

EXERCISE 4 - Lab Procedures

I. Calibrate your reticle

1. Rotate the scanning (4X) objective into viewing position and place a transparent plastic ruler on the stage. Focus the microscope and align the ruler until the millimeter lines come into clear focus in the field of view.
2. Define the distance between 2 of the lines closest together on the reticle as one reticle unit (r.u.).

⇒ **Careful! Do not be distracted by numbers on the reticle, if present. A reticle unit is the *smallest* unit visible on the reticle.**

3. Rotate the ocular and move the ruler until the reticle is aligned across 2 adjacent millimeter marks. Determine the number of reticle units (r.u.'s) which correspond to 1mm as seen through the microscope. Make sure you count the number of r.u.'s from the middle of one mm line to the middle of the next mm line.

Reticle units per millimeter for your microscope using the 4X objective: _____.

Using this information, calculate the number of μm per r.u.

With this microscope, using the 4X objective, one reticle unit corresponds to _____ μm

4. Rotate the nosepiece to bring the 10X objective into aligned position. Note that the reticle appears the same size, with its marks the same distance apart as when the 4X objective was in position. However, the millimeter lines on your ruler appear to be spaced much further apart at higher magnification.

Determine the μm 's per r.u. when using the 10X objective. If the reticle in your microscope is not long enough to reach between 2 adjacent millimeter marks at this magnification, use the following formula to calculate μm 's per r.u.

$$\frac{\mu\text{m}}{\text{r.u.}} (\text{using } 4\text{X obj.}) \times \frac{4\text{X}}{\text{Mag of the new obj.}} = \frac{\mu\text{m}}{\text{r.u.}} (\text{using new obj.})$$

With this microscope, using the 10X objective, one reticle unit corresponds to _____ μm .

Also determine the μm 's per r.u. when using the 40X objective, using the formula above.

With this microscope, using the 40X objective, one reticle unit corresponds to _____ μm .

II. Examining Prokaryotic Cells

1. **Bacterial shapes** - Because bacterial cells are very small, not much more than their basic shape will be visible under the light microscopes available in lab. Usually, bacteria exhibit one of 3 shapes: **bacilli** (rods), **cocci** (spheres), or **spirilli** (spirals).
 - a. Place the prepared slide labeled "bacterial types" on a white background and examine it with your naked eyes. You should see 3 distinct pinkish or purplish spots under the coverslip. Each spot contains bacteria exhibiting one of the 3 shapes listed above.
 - b. Place the slide on the stage of your microscope and examine each of the 3 colored spots. Focus with low power first and then switch to high power. The cells you observe should be stained pink or purple. They will be much smaller than the eukaryotic cells you observed last week. Note that in some cases the cells may be attached to form clusters or chains. Be sure you locate and can recognize all 3 bacterial shapes. For assistance, check photographs that may be available in lab or in your textbook.

- c. In your lab notebook, draw diagrams of the 3 bacterial shapes observed on your slide and label each shape with its name.
 - d. Use the reticle to measure the size of a few bacterial cells with each shape. Record the results in your lab notebook in reticle units, then convert the measurements to micrometers. Make sure you mark down the objective you used when making your measurements.
 - e. When you are finished, return the slide to its original location.
2. **Cyanobacteria** - Cyanobacteria are a fairly common group of bacteria that carry out a type of photosynthesis that produces O₂. (Note: Some bacteria carry out alternative types of photosynthesis that do not produce O₂.) Scientists believe cyanobacteria were largely responsible for converting the early earth's atmosphere from a reducing atmosphere into an oxidizing atmosphere. Cyanobacteria use a pigment called **chlorophyll a** to absorb light energy for photosynthesis. This type of chlorophyll is also found in photosynthetic eukaryotes. However, in cyanobacteria the chlorophyll molecules are not located in chloroplasts, as they are in eukaryotes. Instead, they are found on the surface of **photosynthetic lamellae** located throughout the cytoplasm.
- a. Prepare a wet mount of *Anabaena*, making sure you get some of the green filaments from the culture onto your slide. Examine *Anabaena* using low power first and then high power. On high power you should be able to distinguish the individual cells that make up each filament. For assistance, check photographs that may be available in lab or in your textbook.
 - b. In your notebook, draw a filament of *Anabaena* which clearly shows the shape of the individual cells. Do all the cells look identical? Note any differences on your diagram.
 - c. Use the reticle to measure the size of a few *Anabaena* cells. Record the results in your lab notebook in reticle units, then convert the measurements to micrometers. Make sure you mark down the objective you used when making your measurements.
 - d. When you are finished, remove the coverslip and place it in the discard container provided. Wash the slide, dry it, and return it to its original location.

III. Examining Eukaryotic Cells

1. **Mixed Protozoans (non-photosynthetic protists)** - "Protozoans" is a general term for a variety of mostly unicellular, non-photosynthetic protists.
 - a. Prepare a wet mount of the Mixed Protozoans culture.

⇒ Note: Protozoans are relatively large and tend to settle to the bottom of the culture jar. You will get the best results if you leave the water in the jar undisturbed, and take your sample from the bottom. Squeeze some air out of a dropper BEFORE you place it into the jar, and then slowly suck in some of the water and debris from the BOTTOM of the jar. Do NOT disperse the cells by stirring up the water in the jar or by squeezing air out of the dropper into the water.
 - b. Examine the slide with your microscope. Using the keys and diagrams provided, identify at least 2 different protozoans on your slide.

⇒ Careful: The protozoan culture may be contaminated with small animals called rotifers or other organisms. If you are not sure the organism you are viewing is a protozoan, check with your instructor
 - c. In your lab notebook, draw diagrams of 2 different protozoans and label each with its name. Next, record the length and width of each organism in reticle units, and then convert the measurements into micrometers. Finally, indicate whether each organism is unicellular or colonial.
 - d. When you are finished, discard the coverslip in the container provided. Clean and dry the slide and return it to its original location.

2. **Paramecium (non-photosynthetic protist)** - *Paramecium* is a common protozoan that uses **cilia** for locomotion and feeding. It feeds mainly on bacteria and has a long oral groove lined with cilia that beat food into an oral cavity. At the end of the oral groove, the food is engulfed by **phagocytosis** and becomes enclosed in **food vacuoles** which fuse with enzyme-filled **lysosomes** to be digested. Undigested food is eliminated through an **anal pore**. Like *Euglena*, which you viewed last week, *Paramecium* lives in freshwater lakes and ponds, and depends on contractile vacuoles to remove excess water from its cytoplasm.

- a. View a prepared slide of *Paramecium* with your microscope using low power first and then high power.
- b. In your lab notebook, draw a diagram of *Paramecium*. Make the diagram at least 10cm long. With the help of photographs, diagrams, or models, LABEL the structures listed below:

plasma membrane
cytoplasm
nucleus
nucleolus
nucleoplasm
nuclear membrane
contractile vacuole
oral groove
food vacuole

- c. Next, you will be measuring the length and width of 6 different paramecia in reticle units. After you are finished, you will convert your measurements into micrometers. Prepare a table in your lab notebook to record your data. Give the table a descriptive title.
- d. Using whichever magnification is most appropriate, measure and record the length and width of 6 different paramecia in reticle units. For proper measurement, the length of one *Paramecium* should appear no longer than the total length of the reticle, but its width should appear at least as wide as one reticle unit. Also record the magnification at which the measurements were made.
- e. Convert all measurements to micrometers, and record the results in you table.
- f. When you are finished, remove the slide from your microscope and return it to its original location.

3. **Green Protist Survey Mixture (photosynthetic protists)** - "Green Protists" is a general term for a variety of unicellular and colonial, photosynthetic protists.

- a. Prepare a wet mount of the Green Protist Survey Mixture. Make sure you get some of the green material at the bottom of the culture jar on your slide.
- b. Examine the slide with your microscope. Using the keys and diagrams provided, identify at least 2 different green protists on your slide.

