Introduction to the Compound Microscope
Cell Structure & Function

Laboratory Safety
Lab coat, long pants, closed-toe shoes, safety goggles, and nitrile or latex gloves are required.

**You may want to print this handout in color**

Learning Objectives
1. Properly clean and carry a compound microscope.
2. Focus a specimen using all objectives of a compound microscope.
4. Identify the parts of the compound microscope and explain the function of each part.
5. Define magnification and resolution.
6. Estimate the size of an object when viewed through a compound microscope.
7. Adjust the diopters to fit one's own eyes.
8. Describe the major differences between prokaryotic and eukaryotic cells and be able to distinguish each cell type under the microscope.
9. Compare and contrast animal and plant cells and be able to distinguish each type under the microscope.
10. 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).
11. Examine the diversity in cell size and shape.
12. Properly prepare and view wet mount slides under the microscope.

Part A: Introduction to the Compound Microscope
Introduction
During this course you will need to view biological structures and organisms too small to be seen by the unaided eye. You will have two types of microscopes to assist you in viewing these specimens during this semester. They are the compound binocular microscope, which we will use in this lab, and the dissecting microscope, which we will use later in the semester. The unaided eye has the ability to distinguish between two items if they are at least 0.1mm apart (resolving power) (Vovopich and Moore, 1999). By using a light microscope we are able to distinguish between two items that are at least 0.1µm apart (Vovopich and Moore, 1999)

Care and Cleaning of the 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 place 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 it is important to clean the ocular lens and the objective lens each lab period before and after use. To do this take a piece of lens paper and dampen it with the lens cleaning solution found at each table. It is important to only use lens paper and not paper towels, since they will scratch the lens.
Activity A1: Observation and Identification of the Parts of the Compound Binocular Microscope

Now that you have the microscope carefully placed on your lab table and you have cleaned the ocular lens and objective lens, 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.

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. Take a moment to move the condenser up and down and then position it up close to the stage. For the purpose of this class we do not need to change the position of the condenser. If you have a problem focusing your specimen always check the position of the condenser before calling over the instructor.

4. Above the condenser lies the **stage**. It is mounted at a right angle to the arm and positioned just below the 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 **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. The **fine adjustment knob** is used to move the stage up or down only very slightly. Since these scopes are **parfocal** (all the objective lenses 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.

8. 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. Generally, 4X is referred to as the scanning lens, 10X as the low 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.
9. 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 x 10 or 100X. It is this combined magnifying power that makes this microscope a compound microscope.

Ocular Lens X Objective Lens = Total Magnification

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

<table>
<thead>
<tr>
<th>Ocular Lens Magnification</th>
<th>Objective Lens Magnification</th>
<th>Total Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X</td>
<td>4X</td>
<td>40X</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

**Activity A2: Calibration of the Binocular Compound Microscope**

1. 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.

2. Obtain a slide of a prepared specimen. Place the slide cover slip side up in the spring clip on the stage and find the specimen with the 10X objective.

3. Adjust the eyepieces by looking through the oculars and slide them in and out until you see one image. This intraocular distance will correspond to your interpupillary distance. Record this setting in mm here: ______________________________

4. Adjust the focus point of the ocular on the right eyepiece to correspond to the number written in the above blank by turning the ring at the base of the ocular until the white scale is set to that number. Close your left eye and look through your right eyepiece. Focus the specimen using the fine focus.

5. Cover your right eye and look through the left eyepiece. **DO NOT ATTEMPT TO FOCUS WITH THE FINE FOCUS.** Instead focus the specimen by turning the ring at the base of the left ocular. Once you have it in focus look through both eyepieces to ensure that you have the eyepieces properly calibrated. Write the appropriate number here: R ___________ L ___________

6. Always set the microscope to these values before you use it to observe your specimens.

**Activity A3: Viewing a Specimen**

1. 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.

2. If the 4X objective is not currently in line with the body tube rotate the 4X objective until it clicks into place.

3. Plug in the microscope and turn on the light source.
4. Either on your lab bench or on the table are some letter "e" slides. Open the spring 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 move the slide until the "e" is positioned over the opening above the condenser lens and is illuminated by the light.

5. 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.

6. 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.

7. Draw the letter "e" as it appears in your field of view (through the microscope oculars)

![Draw the letter "e" as it appears in your field of view (through the microscope oculars)](image)

Draw the letter "e" as it appears when you look at it on the stage (without the microscope)

![Draw the letter "e" as it appears when you look at it on the stage (without the microscope)](image)

Does the letter appear different when viewed through the microscope? ____________________________

If so how? ________________________________________________________________

Is the letter larger or smaller when viewed through the microscope? ____________________________

While looking through the oculars move the slide away from you. Which way did the letter move in your field of view? ____________________________

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.

