotic cells (bacteria)?
5. List the ways that eukaryotic cells are similar to the prokaryotic cells (bacteria)? (Think about what features ALL cells share)
6. How does the size of a prokaryote compare to a eukaryote? Explain.
7. Recall from the laboratory exercise some of the way in which the animal cells and plant cells differ under the microscope?
8. What was the highest magnification (lens) for each cell type that you were able to see each of your cells with (scanning lens X objective lens)?

Bacteria:

Plant (Onion):

Animal (Cheek):

9. What stains were used to stain animal cells and plant cells?

Animal:

Plant: