Activity #3 - Microscopic Examination of Cell Structure.

Learning Goals:

- To name the parts of the basic light microscope, and be able to explain their functions
- To view specimens with the compound light microscope in the correct focal plane
- To calculate magnifications and visual field diameters for different objective lenses
- To estimate the lengths of cells and structures in a microscope field
- To understand the relationship between linear dimensions of a cell and its volume
- To understand the similarities and differences between prokaryotes and eukaryotes

Background info:

Unicellular life comes in a variety of shapes and sizes. Among the prokaryotes, there are rod-shaped organisms, spherical, spiral-shaped, and even square ones. Eukaryotic single-celled organisms show tremendous diversity in intracellular organization. In this exercise we will observe several different types of microbes. Because they are too small to be seen with the unaided eye, they must be observed using a microscope.

We have discussed the concept that the cell is the smallest unit that has the characteristics of life. Multicellular organisms that are big enough to see with your naked eye are still made up of many smaller cells. In order to understand how living organisms function, you need to understand how individual cells function. It's important for you to become familiar with the use of the compound light microscope to better understand cell biology. In lab you will learn about two types of cells. **Prokaryotic cells** lack a nucleus and membrane-bound organelles and tend to be 0.5-5 um in size. **Eukaryotic cells** have a membrane-enclosed nucleus that serves to contain and separate the DNA and other nuclear components from the remainder of the cell. Other organelles, such as mitochondria and chloroplasts, are also surrounded by membranes within the eukaryotic cell. Eukaryotic cells are usually between 5-50 um in size. In today's lab you will learn to locate, visualize, and measure individual cells using a compound light microscope.

When observing and comparing cells and subcellular structures, it is important to recognize that these are three dimensional objects. As the linear dimensions increase, surface area will increase even faster, and volume will increase even faster than surface area. Consider the example of a cell in the shape of a sphere. Calculate the surface area and volume of two cells, measuring 1 µm (similar to a medium-sized bacterium) or 10 µm in diameter (similar to a typical eukaryotic cell).

Reminder: What is the mathematical formula for calculating the surface area of a sphere? What is the formula for the volume of a sphere?

Surface area =

Volume =

			Fold increase
Length on a side	1 μm	10 μm	
Surface area			
volume			

What would these differences in surface area and volume mean for nutrient distribution and waste exchange for each of these cells?

Bonus: By what factor would the cell mass change as a cell's length goes from 1 um to 10 um, assuming the cytoplasm would have the same density for both cell sizes?

I. Care and Use of the Compound Light Microscope

Microscope Parts:

Arm: Supports the lenses, stage, and nosepiece and provides a carrying handle. **Take special care when moving or carrying the microscope. Place one hand under the microscope and the other hand should be grasping the arm.**

Base: The bottom of the microscope which sits on the table. It contains the power controls, power cord, and the light source.

Ocular lenses: The lenses you look through. Ocular lenses have a magnification of 10X. Note the ridged **diopter adjustment ring** around the base of the left ocular—this can be used to adjust the microscope focus independently for the left and right eyes (see procedure later in this lab packet).

