Lab 3. Microscopic Observation of Unicellular and Multicellular Organisms

Prelab Assignment

Before coming to lab, read carefully the introduction (pp. 1 – 6) and the procedures (pp. 6 – 17) for each part of the experiment, and then answer the prelab questions at the end of this lab handout. Hand in the prelab assignment just *before* the start of your scheduled lab period.

Goals of this Lab Exercise

After completing this lab exercise you should be able to...

- Identify the parts of a compound light microscope and use a microscope to competently examine biological samples.
- Use a microscope to identify the following organisms or tissues: *Amoeba, Trichonympha, Protococcus, Scenedesmus, Volvox, Spirogyra,* and human cheek epithelium
- Accurately sketch, describe and cite the major functions of the structures and organelles of the cells examined in this lab exercise.
- Determine the diameter of the field of view for the various objectives of a microscope.
- Estimate the size of specimens viewed with a microscope.
- Describe the evidence that supports the theory that over time organisms have evolved from unicellular organisms, to loose aggregates, to simple colonial organisms, to complex colonial organisms, and, finally, to multicellular organisms.
- Describe the similarities and differences in the structures of the animal and plant cells observed in this lab exercise.

Introduction

The Microscope

The microscope is one of the principal tools of the biologist. Without the microscope, many of the great discoveries of biology would never have been made. The light compound microscope, illustrated in Figure 1 on the following page, is the type of microscope most commonly used. Proper, comfortable use of the instrument demands practice. The practice afforded you in this exercise depends upon familiarity with the parts of the microscope and with their interactions.

Magnification and Resolution

In using the microscope it is important to know how much you are magnifying an object. To compute the ***total magnification*** of any specimen being viewed multiply the power of the eyepiece (ocular lens) by the power of the objective lens being used. For example, if the eyepiece magnifies 10x and the objective lens magnifies 40x, then 10 x 40 gives a total magnification of 400x.

The compound microscope has certain limitations. Although the level of magnification is almost limitless, the resolution (or resolving power) is not. ***Resolution*** is the ability to discriminate two objects close together as being separate. The human eye can resolve objects about 100 µm apart (note: 1 µm = 1 micrometer = 1 millionth of a meter). Under ideal conditions the compound microscope has a resolution of 0.2 µm. Objects closer than 0.2 µm are seen as a single fused image.

Resolving power is determined by the amount and physical properties of the visible light that enters the microscope. In general, the greater the amount of light delivered to the objective lens, the greater the resolution. *The size of the objective lens aperture (opening) decreases with increasing magnification, allowing less light to enter the objective lens. Thus, it is often necessary to increase the light intensity at the higher magnifications*.

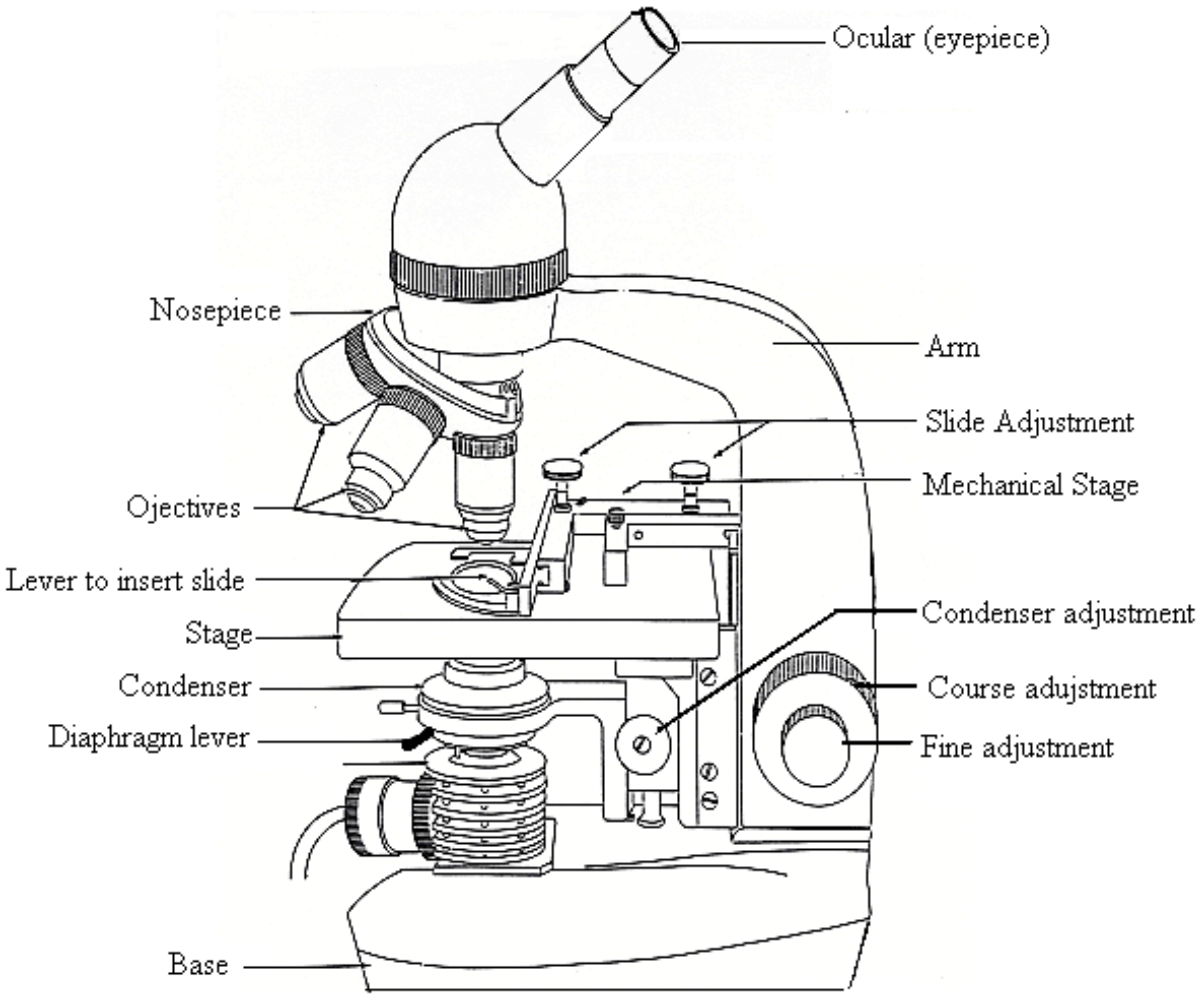


Figure 1. A typical compound light microscope used in many biology labs.

Note each of the following features of the microscope and there individual uses to allow you to take full advantage of the use of a microscope.

- ***Ocular***—contains a lens that magnifies the specimen (usually 10x)
- ***Revolving Nosepiece***—used to change magnifying lenses (objectives)
- ***Objectives***—magnifying lenses usually 4x, 10x, 100x and 100x (oil immersion lens).
- ***Condenser***—adjustable device gathers the light rays from the light source and focuses them onto the specimen
- ***Diaphragm***—this lever controls the amount of light shown onto the specimen
- ***Coarse and Fine Focus Knobs***—used to focus the specimen. The course focus knob is only used with the 4x (low power) objective.

Depth Perception and the Microscope

Any microscopic object viewed has depth as well as length and width. While the lens of your eye fully adjusts to focus on an object being viewed and provides you with a three dimensional interpretation, the lenses of a microscope are focused mechanically and can only "see" in two dimensions, length and width. For example, *if the specimen you are examining has three layers of cells, you will only be able to focus on one cell layer at a time*. In order to perceive the relative depth of your specimen use the *fine adjustment* to focus through the different planes (i.e. the three cell layers) individually to build a three-dimensional picture or interpretation of your specimen.

The Field of View and estimating the Size of Specimens

When you view an object under the microscope you will observe that it lies inside a circular field of view. *Each different magnification has a different sized field of view*. If you determine the diameter of the field of view you can estimate the size of an object seen in that field. *As you increase the magnification, the field of view (and diameter) gets proportionately smaller*. As a consequence, a critter that appears small under scanning power may appear large under high power. The actual size of the critter did not change only the space in which you placed it for viewing.

Refer to ***part B*** of the procedure for a discussion on how to determine the diameter of the field of view and how to estimate the size of a specimen viewed with a microscope.

The Oil Immersion Lens

Although the oil immersion lens (100x) when used properly offers the ability to view objects at high magnification (1000x), the objects viewed in this lab exercise do not warrant its use. As its name implies, an oil immersion lens requires a drop of immersion oil to be in contact between the lens and the slide for the lens to function effectively. Since immersion oil has the same refractive index as glass, it prevents the scattering of light as light passes from the glass slide to the objective lens (also made of glass). ***Poor resolution is the result if the immersion lens is used without oil*** since light will be bent (and thus scattered) as it passes from the slide to air, and then through the objective because air and glass bend light differently as a result of having different refractive indexes.

Care of the Microscope

Your microscope is an expensive instrument that must be given proper care. Always follow these general instructions when using a microscope.

1. Carry the microscope with both hands, one hand under the base, and the other on the arm. When getting ready to put the microscope away, always return it to the low power or scanning power setting.
2. When setting the microscope on a table, always keep it away from the edge.
3. It is generally best to clear your lab table of items that are not being used.
4. The lenses of the microscope cost almost as much as all of the other parts together. Never clean them with anything other than lens paper. Paper towels and other paper tissues will scratch the lens.
5. Please inform the instructor or the biology lab technician of any microscope damage or irregularity in its operation as soon as possible. Do not return a faulty microscope without first informing the instructor or lab tech.
6. You are responsible for the microscope while you are using it. Treat it with care!

Techniques for Setting Up & Viewing Objects with a Compound Light Microscope

Be familiar with the following procedures outlining the correct usage of the microscope *before* coming to lab. The steps that follow should be observed in this lab exercise and all other lab activities in this and other courses/lab experiences.

1. **Place the scanning power or the low-power objective in position** (if not already in position). In changing from one objective to another, you will hear a *click* when the objective is set in proper position.
   - **Make certain that the lenses are clean.** Dirty lenses will cause a blurring or fogging of the image. The high power and ocular lenses are the lenses that most often get dirty.
   - **Cleaning microscope lenses:** Place a drop of lens cleaning fluid on a piece of *lens paper*. Clean lens with a *gentle* circular motion, then dry with a *fresh* piece of lens paper. Always use lens paper for cleaning! Any other material (including Kimwipes) may scratch the lens.

2. **Check the Preliminary Lighting**
   a. Plug in the electrical cord to turn on the substage light. If equipped with a light switch, turn it on too.
   b. Position the ***condenser*** as high as it will go by turning the substage adjustment. This provides for a maximum of light.

> **Important!!!**
> - When looking through the ocular you should see a white circular field of view that is *evenly* illuminated.

   c. Open the ***iris diaphragm*** by using the lever beneath the condenser that is below the stage of the microscope.

3. **Place the slide on the stage for viewing at scanning or low power.**
   a. Make certain that the scanning power objective (4x) or the low power objective (10x) is clicked properly in place.

> **Which objective should you begin your observations with?**
> - If you need to scan the slide to find the location of a specimen use the scanning objective (4x) with its larger field of view.
> - If you have a pretty good idea where the specimen is located on the slide it is O.K. to start with the low power objective (10x).
> - Because of the danger of damaging these lenses and their very small field of view, *never* begin microscopic examinations with the high power (40x) or the oil immersion (100x) objectives.
> - ***See the previous page for information concerning the oil immersion lens***.

   b. Lower the stage away from the objective with the coarse adjustment.
   c. Place a properly prepared slide (see below) on the stage and secure with the stage clips or mechanical stage depending upon which is present on your microscope.
   d. Move part of the slide with the object to be viewed *directly* above the brightly illuminated substage condenser.

4. Focus: Proper Focusing Technique for Scanning and Low Power

a. Viewing the stage from the side, use the ***coarse adjustment knob*** to *raise* the stage until the stop is reached that will prevent further movement of the stage.

b. Looking through the eyepiece (ocular) ***lower the stage slowly by turning the coarse adjustment knob*** away from you until the object is in focus. It should take *less* than a quarter of a turn to bring the image into focus.

Tips:
- When it is difficult to find a specimen to focus on (e.g. when examining amoeba), bring the edge of the coverslip into the center of the field of view, and then try focusing on the edge. Then search the slide for the desired specimen.
- Reduce the light intensity to aid in the observation of viewing clear/transparent objects such as amoeba.
- To avoid eye strain while viewing specimens with a *monocular* microscope (i.e. one with only one ocular or eyepiece), practice keeping *both* eyes open. Many biologists are capable of observing a specimen and sketching it at the same time! Try it out in today's lab!

c. Use the ***fine adjustment*** to bring the object into sharp focus.

d. Adjust the amount of light with the iris diaphragm and intensity of light with the condenser for optimum viewing. Too much or too little light adversely affects the quality of the image viewed!

5. Increasing Magnification: Switching from Low to a Higher Power

a. First, be sure the object that you want to view at a higher magnification is in the *center* of the field of view and *sharply* focused under *low* power.

b. Switch to high power. Watch from the side to make sure that the objective lens does not touch the slide. Since most microscopes are ***parfocal*** *, the object should be in focus, or almost in focus.

* Most microscopes are ***parfocal***, which means that little refocusing is needed when moving from one lens to another. Only *fine* adjustment may be required. If properly focused at low power, and the slide is prepared correctly (i.e. the specimen is *thin* and flattened by a coverslip), you should be able to switch automatically from low power to high without fear of having the high power objective lens scraping or touching the slide.

6. Re-Focus with the Fine Adjustment under High Power

a. **Only use the fine adjustment at high power!** To avoid damaging the lens, *never* use the coarse adjustment when the high-power objective is in place.

b. Adjust the amount and intensity of light for optimum viewing. The amount of light may need to be increased since less light passes through the objective at higher magnification.

c. The ***working distance*** is the distance between the specimen viewed and the objective lens of the microscope. As you increase magnification the working distance becomes less and less. The objective will be almost touching the cover slip when properly focused at high power.

If the image viewed at high power is not sharp try the following:
- The object may not have been focused properly at low power. Repeat steps 4-6.
- Something may be dirty and needs to be cleaned. Common culprits include the high power objective, ocular, cover slip, and/or slide.

7. Remove the slide from the stage

a. Switch the objective to either scanning or low power. Removing a slide while under high power may scratch the lens.
b. Lower the stage using the coarse adjustment.
c. Remove the slide from the stage:
   - **Disposal of Wet Mounts:** Discard the cover slip (plastic coverslips in the trash, glass coverslips in the broken glass container at the front of the lab), rinse the slide at a sink, and then place the slide to dry on the paper towel labeled "Wet Clean Slides" on the instructor's table at the front of the lab.
   - **Prepared slides:** Return to their proper location within the plastic slide container on the lab supplies cart.

Preparing a Wet Mount

- Place a drop of water on a clean slide with a dropper.
- Put the object in the water drop.
- Lower one edge of the coverslip to the edge of the water drop as shown in the illustration (Figure 2). Lower the coverslip slowly to avoid air bubbles. A gentle tapping will usually remove any bubbles that may be present. Blot any excess water with a paper towel. More water can be added with a dropper at the edge of the coverslip. Do not let your specimen dry out.

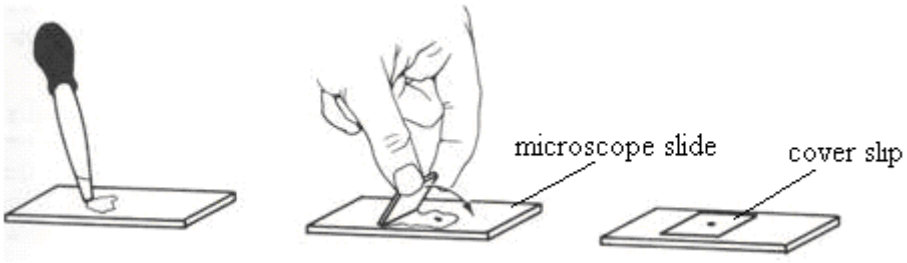


Figure 2. Wet Mount Preparation

PROCEDURE

Important Notes!!

- To get an overview of this laboratory activity and to use your lab time efficiently read the following procedure *before* attending lab. If you and your group members are not familiar with the procedure before coming to lab you will have great difficulty completing this exercise during the lab period.
- As you follow the procedures outlined below, record you observations and responses to the questions on the lab report sheet at the end of this handout.

Part A. Observation of the Letter "a"

Introduction

In this exercise you will learn to use the microscope to examine a familiar object, a self-prepared slide of the letter "a". *Refer to the previous sections, "Setting Up & Viewing Objects Through the Microscope" and "Preparing a Wet Mount" to prepare your slide and observe it with the microscope*. Practice adjusting your microscope to become proficient in locating a specimen, focusing clearly, and adjusting the light for optimum viewing.

MATERIALS

Items *Always* Located in Trays on the Instructor's Table:

Lens Paper and Lens cleaning solution
Metric Rulers
Clean microscope slides
Coverslips
Eye droppers
Forceps (tweezers)
Scissors

Materials Needed for Part A

Letter "a" cut from a Newspaper
Microscope slide and coverslip
Dropper bottle of water
Compound light microscope

PROCEDURE (Perform individually or in teams of two)

1. In Table 1 of the report sheet record the magnification of each of your microscope's objectives. Also note the magnification of the ocular.
2. Determine and then record in Table 1 the *total* magnification when using each objective.
3. Make a wet mount of a letter "a" (lower case letter) that you have cut from a newspaper.
4. Place the wet mount of the letter on the stage of the microscope so that the letter is *right side up* as you look at the slide of the letter with the *naked eye* from *behind* the microscope.
5. Observe under scanning power (i.e. the lowest power objective, 4x), and then low power (10x objective). Keeping the image of the letter within the field of view, use the slide adjustment knobs to move the slide to the left and then to the right. Now move it away from you and then towards you.
6. Answer questions 1-3 on the report sheet
7. Observe under high power. Don't forget to fiddle with the diaphragm and the condenser to get optimum viewing conditions after changing objectives.

Part B. Determining the Size of the Microscopic Field of View

Introduction

Often the size of the objects you are observing under the microscope need to be estimated. Because these objects are usually too small to permit direct measurement, it will be convenient for you to learn a method to indirectly measure them. Measurement of the diameter of the field of view for the different objective magnifications will enable you to estimate the size of the things viewed under the microscope.

MATERIALS

Compound light microscope
Metric Ruler

PROCEDURE (Perform individually or in teams of two)

1. Be sure the *scanning objective* (4x objective) is in position. Place the graduated edge of a plastic metric ruler across the mid-line (diameter) of the field of vision as in Figure 3, below. Bring the ruler into focus. Record in Table 1 of the report sheet the diameter in both millimeters (mm) and in micrometers (μm), the most common units of measurement in microscope work. The following relationships are useful when converting between micrometers and millimeters:

1 mm = 1000 μm **1 μm = 0.001 mm**

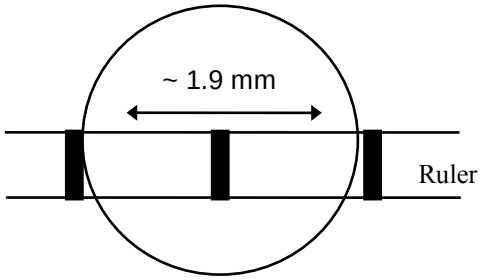


Figure 3. Hypothetical determination of the diameter of the field of view with a metric ruler.

2. Calculate the diameter of the field of view for the other objectives of your microscope and record your results in Table 1 of the report sheet.

How to Calculate the Diameter of the Field of View at Higher Magnifications:

- Once you have experimentally determined the diameter of the field of view for the scanning and/or low powers you can *calculate* the diameters of the fields of view for the rest of the objective lenses. Recall that the diameter of the field of view gets proportionately smaller as the magnification increases. If, for example, the diameter of the field of view were 3.0 mm at a total magnification of 20x, the diameter of the field of view at 100x would be 5 times less, i.e. 0.6 mm or 600 μm. Why? 100x is five times greater than 20x, thus the field of view at 100x must be 5 times less: 3.0 mm / 5 = 0.6 mm, which equals 600 μm.
- Once you know the field of view diameter, you should be able to *estimate* the size of any organism found within that field. See questions 6-8 on the Prelab Exercises to get some practice calculating field diameters and estimating the sizes of critters viewed with a microscope.