⇒ Careful: The green protist culture may be contaminated with *Anabaena* or other organisms. If you are not sure the organism you are viewing is a green protist, check with your instructor

What gives these organisms their green color? _____

What does this tell you about their method for obtaining organic compounds? _____

- c. In your lab notebook, draw diagrams of 2 different green protists and label each with its name. Next, record the length and width of each organism in reticle units, and then convert the measurements into micrometers. Finally, indicate whether each organism is unicellular or colonial.
- d. When you are finished, discard the coverslip in the container provided. Clean and dry the slide and return it to its original location.

4. **Euglena (photosynthetic protist)** - *Euglena* is a common green protist that uses a flagellum for locomotion. Last week, you viewed a wet mount and a prepared slide of *Euglena*.

- a. View a prepared slide of *Euglena* with your microscope using low power first and then high power.
- c. You will be measuring the length and width of 6 different *Euglena* in reticle units. After you are finished, you will convert your measurements into micrometers. Prepare a table in your lab notebook to record your data. Give the table a descriptive title.
- c. Using whichever magnification is most appropriate, measure and record the length and width of 6 different *Euglena* in reticle units. For proper measurement, the length of the *Euglena* should appear no longer than the total length of the reticle, but its width should appear at least as wide as one reticle unit. Also record the magnification at which the measurements were made.
- d. Convert all measurements to micrometers, and record the results in your table.
- e. When you are finished, remove the slide from your microscope and return it to its original location.

5. **Onion Bulb Cells (non-photosynthetic plant cells)** – These plant cells live underground and, unlike most plant cells, are not photosynthetic.

- a. Place a drop of Lugol's solution on a CLEAN microscope slide.
- b. Use forceps to peel the very thin and transparent "skin" from the inner (concave) surface of the onion bulb. Carefully spread the onion skin in the Lugol's solution on the slide so that it lies flat and in a single smooth layer. Cover it with a coverslip.
- c. Examine your slide with both low and high power. The onion cells should resemble the photographs provided in lab. The "lines" that form the box-like grid are nonliving **cell walls** composed chiefly of **cellulose**. The cell wall surrounds the **plasma membrane**, which encloses the **cytoplasm**. The central part of the cell is filled with a large membranous sac called the **central vacuole**, which is filled with water and salts. The vacuole squeezes most of the cytoplasm, organelles, and the nucleus into a thin layer around the edge of the cell. This is difficult to observe under the light microscope, but can easily be seen in the plant cell models, photos, and diagrams available in lab. It is also difficult to observe any membranes in this preparation, but you should be able to determine where they are with the help of photographs, diagrams, or models.
- d. As you examine each cell, locate the **nucleus**, which will appear as a bright yellow, round structure.

Why does the nucleus appear yellow? _____

Inside each nucleus there will be one or more tiny circles called **nucleoli** (singular is **nucleolus**). Note that in some cells the nucleus appears to be located near the center of the cell (where the vacuole is supposed to be) rather than squeezed along the side of the cell. Explain this apparent discrepancy.

- e. In your lab notebook, draw ONE onion cell. Make the diagram at least 10cm long. With the help of photographs, diagrams, or models, LABEL the structures listed below:

cell wall
plasma membrane
cytoplasm
central vacuole
nucleus
nucleolus
nucleoplasm
nuclear membrane

- f. Measure the length and width of an onion cell using the reticle. Convert the measurements to micrometers and record the result next to your diagram.
- g. When you are finished, discard the coverslip in the container provided. Clean and dry the slide and return it to its original location.

6. *Elodea* Cells (photosynthetic plant cells)

- a. Place a drop of water on a CLEAN microscope slide.
- b. Use a pair of forceps to remove a leaf from the *Elodea* plant, place the leaf in the drop of water, and cover it with a coverslip.
- c. Examine the leaf under low power and high power. The *Elodea* cells should resemble the photographs provided in lab. The small, green, circular objects are **chloroplasts**. Why do some of the chloroplasts appear to be in the center of the cell where the central vacuole is supposed to be?