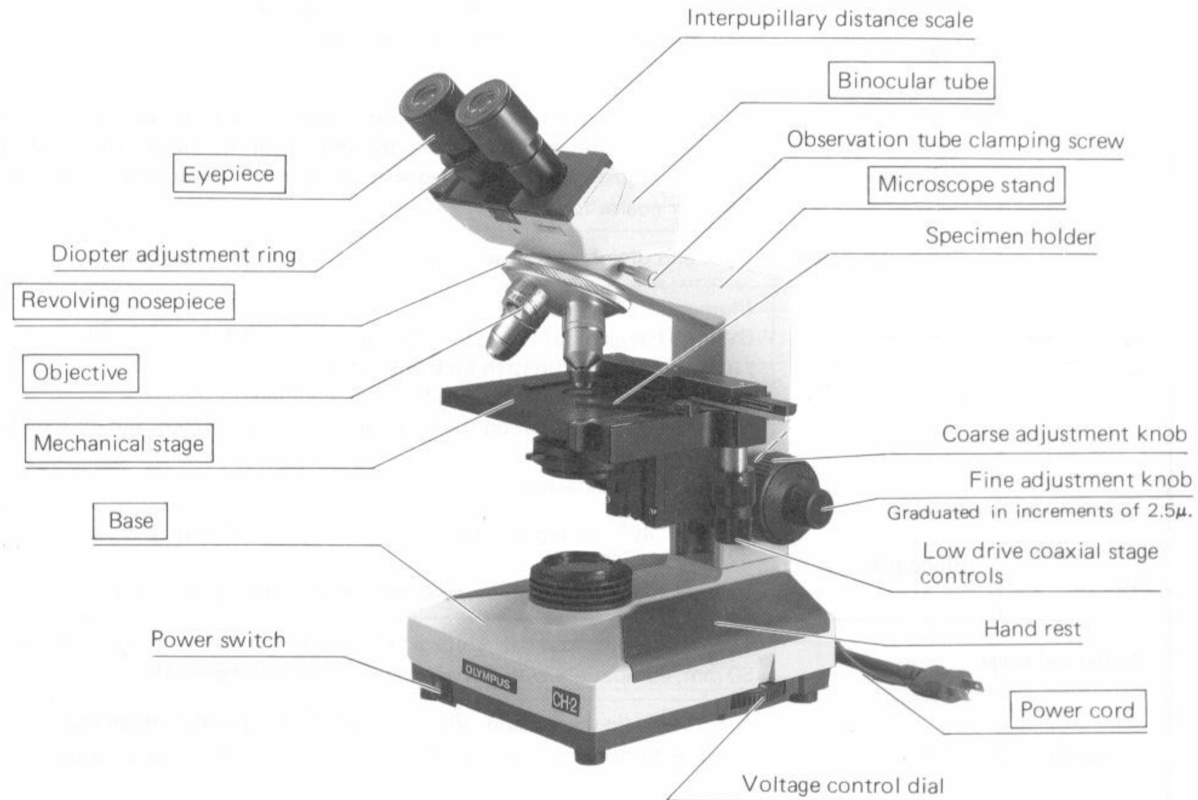

Image from Olympus Model CHT microscope instruction booklet.

Objective lenses: There are 4 objective lenses on your microscope each with a different magnifying power. You will find this number stamped on the side of the objective lens. Multiply the magnification of the ocular lens times the magnification of the objective lens to calculate the total magnifying power of the combined lenses. The 4 lenses are:

- **4X or scanning objective**. Use this for scanning the whole slide and pinpointing the area to be observed. This is the shortest of the objective lenses so there is a greater distance between slide and the objective. When the slide is in focus, there is still space between the slide and the objective lens so it won't be possible to rotate the objective lens into the slide and break it. It is possible to bash into the slide with the higher objectives and damage the scope**. That is why this and the 10X objective are the only objectives you can use with the coarse adjustment knob**. Always start and finish your microscope use with the 4X objective.

- **10X or low power objective**: Allows you to view objects in greater detail. This is a longer lens so you need to be careful using it.

- **40X or high dry objective**: Allows you to view objects in even greater detail. This is a longer lens so you need to be careful using it. **Use only the fine adjustment knob with this lens**.

- **100x or oil immersion objective.** The refractive index for air is 1.00. The refractive index for glass is 1.25. In order to prevent the bending of light rays as they are passing from air through glass, immersion oil is placed directly on specimens to be examined with the 100X objective. The refractive index of the immersion oil is the same as for glass, so the path of light rays is not bent when moving from one medium to another. One unfortunate aspect of using oil is the potential damage that can be done to the 100X objective lens if the oil is not properly removed from the lenses after use. **Therefore, remember to thoroughly clean the oil objective with lens tissue after each use. Use only the fine adjustment knob with this lens**.

Nosepiece: Holds the objectives and rotates them into position. Start with the 4X objective in the viewing position and rotate in a clockwise circle to the next highest powered objective. To prevent damage to your slide and to the microscope, **Never rotate the nosepiece unless you are looking directly at the slide and objective lenses with your naked eye!**

Mechanical stage: A movable platform that holds the slide and brings it up to viewing distance.

Stage clips: Stage clips hold the microscope slide in place on the mechanical stage with a spring-loaded clamp. Open the movable arm of the clip and place the slide between the clips. **Gently** release the arm. Snapping the arm onto the slide can chip the slide. **Never lift the stage clips up. The slide fits <u>between</u> the clips, not under the clips.**

Coarse adjustment knob: This is the large knob which is located on both sides of the microscope arm. It rapidly raises the mechanical stage up to viewing distance and brings the slide into focus. Use only with the 4X objective.