Part C. Depth Perception: Depth of Field

Introduction

It is important to remember that by using the coarse and fine adjustments you bring the microscope into focus at many different levels. At each setting you can see clearly only one plane of the object. To see other planes clearly, e.g. to see different layers of cells in a sample of tissue, you must change the focus with the fine adjustment.

MATERIALS

Compound microscope
Clean microscope slide and coverslip
Red, yellow, and green pieces of thread
Dissection needle and forceps to manipulate thread on the slide

PROCEDURE (Perform individually or in teams of two)

1. Obtain a prepared slide with three colored crossed threads. If a prepared slide is not available make a wet mount with three colored threads (red, yellow, and green) that cross at one or nearly point under the cover slip. Place the threads on the slide in this order: red, followed by yellow, with green on top.
2. **Determination of the relative depths of field:**
   - Observe the region where the threads cross with the scanning objective (4x). Notice as you rotate the fine adjustment knob back and forth slowly, you can see different depths and get a three-dimensional (i.e. length, width and depth) view.
   - How many threads can you bring into focus simultaneously? Record your response in Table 2 of the report sheet.
   - Repeat with the low power objective (10x), and finally with the high power objective (40x). Don't forget to fiddle with the diaphragm and the condenser to get optimum viewing conditions after changing objectives.

3. **As viewed under the microscope, determine the order the threads *appear* to be in**: With the low power objective (10x), *lower* the stage with the course or fine adjustment until the threads are just out of focus. Slowly bring the threads into focus with the fine adjustment. Which thread comes into focus first? Continue to raise the stage with the fine adjustment. Which thread comes into focus last? Use this information to help answer question 4 on the lab report sheet.

Parts D - F. The Organization of Cells

Introduction to Parts D-F

In this exercise you will observe the cell structures that are common to *all* eukaryotic cells: plasma **membrane** (also known as a cell membrane), **nucleus** (a membrane bound structure that contains the genetic material), and the **cytoplasm** (the area of the cell between the plasma membrane and the nucleus; all other cell organelles float within the cytoplasm). However, all cells are not alike. The organelles found within a specific cell are dependent on the function of the cell. Likewise, the functions a cell are dependent upon the organelles present!

Moreover, some organisms are **unicellular** (composed of one cell), with all living functions (e.g. digestion, respiration, excretion, reproduction, etc.) performed by the organelles in that one cell. Other organisms are **aggregates** (random, temporary, clusters of cells). Organisms that are a permanent cluster containing predictable and consistent number of cells called **colonies**. **Simple colonies** have no physiological inter-connection but maintain a predictable multicellular structure. **Complex colonies** have cells that are physiologically inter-connected, with moderate specialization of groups of cells. **Multicellular** organisms have large numbers of cells with specialized structure and function. No one cell of a multicellular organism can exist long by itself since it requires other cells, tissues, and organs to carry out the many life processes that it by itself can not perform. There is strong evidence that suggests that the first organisms to evolve were unicellular organisms, from which evolved all of the more complex forms of life: aggregates, colonial organisms, and, finally, the multicellular forms of life.

In the following exercises, you will examine selected unicellular, aggregate, colonial, and multicellular organisms.

Part D. Observation of Unicellular Eukaryotic Organisms: Amoeba and *Trichonympha*

Introduction

Unicellular eukaryotic organisms are found in the **Kingdom Protista**. Organisms in Kingdom Protista are either **photosynthetic autotrophs** (can make their own food via photosynthesis) and/or **heterotrophs** (obtain food their food by the consumption of other organisms or their by-products).

In this part you will observe the following heterotrophic protists: *Amoeba,* commonly found in ponds, and ***Trichonympha*****,** a protist found in the gut of termites. Termites lack the enzymes necessary to digest wood and thus require *Trichonympha's* wood digesting enzymes to make the nutrients in the wood available to them. *Trichonympha* is so well adapted to the environment inside a termite's gut that it cannot survive outside of it! This symbiotic relationship is thus mutually beneficial to both organisms.

MATERIALS

Compound microscope
Dissecting Microscope
Clean microscope slides and cover slips
Dissection needles
Forceps
Insect ringers solution
Cultures:
Amoeba
Living termites

PROCEDURE (Perform in teams of two)

Preparation of an *Amoeba* Wet Mount (Partner #1 sets up)

1. Partner #1 sets up the *Amoeba* wet mount while partner #2 prepares the Wet Mount of *Trichonympha* as outlined below.
2. Place the *Amoeba* culture under the dissecting microscope and focus on the bottom of the dish. The amoeba should appear as a light, irregular shaped organisms on the bottom.
3. Use the pipette labeled for use with this culture to transfer a drop of several *Amoeba* to a clean slide and cover with a clean coverslip.

Handy Dandy Tips:

- **Expel the air from the pipette *before* placing it in the culture dish:** *Amoeba* are delicate and easily displaced from their position in the culture dish. Don't blast them away with the pipette!
- **Avoid contaminating the cultures:** Do not interchange pipettes between cultures!

4. Observe with a compound microscope as described below and then observe the Wet Mount of *Trichonympha* prepared by your partner.

Examination of living *Amoeba* (Performed by Both Partners)

1. Use low or scanning power of your compound microscope to scan the entire slide to locate an amoeba. They are often found near debris and other protists on which they feed. If you have troubles finding any amoeba try make a fresh slide and/or consult your instructor and/or other students for help.

> **Tip:** Use the diaphragm and/or condenser to create contrast (i.e. significantly decrease the amount of light). If too much light is used, finding an amoeba will be like trying to find a white goose in a snowstorm!

2. Center the amoeba in the field of view, then switch to high power.
3. In the appropriate space on your report sheet, use a sharp *pencil* to make an **accurate sketch** of *your* amoeba at high power. Estimate and indicate the approximate size of the amoeba in micrometers.
4. **Identify and *neatly* label on your sketch the following structures:**
   - **Plasma membrane:** the boundary that separates the organism from the surroundings.
   - **Cytoplasm:** the fluid portion of the cell between the plasma membrane and the nucleus; Contains the cell organelles and has many nutrients, enzymes, etc. dissolved in it.
   - **Nucleus:** the grayish/darkish, granular, oval to football-shaped structure; Contains the genetic material, DNA, which directs the activities of the cell; You may see the nucleus moving about the cytoplasm.
   - **Pseudopodia** or "false feet": finger-like projections of the cytoplasm; Involve in movement and for capturing and engulfing food during the process of **phagocytosis** (literally: cell feeding)
   - **Food vacuoles:** the many small, dark, irregularly shaped vesicles within the cytoplasm; Contain partially digested and undigested food particles.
   - **Contractile vacuole(s):** clear, spherical vesicles of variable size that pump excess water out of the cell; Watch one as it gradually enlarges as it fills with excess water that has entered the cell, and then decreases in size as it pumps the water out of the cell. It may contract at the rate of 1-3 times per minute. However, its activity is quite variable, as it is dependent on the solute content of its surroundings: very active in a dilute environment (e.g. low salt concentration), less active in a high solute environment (e.g. high salt concentration).