The nucleus in these cells is much more difficult to see than it was in the onion cells. Explain why:

- d. Cells are 3-dimensional; therefore, they have depth as well as width and length. Choose an individual cell and adjust the fine focus up and down (never use the coarse adjustment on high power). Notice the location of the various cell structures as you focus on the upper, middle, and lower part of the cell.
- e. Continue to focus carefully up and down through the *Elodea* leaf. Notice the overlapping cells. How many cell layers comprise the leaf's thickness?

- f. You may see the chloroplasts in some cells moving in a circular fashion around the edge of the cell, just **INSIDE** the plasma membrane. [Note: You can often stimulate this motion by increasing the diameter of the diaphragm.] It is actually the movement of the cytoplasm that carries the chloroplasts around the cell. This phenomenon is called **cyclosis** or **cytoplasmic streaming**. Is the direction of movement the same in all the cells? Explain:

- g. In your lab notebook, draw ONE *Elodea* cell. Make the diagram at least 10cm long. With the help of photographs, diagrams, or models, LABEL the structures listed below. If you observed cytoplasmic streaming, draw arrows to show the direction of streaming.

- cell wall
- plasma membrane
- cytoplasm
- chloroplasts
- central vacuole
- nucleus
- nucleolus
- nucleoplasm
- nuclear membrane

- h. Measure the length and width of an *Elodea* cell using the reticle. Convert the measurements to micrometers and record the result next to your diagram.
- i. When you are finished, discard the coverslip in the container provided. Clean and dry the slide and return it to its original location.

7. Human Cheek Cells (animal cells)

- a. Place a drop of Lugol's solution on a CLEAN microscope slide.
- b. Remove a CLEAN toothpick from its package and use it to gently, but firmly, scrape the inside of your cheek. Be careful not to gouge your cheek. Some cheek cells will be in the saliva which sticks to the toothpick.
- ⇒ **CAUTION – HUMAN BODY FLUIDS MAY TRANSMIT DISEASE. USE ONLY A TOOTHPICK WHICH YOU HAVE PERSONALLY REMOVED FROM AN UNOPENED PACKAGE. DO NOT GIVE YOUR TOOTHPICK TO ANOTHER STUDENT. DISCARD THE TOOTHPICK IN THE DESIGNATED CONTAINER AS SOON AS YOU HAVE PREPARED YOUR SLIDE. DO NOT HANDLE A CHEEK CELL SLIDE THAT WAS PREPARED BY ANOTHER STUDENT!**
- c. Stir the scrapings into the drop of Lugol's solution on the slide, and then discard the toothpick in the container provided.
- d. Cover the drop of Lugol's solution with a coverslip and examine it using low power on your microscope. The cells will look yellow (why?) and should resemble the photographs of cheek cells available in lab. Note that groups of cheek cells may be clumped together.
- e. Move your slide so that one particularly clear cell is in the middle of the field of view. Now switch to high power and examine the cell more closely. Compare the cheek cell with the previous cells you have viewed. Note that animal cells lack a cell wall, chloroplasts, and the large central vacuole characteristic of plant cells.
- f. In your lab notebook, draw ONE human cheek cell. Make the diagram at least 10cm long. With the help of photographs, diagrams, or models, LABEL the structures listed below.

plasma membrane
cytoplasm
vacuole
nucleus
nucleolus
nucleoplasm
nuclear membrane

- g. Measure the length and width of a human cheek cell using the reticle. Convert the measurements to micrometers and record the result next to your diagram.
- h. When you are finished, place your slide in the container of disinfectant provided.

Clean up

Turn off your microscope, unplug it, wrap the power cord around the base, rotate the scanning objective into viewing position, and return the microscope to the microscope cabinet.

All disposable glassware goes into the special glass disposal receptacle.

Wipe off your work space with a damp paper towel. Leave your work area in the same order that you found it in. Make sure everything that you have used is clean, put away, or discarded. Ask your instructor to check your work area before you leave.