Why do you think that is needed? __________________________________________________________

Is the letter larger or smaller when viewed through the microscope? ________________

While looking through the oculars move the slide away from you. Which way did the letter move in your field of view? __________________________________________________________

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? ________________

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.

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? ________________

Activity A4: Estimating the Diameter of the Microscope Field

1. Obtain a grid slide and place the slide on the stage and position the grid over the opening in the stage. The grid on this slide is composed of 2 mm length sides. Put the 4X objective in line with the body tube and use the coarse adjustment knob to bring the grid into focus.

Example of a grid slide being viewed under the microscope.
2. Using the stage adjustment knobs move the slide until one grid line touches the edge of the field of view on one side. Now count the number of squares you can see across the diameter of the field of view. If only part of a square is in view, then estimate what part of the millimeter is represented by that partial square. Record the value here in mm and then convert the value to μm.

4X: ______________ mm ______________ μm.

Repeat the process for the 10X recording your results here:

10X: ______________ mm ______________ μm.

Since the diameter of the field of views for the 40X and 100X are too small for the grid slide, these diameters must be calculated using the measurements from the 4X or 10X field of views. The formula for calculating the diameter of an unknown field (B) based on a known field (A) is below.

\[
\text{Diameter of field B} = \frac{\text{(Diameter of field A)} \times \text{(Total magnification of field A)}}{\text{Total magnification of field B}}
\]

**Example:** The diameter of field A is 3 mm and the total magnification is 60X. The total magnification of field B is 90X. What is the diameter of field B?

\[
\text{Diameter of field B} = \frac{(3 \text{ mm}) \times (60\text{X})}{90\text{X}} = 2 \text{ mm}
\]

Based on this formula and your data for 10X calculate the diameter of field for:

40X: ______________ mm ______________ μm.

100X: ______________ mm ______________ μm.

3. Estimate the length (longest dimension) of the following microscopic objects. Base your calculations on the field sizes you have determined for your microscope.

a. Object seen in low-power field:
   approximate length: ______________ mm

b. Object seen in high-power field:
   approximate length: ______________ mm
   or ______________ μm

c. Object seen in oil immersion field:
   approximate length: ______________ μm.
Part B: Cell Structure & Function

Introduction

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 B1: How to Prepare Wet Mount Slides

Wet Mount Procedure

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.

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 (leaf, potato slice, 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.
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!**

7. **Disposal:** When you are finished with your wet mount slide, you can re-use the same slide and coverslip to make additional wet mounts. Carefully rinse slide and coverslip under the sink and dry to re-use. **Make sure coverslips do not get washed into the sinks!** Once you are finished for the day, coverslips should be disposed of in the cardboard glass disposal near the instructor bench (Do not place glass in the regular trashcan!). Slides should be cleaned, dried and returned to slide boxes.

**Pond Water Slide**

Use the instructions given above to make a slide of the pond water at the instructor bench. Pond water contains various prokaryotes and eukaryotes, but the specific organisms will depend on the site of collection. You will have better luck finding specimens if you pipet water close to any solid material that may be present (plant matter, etc).

Record your observations in the circle provided.

Activity B2: Wet Mount Slides of Plant and Animal Cells

**Slide 1: Plant Cells – Elodea Leaf**

*Elodea* is an aquatic plant in which many of the basic features of plant cells are easily seen under the microscope. Examine a single leaf of *Elodea* under the microscope according to the instructions below.

1. Obtain a single *Elodea* leaf from the main plant. Younger (smaller) leaves work best.
2. Place the leaf on a clean slide and add a drop of water and a coverslip according to the wet mount preparation instructions.
3. View the slide at low and high magnifications, noting the shape of the cells, the **cell walls** and the **chloroplasts**. The central vacuole and nucleus may also be visible. As the slide warms up, you may be able to see the chloroplasts moving around the central vacuole in a process known as cytoplasmic streaming.
4. **What is the function of the chloroplasts?**

*Total Magnification: _____ x*
5. Compare your observations of *Elodea* with the picture shown below. Record your observations in the circle provided.

![Elodea Image]

6. Estimate the size of the plant cells and record your estimate here. ________ μm (Hint: What is the size of your field of view? What portion of your field of view is taken by the cell? See page 6 for review)

**Slide 2: Plant Cells – Potato**

Potatoes are modified underground stems used for carbohydrate storage. Starch is stored in organelles called *amyloplasts*, which will be visible under the microscope after staining the potato with iodine.