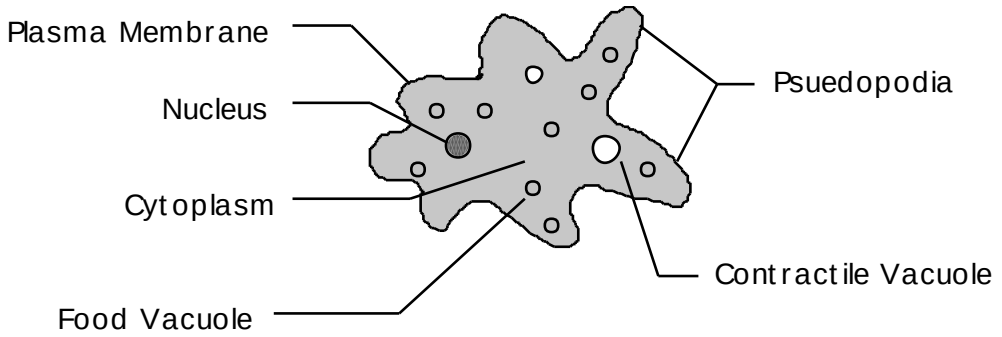


Figure 4. The shape of an amoeba constantly changes as it uses pseudopodia to both move and ingest food by phagocytosis (Latin for "cell feeding").

Preparation of a *Trichonympha* Wet Mount (Partner #2 sets up)

1. One partner makes this slide while the other partner prepares the *Amoeba* slide.
2. On a clean microscope slide place 2 or 3 drops of insect ringers solution (a saline solution that is isotonic to the fluids within the termite; i.e. It has the same solute concentration as the termite's internal fluids)
3. Use forceps to place a termite into the ringer's solution and then transfer the slide to the stage of a dissecting microscope. Use a desk lamp to provide light from the side.
4. Use forceps and/or dissecting needles to pull the termite apart from end to end.
5. Find the termite's digestive system (a long dark tube).
6. Leaving the digestive system on the slide, remove all of the larger parts of the critter from the slide and discard in the trash.
7. Use a dissecting needle to squash the intestine to release the *Trichonympha* and the many other protists and bacteria that inhabit the gut of the termite.
8. Cover with a clean coverslip. Use a paper towel to dry the bottom of the slide and/or the parts of the slide not under the coverslip before transferring the slide to a compound microscope.
9. Observe with a compound microscope as described below.

Examination of living *Trichonympha* (Performed by Both Partners)

1. Use low power of your compound microscope to scan the slide to locate *Trichonympha*. There should be dozens of them present as well as many other smaller protists (unicellular eukaryotic organisms) and several species of bacteria (prokaryotic cells), the smallest of critters present.
2. Center several *Trichonympha* in the field of view and switch to higher powers.
3. In the appropriate space on your report sheet, use a sharp *pencil* to make an **accurate sketch** of one of *your Trichonympha* at high power.
4. Estimate and record the approximate size of *Trichonympha* in micrometers.
5. **Identify and *neatly* label on your sketch the following structures:**
   - **Plasma membrane**, **Cytoplasm**, and **Nucleus**
   - **Flagella**: Long hair-like structures attached to the outward side of the plasma membrane. Although used for locomotion in other species, the exact role in *Trichonympha* is not fully understood since there is little room for movement within the very crowded digestive tract of the termite.
   - **Wood particles**: Usually located towards the larger end of the cell that lacks the flagella. Acted upon by the enzyme cellulase, which digests the major component of wood, cellulose (a polysaccharide), into glucose, a monosaccharide that can be used as a source of energy and as a raw material in biosynthetic reactions by both *Trichonympha* and the termite.
6. As described above, observe the Wet Mount of *Amoeba* prepared by your partner.

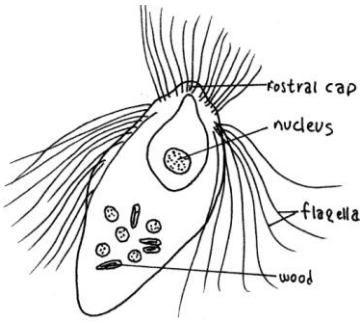


Figure 5. *Trichonympha* is a protist that dwells in the digestive tract of termites where they aid in the digestion of wood particles ingested by the termites.

Part E. Aggregate and Colonial Organisms: Observation of *Protococcus*, *Scenedesmus*, and *Volvox*

Introduction

In contrast to unicellular organisms, colonial organisms are critters consisting of a group of cells that are to small extent dependent upon one another. The organisms observed in this part of the lab, *Protococcus, Scenedesmus,* and *Volvox*, show an increasing degree of interaction and dependence on their constituent cells. Refer to the captions and illustrations of Figures 6-8 as you observe these organisms under the microscope.

Figure 6. ***Protococcus*** is a terrestrial algae (often incorrectly referred to as "moss") commonly found growing on the bark on the north side of trees in the Pacific Northwest. *Protococcus* is a loose aggregate of a variable number of cells with no permanent connections between them. The greenish color of the cells is due to *chlorophyll*, the primary pigment responsible for capturing light energy from the sun during photosynthesis. Although not visible under normal conditions, the plasma membrane is located just inside the cell wall.

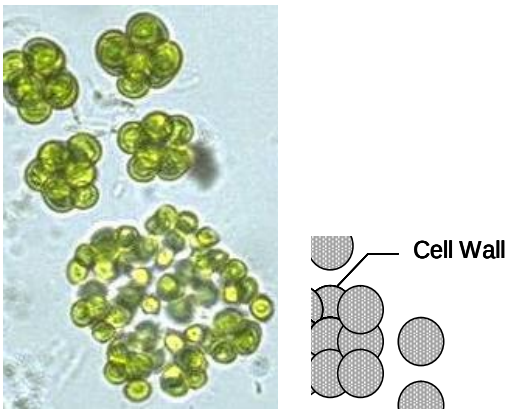


Figure 7. ***Scenedesmus*** is an aquatic algae commonly found in polluted water and in aquaria. It is a simple colony often consisting of four to eight cells that are permanently united by their cellulose cell walls. Although not visible under normal conditions, the plasma membrane is located just inside the cell wall.