Fine adjustment knob: This is the small knob located in front of the coarse adjustment knob. It barely moves the mechanical stage. Use it to fine tune your focus with any objective.

Mechanical stage control knobs: They are located on the right hand side of the mechanical stage and move the microscope slide position. The top knob controls front to back movement and the bottom knob controls left to right movement.

Condenser: The lens system below the stage that focuses the beam of light from the light source up to the slide. It contains the iris diaphragm. There is a height adjustment knob for the condenser on the left side of the stage. Students frequently mistake this knob for the stage control knobs. You shouldn't need to use this knob if the condenser is set high in the stage.

Iris diaphragm: It is located in the condenser and has a lever that opens and closes it. Use this lever to adjust the light level up and down when you change

magnification. As you go up in magnification you usually need to increase the light to the specimen. Open the iris diaphragm by moving the lever all the way to the left when you begin viewing your slide.

Light source: A light bulb located in the base that provides light for viewing the specimen.

Power switch: Located on the front of the microscope base. **Make sure the rheostat is turned down before you turn on the power switch**. This helps lengthen the life of the bulb.

Rheostat: A dial located on the right side of the microscope base that adjusts the intensity of the light. Set it on 7 or 8 to start viewing your slide. Most light adjustments after that should be made with the iris diaphragm.

General Information and Terminology

Objectives lenses have different lengths and the distance between the objective and the slide is called the **working distance**. The lowest power objective is shorter than the higher ones so there is a greater working distance to the slide. You can use the coarse adjustment on 4X because the working distance is so large that you can't lower the objective into the slide and break it. This is not true with the higher objectives. **If you use the coarse adjustment on the higher powers, you can break the slide, scratch the objective, and damage the microscope. Please be careful—only use the coarse focus adjustment knob with the 4X objective!**

Let your instructor know immediately if you break a slide so that your microscope can be checked. If small pieces of glass get into the controls they can strip the gears and ruin the scope. Never force any part of a microscope that isn't moving easily. Let us know that there is a problem.

Ideally, microscope objectives are **parfocal**. This means that once an objective has been focused, you can rotate to the next highest objective and the image will stay in coarse focus. You will only need to adjust the fine focus knob to see a clear image.

Ideally, microscopes are also **parcentral**. This means that if you have your slide specimen in the center of your field of view it will remain in the center of view as you go up in magnifying power.

The **field of view** (**diameter of field**) is the circle of light you see when you look through the microscope. As you go up in magnification, your field of view gets smaller. If your specimen isn't centered it will be outside your field of view when you increase the magnifying power. If you lose your specimen at the higher powers, go back to the 4X objective and find your specimen and start over with it well centered. Center the specimen each time you increase magnification.

The amount of light reaching your specimen decreases when you increase the power of your objectives. Open your iris diaphragm to let in more light if needed, as you move to higher powered objectives.

Microscope slides have depth. The **depth of field** is the distance you can move through the specimen and have it remain in focus. With a thick specimen you may have to adjust the fine focus repeatedly to see the different features of a specimen. Sometimes when you are having trouble focusing you may be focusing on the cover slip instead of the specimen. It is important to understand how the depth of field affects viewing your specimen.

You may notice a needle in your field of view that does not move when the stage is moved. The pointer may be used to show someone else a particular item of interest on a slide. Microscope pointers are built into one of the oculars.

A. Procedure for the Care and Use of the Compound Light Microscope

Get a slide of the letter "e" from the front desk to use with these general instructions for using your microscope.

1. Clear your work area. Remove the microscope from the cabinet always supporting it with 2 hands; one holding the arm and the other supporting the base. **Gently** place it on the table. Always pick the microscope up when moving it. Dragging it across the counter causes vibrations that can break some small parts inside the scope.

2. Adjust your chair up or down for comfortable viewing.

3. Plug in microscope. Put plastic cover back in cabinet. Make sure cabinet door is closed.

4. Make sure the 4X objective is in place, the mechanical stage is lowered all the way down as you look at it with your naked eye, and the iris diaphragm is all the way opened (lever all the way to the left).