Examine a stained potato slice under the microscope according to the instructions below.

1. Obtain a clean slide and a cover slip.
2. Using a scalpel, carefully shave off a very thin slice of a potato and place on the slide.
3. Add a drop of iodine solution and apply the cover slip.
4. Examine the potato at low and high power. Amyloplasts contain starch, which will turn a purple or bluish-black color when stained with iodine. Note the cell shape and the size and abundance of amyloplasts within the cells.

7. Compare your observations with the picture shown below. Record your observations in the circle provided.

![Potato Image]

8. Estimate the size of the potato cells and record your estimate here. ________ μm
Slide 3: Animal Cells – Human Cheek 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.

Examine your cheek cells under the microscope according to the instructions below:

1. Obtain a clean slide and a cover slip.
2. Gently rub the inside of your cheek with a toothpick and smear the collected fluid onto the slide. Discard the toothpick in the trash.
3. Add a drop of diluted methylene blue stain to the slide and apply the cover slip.
4. View the slide at low and high power. Note the cell shape, plasma membrane, cytoplasm, and nucleus.
5. Compare your observations with the picture shown below. Record your observations in the circle provided.
6. **When you are finished with the cheek slide, place the cover slip in the glass disposal and put the slide in the container of bleach at the instructor bench.**

7. Estimate the size of the cheek cells and record your estimate here. ________ μm

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Activity B3: Prepared Slides

Slide 4: Animal Cells – Neurons

For this activity you will be using a previously prepared and stained slide of animal nervous tissue. This tissue contains two types of cells: neurons and glial cells. Neurons are the primary cells of the nervous system. They communicate with each other via electrical and chemical signals that are sent and received via cellular processes that project outward from the main cell body. Glial cells function in various ways to support the action of neurons.

Examine the neural tissue slide under the microscope according to the instructions below:

1. Obtain a prepared neuron slide from the slide box at your table.
2. Examine the slide at low and high magnifications. Note the large neurons and their long, slender cellular processes. Note the much smaller glial cells that surround the neurons.
3. Note the spherical nucleus near the center of each neuron cell body. Within the nucleus is a darkly stained nucleolus.
4. Compare your observations with the picture shown below. Record your observations in the circle provided.
5. Estimate the width of one of the neuron cell bodies and record your estimate here. ________ μm

**Slide 5: Prokaryotes - Bacteria**

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.

Examine the prepared slide of bacteria under the microscope according to the instructions below.

1. Obtain a bacterial shapes slides from the slide box at your table.
2. Using the 10x objective lens, look for areas of the slide that show a pink or purplish stain. At this magnification, the bacterial cells will be barely visible as small dots.
3. Move to high power. Bacterial cells typically come in one of three shapes: spheres (coccus), rods (bacillus), or spirals (spirillum). You will see all three shapes on your slide in three distinct areas.
4. Note the size of the bacterial cells. How does it compare to the eukaryotic cells you examined earlier?

___________________________________________________________________________________

5. Do these cells have nuclei? ___________________________________________________________

6. Compare your observations with the picture shown below. Record your observations in the circle provided.
**Pre-Lab Activity**
Prior to this lab you should complete the following:
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.

<table>
<thead>
<tr>
<th>Part</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oculars</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>Condenser</td>
<td></td>
</tr>
<tr>
<td>Iris Diaphragm Lever</td>
<td></td>
</tr>
<tr>
<td>Objectives</td>
<td></td>
</tr>
<tr>
<td>Fine Adjustment Knob</td>
<td></td>
</tr>
<tr>
<td>Coarse Adjustment Knob</td>
<td></td>
</tr>
<tr>
<td>Rotating Nose Piece</td>
<td></td>
</tr>
</tbody>
</table>

![Microscope Image]
2. Read Sections 7.1 Bacterial and Archaeal Cell Structures and Their Functions and 7.2 Eukaryotic Cell Structures and Their Functions in your Biological Science textbook (Freeman, 6th edition pages 143-153). Be sure to review Table 7.1 (page 153).

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

Generalized Plant Cell:

Generalized Animal Cell:

Generalized prokaryotic Cell
**Post-lab Activity**
Fill in the following table with the information you have learned in lab.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Prokaryote or eukaryote?</th>
<th>Cell size (μm)</th>
<th>Cell wall present?</th>
<th>Visible internal structures (list)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elodea leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human cheek cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
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</tbody>
</table>