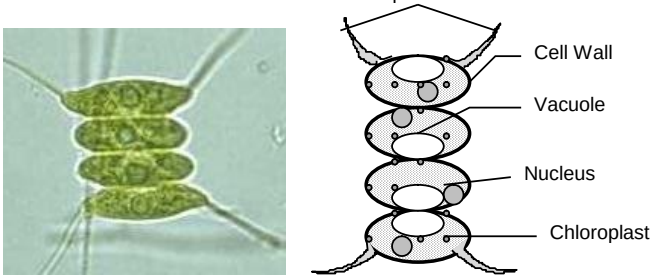
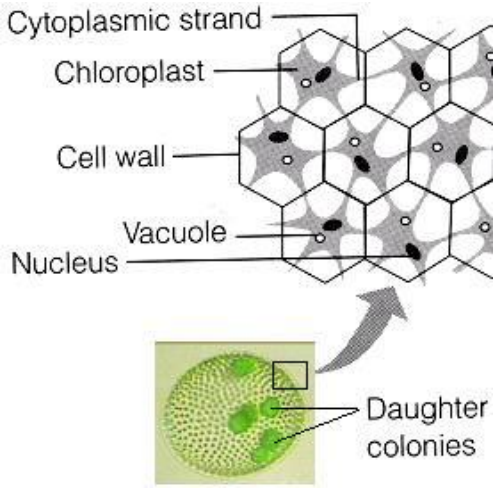


Figure 8. ***Volvox*** is an aquatic alga commonly found in ponds, lakes, and aquaria. It is a complex colony of 500 to 500,000 cells (depending on the species) that are permanently interconnected by cytoplasmic strands to form a spherical colony. Often found on *Volvox* colonies are daughter colonies, small clusters of cells that will eventually leave the parent colony to form an independent colony.



MATERIALS

Clean microscope slides and cover slips
Clean depression microscope slide
Dissection needles
Forceps

Cultures:
Protococcus
Scenedesmus
Volvox

PROCEDURE (Perform in groups of 3 or 4)

Observation of *Protococcus*: A Loose Aggregate (Partner #1 sets up)

1. Obtain a small piece of bark from the supply cart and use a dissecting needle to *gently* brush off a small amount of the green growth into a drop of water on a clean microscope slide. Be gentle! Recall that *Protococcus* forms *loose* aggregates of cells that are easily separated from each other.
2. Complete the Wet Mount by covering with a coverslip. Return the bark to the lab cart.
3. Observe several *small* aggregates of cells under low and then under high power. Cellular detail will be difficult to observe if the aggregates are too large and clumped together.
4. In the appropriate space on your report sheet, use a sharp *pencil* to make an ***accurate sketch*** of a small aggregate of *Protococcus*.
5. Estimate and record the approximate size of a single *Protococcus* cell in micrometers.
6. ***Identify and neatly label on your sketch the following structures*:**
   - **Cell Wall:** Provides support and protection to the cell; Made of cellulose.
   - **Cytoplasm**
   - **Any other cell structures that may be visible**
7. Observe the wet mounts of *Scenedesmus* and *Volvox* that were set-up by your lab partners.

Observation of *Scenedesmus*: A Simple Colony (Partner #2 sets up)

1. Use the pipette labeled "*Scenedesmus*" to obtain a drop from the *Scenedesmus* culture. Place the drop on a clean microscope slide and cover with a clean coverslip.
2. Observe under low and then with high power.
3. In the appropriate space on your report sheet, use a sharp *pencil* to make an ***accurate sketch*** *Scenedesmus*.
4. Estimate and record the approximate size of a *Scenedesmus* colony in micrometers.
5. ***Identify and neatly label on your sketch the following structures*:**
   - **Cell Wall**
   - **Cytoplasm**
   - **Nucleus**
   - **Vacuole:** A clear membrane bound structure that stores sugars produced by photosynthesis
   - **Chloroplast:** Uses carbon dioxide, water, and light energy to produce sugars during the complex process of photosynthesis.
   - **Spines:** Transparent projections found at each end of the colony.
   - **Any other cell structures that may be visible**
6. Observe the wet mounts of *Protococcus* and *Volvox* that were set-up by your lab partners.

Observation of *Volvox*: A Complex Colony (Partner #3 sets up)

1. Use the pipette labeled "*Volvox*" to obtain a drop from the *Volvox* culture. Place the drop on a clean *depression* slide and cover with a clean coverslip. A depression slide is used to prevent the rather large colony from being crushed by the coverslip.
2. Observe under low and then with high power.
3. In the appropriate space on your report sheet, use a sharp *pencil* to make an ***accurate sketch*** of a *Volvox* colony.
4. Estimate and record the approximate size of a *Volvox* colony in micrometers.
5. Identify on individual cells and neatly label on your sketch the following structures:
   - **Cell Wall**, **Chloroplast, Nucleus, and Vacuole**
   - **Flagella:** Responsible for movement of the colony; Two flagella per cell.
   - **Cytoplasmic strand:** Interconnections between adjacent cells of the colony.
   - **Daughter Colonies:** Small spherical clusters of cells that are formed by the asexual reproduction of the colony's cells; eventually released from the colony to form a new colony.
   - **Any other cell structures that may be visible**
6. Observe the wet mounts of *Protococcus* and *Scenedesmus* that were set-up by your lab partners.

Part F. Multicellular Organisms: Observation of *Spirogyra* and Human Epithelial Tissue

Introduction

Multicellular organisms have evolved greater structural complexity by combining cells into larger units: Tissues, organs, and organ systems. In short, there is a division of labor between many tissues and organs to carry out all of the life processes that normally occur in "*simple"* unicellular organism!

The cells of multicellular organisms are specialized to carry out a specific function. A **tissue** (e.g. Muscle tissue) consists of a many cells of a similar type that work together to perform a common function. Different types of tissues are organized together to form functional units called **organs** (e.g. The heart consists of cardiac muscle tissue, nervous tissue, vascular tissue, epithelial tissue, etc.). **Organ systems** consist of different organs operating together to perform various tasks (e.g. the digestive system involves several organs cooperating with each other: Liver, gall bladder, stomach, large and small intestines, etc.)

In this part you will observe cells at the tissue level: Epithelial cells that line the inside of the mouth, and the equivalent type of cell in plants, plant epidermal cells.

MATERIALS

Clean microscope slides and cover slips
Dropper bottle of physiological saline
Toothpicks
Methylene blue
Freshwater algae: *Spirogyra*

PROCEDURE (Perform in groups of two)

Observation of Epithelial Tissue: Cheek Cells (Partner #1 Sets-up)

1. Place a drop of physiologic saline in the center of the slide. Using the flat end of the toothpick, *gently* scrape the inner lining of your cheek. Agitate the end of the toothpick containing the cheek scrapings in the drop of saline.
2. Add a tiny drop of methylene blue stain to the preparation and stir again with the toothpick. Cheek epithelial cells are nearly transparent and thus difficult to see without the stain, which colors the nuclei of the cells and makes them look much darker than the cytoplasm. ***Discard the used toothpick in the disposable autoclave bag provided at the supplies area.***
3. Add a coverslip and observe under low and then under high power.