5. Clean all lenses with lens paper before use. The lens paper can be found in the drawer above your microscope cabinet. **Never clean microscope lenses with anything but lens paper. Other types of paper or tissue contain small sharp fibers that can permanently scratch the high-quality optical glass in the eyepieces or objective lenses.**

6. Turn on the light with the switch. Turn up the light with the rheostat to a setting of 7 or 8. Open the iris diaphragm. Once the rheostat is set, leave it alone and use the iris diaphragm to adjust light if more adjustment is necessary.

7. Rotate the nosepiece around until the 4X objective locks into place.

8. Clean your microscope slide with **lens paper** before use. Open the movable arm of the mechanical stage and position the slide between the arms and **gently** release the arm. A hard release of the arm can chip the slide. Use the mechanical stage control knobs to position the area of the slide to be viewed over the circle of light on the microscope stage.

9. Adjust the width between the ocular tubes by slowly moving them apart and together until one circle of light is visible through scope. Look at the scale between the ocular tubes and record this number to save time in setting up your microscope in the future. This represents your interpupillary distance. Record it here:

   My interpupillary distance is ______________mm.

10. Look at the stage **with your naked eye**. Using the coarse focus adjustment knob, bring the mechanical stage up towards the objective lens until the lens is almost—but not quite--touching the slide. Make a note of the relationship between the direction on the focus knob motion and the motion of the stage:

    Which way do you have to move the knob to move the stage toward the objective? _________________________

    Away from it? ______________________

    Note: always begin adjusting the microscope for each new slide by **looking with your naked eye** as you bring the objective towards the slide. This is the best way to avoid breaking slides and damaging the microscope!

11. Look through the oculars and slowly turn the coarse adjustment knob to bring the mechanical stage down away from the objective lens, and the slide into focus. The slide will be about an inch (2.5 cm) away from the 4X objective when it is in focus. Use the fine adjustment knob to fine tune the focus. For some objects less light may result in a better image; experiment with the iris diaphragm.

    If you wear glasses, you may wish to remove them to avoid scratching them—we will adjust the eyepieces separately for the strength of each eye below. If you wear contact lenses, leave them in.

12. **Use the coarse adjustment knob only with the 4X objective**. Never use it with the higher powered objectives. **Use only the fine focus for the higher powered objectives**. If you cannot get the slide in focus with fine adjustment on the higher powers, go back to the 4X objective and start over.

13. Adjusting the eyepieces for comfortable two-eyed viewing:

    Adjust the eyepieces for your own eyes: Close your left eye; look through only the right eye. Remember which way you need to turn the focus knob to bring the slide down away from the objectives. CAREFULLY use the focus knob to move the slide down away from the objectives until it is in fine focus for your right eye. Now close your right eye, and open your left eye. Rotate the left eyepiece's ridged diopter adjustment ring to bring the left eyepiece into focus on the slide.

    Finally, open both eyes and make any fine adjustments to the left eyepiece to make viewing the slide comfortable. If you feel like you are crossing your eyes, or like moving your eyes around the slide is making you dizzy, you need to repeat the eyepiece adjustment procedure until both objectives are in focus for your eyes.

14. Looking directy at the slide and objective lenses **with your naked eye**, carefully rotate the nosepiece around until the 10X objective locks into place. Use only the fine adjustment knob to bring the specimen back into focus.

15. Looking directly at the slide and objective lenses **with your naked eye,** carefully rotate the nosepiece until the 40X objective locks into place. Use only the fine adjustment knob to bring the specimen back into focus.

16. Looking directly at the slide and objective lenses **with your naked eye**, rotate back to the 4X objective, lower the mechanical stage (NOT the condenser!) all the way down, and return your slide to the appropriate tray at the front desk

B. Orientation of the Image

1. Get a slide of the letter e from the front desk and position it in the stage clips so that it is readable. Look at the slide and draw the letter e as you see it with your naked eye.

   This is what the letter looks like with the naked eye:______________

2. Now look through the microscope and bring the letter e into focus with the 4x objective and then bring into focus using the 10x objective. Draw the letter e as it appears through your microscope.

   This is what the letter looks like through the microscope:________________

3. How is the image oriented through the microscope as compared to its orientation on the slide? Is it upside down or right side up? Is it facing the same way or is it backwards? Circle the correct answers in the text above.

4. Use the stage control knobs to move the slide to the right while looking through the scope.

   In which direction does the specimen move when the stage moves right? ____

5. Use the stage control knobs to move the slide away from you while looking through the scope.

   In which direction does the image move when the stage moves away?_______

6. Remove the slide and put in the appropriate tray on the front desk.

II. Making Microscopic Measurements

A. Determining the Diameter of Field

1. Get a slide of a transparent ruler from the front desk and position it in the stage clips. What is the total magnification of the scope when using the 4X objective? Remember that the eyepieces add another factor of 10 to the magnification.

   Total magnification when using the 4X objective and 10X eyepiece is _________.

   Bring the ruler image into focus with the 4X objective. Position the metric side of the ruler in the center of the field of view ("field") with one black millimeter line to the very left of your field view. Count the millimeter lines in your field. This is the scanning-power diameter of field.

How many millimeters wide (the diameter) is your field? (Estimate the amount of space between the lines.) ________

Convert this number to micrometers [there are 1000 micrometers (μm) in a millimeter (mm)]. The 4X field diameter is ________________um.

2. Rotate the 10X objective into position as you look at the slide and objectives **with your naked eye**. Then examine the slide using the eyepieces.

   What is the total magnification when using the 10X objective? __________

   Position the ruler so that one black line is to the left of your field. What is the diameter of your field with the 10X objective (Don't forget the units)? __________

   Can you confidently determine the low-power diameter of field?

3. Use the following equation which uses the scanning-power diameter of field to calculate the low-power diameter of field.
   Note: Be sure to use the **total** magnifications (40X for the 4X objective, 100X for the 10X objective).

   Diameter1 x mag1 = diameter2 x mag2

   So in this case, to calculate the 100X field diameter, the equation would be

   ________________ x __40X__ = ___________________ x _100X___

   In plain English:
   40X field diameter x 40X mag. = 100X field diameter x 100X mag.

4. To compute the higher-power diameters of field, repeat the above equation substituting the higher-power magnification for the low-power magnification.

Table 1. Relationship between Magnification & Field Diameter

Objective lens	Total Magnification	Diameter of field (μm)
4x		
10x		
40x		
100x		

What happens to the diameter of field as you increase the magnification by a factor of 10, numerically?___________________________

5. Measuring objects using the field diameter

Now that you know the diameter of field with each objective, you can determine the length of an object using one of the following methods:

- Estimate the fraction of the field that is taken up by your specimen and use the formula below to estimate the size of your specimen.

  fraction of field taken up by specimen x diameter of field = ___________

- Divide the diameter of field by the number of cells that cross the diameter of field

$$\frac{\text{Diameter of field}}{\text{Estimated number of cells that would fit across the diameter of field}} = \text{length of object}$$

6. Return your slide to the appropriate tray at the front desk.

III. Prokaryotic Versus Eukaryotic Cells

Microbes may be viewed in either a wet mount or a stained mount. A **wet moun**t enables microbes to be observed in their natural (living) state, so that characteristics such as motility, morphology, and intercellular organization can be observed. Simple staining procedures use a single stain to help visualize the individual cells of microorganisms that are deposited on a slide by smearing. Most dyes used in this procedure (crystal violet, methylene blue, or basic fuchsin) contain chromophoric (colored) cations (positively charged ions). Because the surfaces of bacterial cells have a slightly negative charge, the positively charged dyes have an affinity for the cells. The dyes that interact in this fashion with the bacterial cells are referred to as **basic dyes.** Other dyes that are anionic, that is, those in which the chromatophore carries a negative charge, are useful in other staining techniques. In this lab you will prepare and view microbes in both wet and dry mounts.

The **Gram stain** was developed in 1884 by Hans Christian Gram, a Dutch bacteriologist, to help study a group of spherically shaped bacteria isolated from human lung tissue. The stain differs from other simple stains performed previously in that the Gram stain is used to differentiate types of bacteria depending on their differing abilities to retain a particular stain. It is therefore referred to as a differential staining technique.