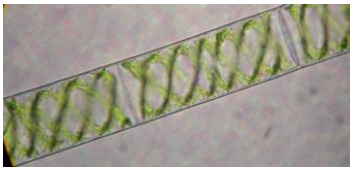
> **Tips:**
> Although the cells form a solid sheet of cells in your mouth, the scraping of the toothpick probably caused the cells to separate from each other. Try to find a cluster of two or three cells whose shapes have not been totally distorted. Avoid observing clumps of cells that show little cellular detail

4. In the appropriate space on your report sheet, use a sharp *pencil* to make an ***accurate sketch*** of a cluster of two or three cells (or one cell if 2 or 3 cells can't be found).
5. Estimate and record the approximate size of a single epithelial cell in micrometers.
6. Identify on individual cells and neatly label on your sketch the following structures:
   - **Plasma Membrane**, **Cytoplasm, and Nucleus**
   - **Any other cell structures that may be visible**
7. Observe the Wet Mount of *algae* that was set-up by your lab partner.

Observation of Epidermal Tissue: *Spirogyra* (Partner #2 Sets-up)

1. Use a pipette to place a sample of the freshwater algae, *Spirogyra*, on a clean slide, cover with coverslip, and observe with low and high power.

> **Tip:** Use a dissecting needle to straighten and untangle the strands of *Spirogyra* on your microscope slide



2. In the appropriate space on your report sheet, use a sharp *pencil* to make an ***accurate sketch*** of <u>*one strand*</u> of *Spirogyra.*
3. Estimate and record the approximate length and width in micrometers of a single cell.
4. Identify and neatly label on your sketch the following structures:
   - **Cell Wall:** Often coated with slime in filamentous algae such as *Spirogyra*.
   - **Cytoplasm:** the internal aqueous environment inside the cell.
   - **Nucleus:** Embedded within the cytoplasm of *Spirogyra;* Very light in color and therefore difficult to find if the diaphragm is opened too wide and/or the condenser is not adjusted properly.
   - **Chloroplast:** In *Spirogyra*, it appears as a long spiral band that runs the length of the cell.
   - **Vacuole:** Clear and large membrane-bound organelle within the cell that stores the sugars produced by photosynthesis. Being clear it is difficult to observe in *Spirogyra* cells unless the diaphragm is used to decrease the light intensity and the condenser and fine adjustment are properly adjusted.
5. Observe the Wet Mount of human cheek cells that was set-up by your lab partner.

6. **After competing part F: Clean up!!**
   - Rinse of slides and return them to the front table to dry.
   - Dispose of used coverslips properly: plastic in the trash, glass ones in the broken glass container by the front desk.
   - Rotate the nosepiece of your microscope to low power, wind-up the cord, ensure the microscope is clean and dry, and then return it to the cabinet.
   - Clean and dry your lab table.
   - Make sure the lab supply carts are neat, clean, and orderly

Gentlemen, if your wife wants to learn to drive,
please don't stand in her way.
☺

If love is blind, why is lingerie so popular?
☺

After twelve years of therapy my psychiatrist said something
that brought tears to my eyes. He said, "No hablo Ingles."
☺

Lab 3 Report Sheet
Microscopes and Cells
Biol 211

Name _____
Group Number _____ **Date** _____

Results

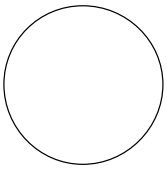
Table 1. Summary chart for *your* microscope

	Low Power	Medium Power	High Power	Oil Immersion
Magnification of Objective Lens	**x**	**x**	**x**	**x**
Total Magnification	**x**	**x**	**x**	**x**
Field Size (diameter)	mm	mm	mm	mm
Field Size (diameter)	µm	µm	µm	µm

1. When you move the letter to the left, which direction does it appear to move within the field of view?

2. When you move the letter away from you, which direction does it appear to move within the field of view?

3. Describe the orientation of the letter "a" as viewed under the microscope. In the circle below use a *sharp* pencil to make a *simple* sketch of the letter as viewed under low power. The circle represents the field of view. Indicate the magnification in the space provided.



Letter "a" as viewed at
_____x magnification

Orientation of Letter:

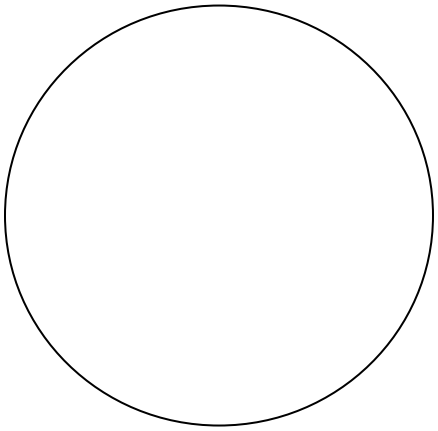
Part C. Depth of Field

Table 2. Depth of field observations from part C.

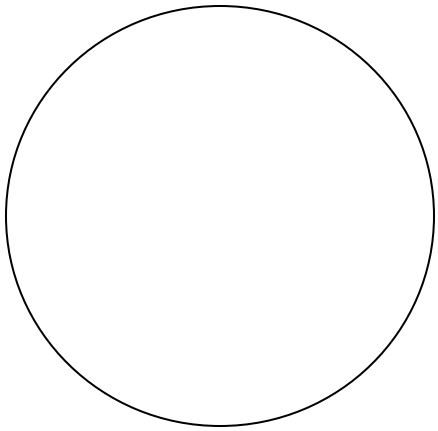
	Objective used to observe threads			
	4x	**10x**	**40x**	**Misc. Observations and/or comments**
How many of the threads were completely in focus?				
Which objective had the shortest depth of field?				
Which objective had the greatest depth of field?				

Part D. Observation of Unicellular Eukaryotic Organisms: *Amoeba* and *Trichonympha*

(Don't forget to label the required cell parts!)



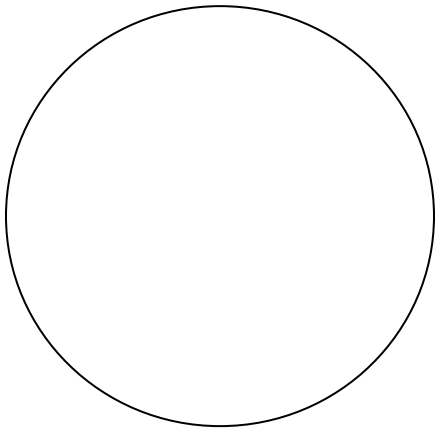
Amoeba **as viewed at _____x Estimated size: _____**