The Gram stain is one of the most frequently used techniques in microbiology, and mastering it can be difficult. In order to become proficient, one must pay careful attention to the procedure and practice it often. The technique separates bacteria into two groups: Gram-positive and Gram-negative. The first step of the Gram stain involves staining the fixed smear of organisms with a primary stain of crystal violet. This is followed by the application of Gram's iodine stain, also known as the mordant (a substance capable of intensifying or deepening the reaction of the specimen to a stain). The next step in the procedure is the most critical and involves washing the stained smear with a decolorizing agent, usually 95% ethanol or isopropyl alcohol. The last step employs a counterstain known as safranin. Gram-positive organisms will not be easily decolorized and thus retain the purple stain of crystal violet. On the other hand, the Gram-negative organisms will be decolorized by the alcohol so that they then can take up the safranin counterstain. Gram-negative organisms will appear pinkish or red. If too much alcohol is applied, it is possible to decolorize almost all cells. Careful adherence to the procedure will help to ensure successful results.

The results of the Gram stain depend on the age of the culture and the amount and timing of alcohol decolorization. The age of the culture is an important factor in the outcome of the Gram stain. Most Gram-positive organisms will lose their Gram-positivity with age. **The Gram stain should always be performed on vigorous, actively growing cultures** (18- to 24-hour cultures usually will give excellent results). To further complicate interpretation of results, some organisms turn out to be **Gram-variable** following Gram staining. This means that some of the cells in the population will stain purple (gram-positive) and other cells will stain red (gram-negative). Only repeated staining of the same culture at different times will verify the existence of a truly Gram-variable culture. Gram-variability is relatively rare; mixed results in the Gram stain most probably occur from working with an impure culture, or either underdecolorizing or overdecolorizing with ethanol.

The basis of the Gram stain resides in the differences in cell wall composition of Gram-positive and Gram-negative bacteria. Be sure to read your textbook for a complete discussion of the topic. Briefly, **Gram-positive organisms** have a thick cell wall composed of peptidoglycan, a polymer containing sugars and amino acids., and a molecule called teichoic acid. On the other hand, **Gram-negative organisms** have a much thinner cell wall and have an outer membrane composed of lipopolysaccharide and lipoprotein. On proposed mechanism is the shrinkage of pores in the thick peptidoglycan layer of cell walls of Gram-positive organisms due to exposure to the alcohol decolorizer. The shrinkage of the pores leads to retention of the dye complex in gram-positive cells; whereas, the higher porosity of the thin peptidoglycan layer of Gram-negative organisms leads to more rapid loss of the dye complex.

Differences in cell wall composition of Gram-positive and Gram-negative organisms can account for the differences in susceptibility to some antibiotics. This is important if a doctor is trying to determine the best treatment for an infection.

A. Preparation of smears and Gram stains. Slides of *Escherichia coli, Bacillus subtilis, Lactotococcus lactis, and Rhodospirillum rubrum* have been prepared for you to save time during today's lab. You should, however, be familiar with the techniques used to prepare the specimens you will observe in Part B.

1. With a sterilized loop, place a drop of liquid culture to be stained on an appropriately labeled clean glass slide. If the smear is too dense (i.e., contains a large number of organisms), staining and differentiating the organisms will be difficult. Make sure to **spread out** the liquid on the surface of the slide to facilitate drying and distribution of the organisms.
2. Allow the smears to dry at room temperature. Do not blow on the slide. Remember, we are laden with "germs" and are not interested at this point in determining the types of microbes in human breath!
3. Gently heat-fix the organisms to the slide by passing the dried smears through the flame of the Bunsen burner. A clothespin or forceps should be used to prevent burning of fingers. Caution must be exercised not to overheat the slide. "Cooked" slides may give inaccurate Gram stain results. Remember to heat-fix gently, and not cook the bacteria on the slide.
4. Place smears on a staining tray rack or over a sink rack and cover the smear with the crystal-violet stain. Allow the stain to react for **1 min.**
5. Using the wash bottle provided, rinse the slide with a small amount of water. Make sure to remove excess water by shaking the slide.
6. Cover the smear with the Gram's iodine stain and allow to react for **1 min.** Wash off the stain with water.
7. Holding the slide at a 45" angle over the sink or staining tray, apply the decolorizer dropwise at the top of the slide and allow the alcohol to run off the slide. Add a maximum of 4 to 5 drops of alcohol to the slide to prevent overdecolorization. Immediately rinse the slide with water. Remove the excess water by shaking.
8. Counterstain by flooding the smear with safranin for **1 min.**
9. Rinse the water and blot dry by placing between pages of the bibulous pad. Wipe the **bottom** of the slide to remove excess stain that may decrease visibility.

Critical precautions to take when performing Gram stains:

- Do not overdecolorize slides with alcohol.
- Make sure all stains are fresh.
- Use actively growing cultures.
- Prepare thin smears containing a sparse number of organisms.

- Do not overheat the slide during the fixation step as structural morphologies of the cells may be altered: Overheating during the fixation step may influence the structure of the cell wall and, as a result, alter the outcome of the stain.
- Do not allow the stains to react with the smear for longer than the specified time.
- Use as little water as possible when rinsing your slides.

B. Observation of Gram stained bacteria.

Each person in a group of four is responsible for setting up one slide on the microscope using the oil immersion lens. All group members must view each slide to be able to compare the sizes and shapes of the organisms.

1. Observe the slide with the 10X and 40X objectives.

2. **Using the 100X (oil immersion) objective**. To use the 100X objective, after you have finished viewing the specimen with the 40X objective, rotate the nosepiece halfway between the 40X and 100X objectives, place a drop of immersion oil onto the specimen and rotate the 100X objective into position. Check to be sure that the oil is contacting both the lens and the slide.

   After each observation at 100X, be careful to wipe the oil off of the 100X objective using <u>LENS TISSUE</u>. This will help to prevent transfer of oil to the other, non-oil objectives (the 4X, 10X, and 40X).

3. Sketch the organisms' shapes and estimate their sizes. Record your data in Table 3.

4. Gently but thoroughly wipe the oil off of the 100X objective using **lens tissue**.

C. Prepare wet mounts of *Escherichia coli, Rhodospirillum rubrum, B subtilis* or *Micrococcus luteus.* Remember to label each slide clearly to avoid confusion later on. Each person in a group of four is responsible for preparing and setting up one slide on the microscope. All group members must view each slide to be able to compare the sizes and shapes of the organisms.

1. if using bacteria from agar, place a drop of dH_2O on the center of slide, touch a toothpick to bacteria on the agar and mix with the drop of water. If using suspended liquid cultures; place a drop of liquid culture onto the slide. ,

2. Cover with a glass coverslip, using a kimwipe to absorb any excess liquid.

3. Observe the slides with the 10x and 40x objectives, and sketch them in Table 3. DO NOT observe these wet mount slides with the 100X objective.

D. Eukaryotic Cells: Unicellular organisms

Prepare wet mounts of *Amoeba proteus, Euglena gracilis, Paramecium caudatum or Saccharomyces cerevisiae* as described above. Remember to label each slide clearly to avoid confusion later on. Each person in a group of four is responsible for preparing and setting up one slide on the microscope. All group members must view each slide to be able to compare the sizes and shapes of the organisms. Observe with the 4X, 10X, or 40X objectives (NOT the 100X!) and record your observations in Table 4. Look for cytoplasmic streaming in the living *Amoeba*.

E. Animal cells: human cheek cells

1. Get a clean slide from the box on your lab table.
2. Place a drop of methylene blue stain on your slide
3. Take the broad end of a toothpick and gently scrape the inside of your cheek
4. Stir the scrapings into the drop of methylene blue and top with a coverslip.
5. Put the toothpick in the beaker of bleach water located on your lab table. Human cells are potentially hazardous and must be disposed of properly.
6. Observe the cells under the microscope at all magnification levels.
7. Make a drawing of the cheek cells and label the plasma membrane, cytoplasm, and the nucleus in Table 4 using the 40x objective.
8. When you have finished with your slide put it in the beaker of bleach.

F. Plant cells: *Elodea* Check to make sure you have Elodea leaves available that have been separated from their roots for less than 4-5 hours.