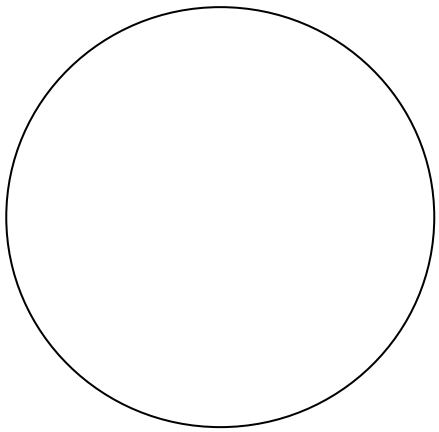
Trichonympha Amoeba **as viewed at _____x Estimated size: _____**

Part E. Aggregate and Colonial Organisms: Observation of *Protococcus*, *Scenedesmus,* and *Volvox*

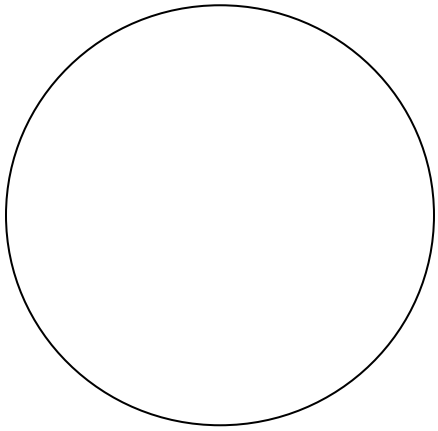
(Don't forget to label the required cell parts!)



Protococcus **as viewed at _____x Estimated size: _____**



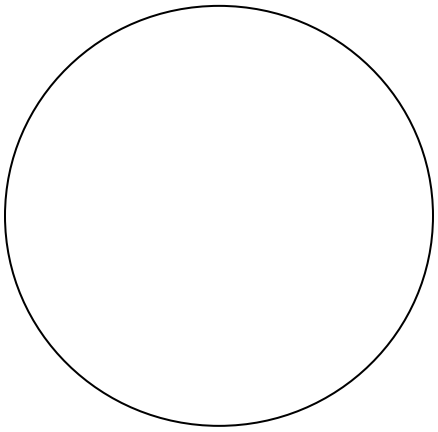
Scenedesmus **as viewed at _____x Estimated size: _____**



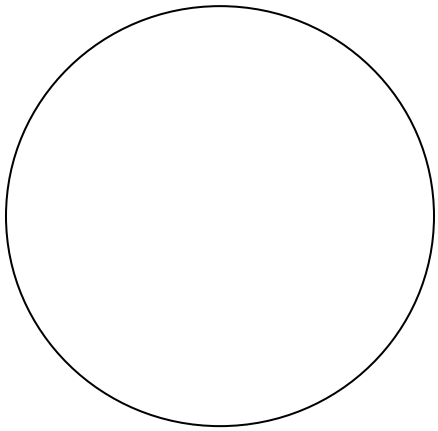
Volvox **as viewed at _____x Estimated size: _____**

Part F. Multicellular Organisms: Observation of *Spirogyra* and Human Epithelial Tissue

(Don't forget to label the required cell parts!)



Spirogyra **as viewed at _____x Estimated size: _____**



Human cheek cells **as viewed at _____x Estimated size: _____**

Review and Application Questions

1. Use your *observations from this lab exercise only* to compare and contrast plant and animal cells from a structural point of view. i.e. Which cell structures do the plant and animal cells observed in this lab exercise have in common, and which cell structures are unique to plant, cells?

2. Explain how the cell structures that are unique to plants are important to the biological success of a plant. *Discuss only those structures observed in this lab exercise.*

3. The fossil record and biochemical evidence indicates that life first appeared as structurally simple unicellular organisms some three billion years ago and then evolved over approximately the next 2.4 billion years to form successively more structurally complex organisms. *Based on your observations in this lab exercise,* describe this evolutionary trend. Use examples from your microscopic observations to illustrate you response.

4. Hypothesize why vacuoles found plant cells are usually much larger than those found in animal cells. Hint: Plants are autotrophs.

5. Why were only a few of the many organelles present in the cells observed in this lab exercise visible? What steps would you take if you wanted to view these structures?

6. Using your textbook as a reference, make a list of the cellular organelles that were *not* visible with your compound light microscope and indicate their primary functions.

Lab 3: Prelab Questions **Name** _____
Microscopes and Cells **Group Number** _____ **Date** _____
Biol 211

Note: Do the prelab reading at the beginning of this lab handout *before* attempting to answer the questions that follow! Hand in this assignment just *before* the start of your scheduled lab period.

1. Why is it necessary to center your object (or the position of the slide you wish to view) before changing to high power?

Complete the table below and use the data to answer questions 2 - 3.

Microscope Number	Objective Lens	Ocular Lens	Total Magnification
1	25x	5x	
2	15x	10x	
3	20x	10x	
4	40x	5x	

2. Given that each slide had the same density of microbes, with which microscope would you expect to *observe* the greatest number of microbes at any given instant? Why?

3. If a slide showing the same organism is examined with each of the microscopes, above, with which...
- two microscopes will the microbe *appear* to move with the *same* degree of rapidity? Why?

- microscope will it *appear* to move the *slowest*? Why?

4. What is meant by resolution (resolving power) of a microscope?

5. After switching from one objective to another, why is it often necessary to readjust the diaphragm and the condenser?

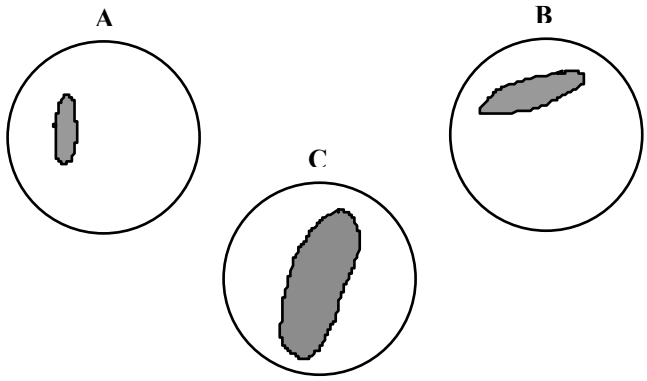
Use the data in the table below for a *hypothetical* microscope to answer questions 6-8

Objective Used	Total Magnification	Diameter of field of View
Low power	30x	6000 μm
Medium power	150x	
High power	300x	
Oil immersion	1500x	

6. Calculate the diameter of the field of view at medium, high, and oil immersion. ***Record your answers in the table above, and show and/or explain your work below***.

7. You observe an object whose length is 1/4 the diameter of the medium power field of view of the hypothetical microscope in the table above. What is its length in microns? _____ In millimeters, mm? _____ ***Show and/or explain your work below.***

8. Calculate (i.e. estimate) the length of the following microscopic objects. Base your calculations on the field sizes you have determined for the hypothetical microscope, above.



a. Object seen in low-power field: **A**

Calculated length: _____ mm

_____ μm

b. Object seen in high-power field: **B**

Calculated length: _____ mm

_____ μm

c. Object seen in oil immersion field: **C**

Calculated length: _____ mm

_____ μm