1. Get a clean slide from the box on your lab table.
2. Obtain a single *Elodea* leaf from the tip of the plant located at the front desk.
3. Place it on your slide with a drop of water and cover with a coverslip.
4. Observe the leaf under the microscope. Note that the leaf has thickness so you may need to refocus to see the different layers. Focus on the edge of the leaf to get the best image. Focus using the 4x, 10x, and 40x objectives.
5. After the cells have warmed up a bit from the light, you should be able to see cytoplasmic streaming starting. Watch the chloroplasts along the wall of the cell. They will start circling the cell wall.
6. Make a drawing of the Elodea at 40x magnification and label the cell wall, chloroplasts, central vacuole, and cytoplasm.
7. To visualize the difference between the cell membrane and the cell wall, put 1 drop of 10% sucrose at the edge of the coverslip. Watch as the cell membrane shrinks back away from the cell wall. Draw the shrunken cell in Table 4.
8. When you have finished with your slide, throw away the coverslip and leaf. Wash your slide at the sinks in the back of the room, dry it with paper towels and return it to the slide box on your lab table.

G. Putting away the microscope

- Clean all lenses with lens paper, and the stage with kimwipes.
- Remove your slide from the stage and return it to the appropriate tray at the front desk.
- Turn the rheostat all the way down and turn off the power switch.
- Rotate nosepiece until the 4X objective locks into place.
- Lower the stage all the way.
- Adjust mechanical stage so the arm isn't sticking out.
- Unplug cord and tuck it between the base and stage.
- Replace dust cover.
- Using 2 hands, return the microscope to its cabinet. Be careful not to hit the oculars when replacing or removing the microscope from the cabinet.

H. The importance of volume

Recall the formula to calculate the volume of a sphere:_______________
If a bacterial cell is 5 um in diameter, and a human cell is 25 um in diameter, assuming both are spherical, calculate the volume of each cell below.

Table 2. Relationship between diameter and volume.

	bacterium	human	human:bacterium ratio
diameter (μm)	5 μm	25 μm	5:1
volume (μm^3)			
volume:diameter ratio			XXXXXXXXXXX

Do you think a human cell could use the same mechanisms as a bacterial cell for moving things around its cytoplasm, given the huge difference in cell volume?

Table 3 – Microscopic Observations of Prokaryotes

Type of cell	dimensions (units)	organelles present	diagram(s)
Escherichia coli (or Serratia fonticola)			Gram stain wet mount
Bacillus subtilis Gram stain only			(no wet mount)
Lactococcus lactis (or Micrococcus luteus)			Gram stain wet mount
Rhodospirillum rubrum Gram stain only			Gram stain (no wet mount)

Table 4 – Microscopic Observations of Eukaryotes

Type of cell	dimensions (units)	organelles present	diagram(s)
Amoeba proteus wet mount only			
Euglena wet mount only			
Paramecium caudatum wet mount only			
human cheek cell wet mount with methylene blue			
Elodea wet mount			wet mount + sucrose

Summary Questions

1. Make sure you can identify and name the parts of the microscope.

2. Make sure you have enough practice to be good at finding the correct focal plane for observing your slides. You will be tested on this ability during the Laboratory Practical Exam later in the semester. Practice makes proficient!

3. How does the distance between the slide and the objective vary when objectives of different magnification are used?

4. How does the diameter of the field of view and the amount of light in the field change with an increase in magnification?

5. If you use a 63X objective and 10X eyepieces, what is the total magnification?

6. If the field diameter using a 5X objective is 100um, what is the field diameter using a 20X objective? (Hint: don't forget to account for the total magnification!)

7. If 25 cells fill the field diameter using the 20X objective in the previous question, how long is each cell, in um?

8. Draw one cubic centimeter here. →
   This is equivalent to one milliliter.

9. If you filled the cube in question 8 with water, how much would that water weigh?

10. If a bacterial cell is 2 um in diameter, and a human cell is 20 um in diameter, what is the ratio of the volume of the human cell to the volume of the bacterial cell?

10. Do all cells contain the same kind of organelles?

11. What structures or characteristics do prokaryotic and eukaryotic cells have in common?

12. How do eukaryotic and prokaryotic cells differ?

13. How do plant, animal, and protist cells differ?

14. Calculate the volume of a rod-shaped bacterium (rounded ends) that is 3 μm long and 1 μm in diameter. Hint: the middle of the bacterium is like a cylinder, and the two rounded ends are like two halves of a sphere.

15. How many of the bacteria above could fit into a spherical white blood cell (d = 15 μm)

16. 1.0 mL of culture containing 3×10^9 bacteria is centrifuged, what would be the pellet volume, using the individual cell volume calculated in #14 above?

17. What fraction of the total culture do the bacteria make up in question #16 above?