Microscopy and Cellular Morphology

As we discussed in class, many organisms on the planet exist as single cells and are referred to as microorganisms – bacteria, protozoans, among others. When a single microorganism is placed on a suitable solid medium, it may grow and divide producing a large population of similar/identical cells. This visible population is referred to as a colony. Although the colony is visible to the naked eye, what we cannot see are the individual microbial cells. By definition, microorganisms are organisms/cells that individually are to small to be seen by the unaided human eye. It is therefore necessary to employ an optical system for magnification in order to observe microbes. Magnifying devises range from a simple hand lens or magnifying glass with magnification powers of 4X –l0X to the electron microscope with a useful magnification of 100,000X or more. Between these two extremes are various types of light microscopes, bright-field, dark-field, and phase-contrast, with magnification powers ranging from 40X to 1,000X.

In this exercise, you will use a compound bright-field microscope to observe several major types of microorganisms. Once you have completed this exercise, you should have gained an appreciation for the complexity and beauty that exits within the microbial world. The size, shape, and other distinctive features of a cell are referred to as cellular morphology.

In this exercise we will learn about the compound light microscope and explore the cellular diversity of algae and protozoans. We will also examine specimens derived from our teeth and gums, which will provide a contrast between our epithelial cells and the bacteria that inhabit this region of our body. We will also observe selected known bacteria and fungi, to further give you a change to explore the diversity of microbial life and become proficient with the use of the microscope.

Objectives:

1. To be become familiar with the major parts of a light microscope, their function, and the proper use of a compound light microscope.
2. Prepare and observe wet mounts of living algal and protozoan specimens.
3. To identify various cellular structures within algal and protozoan cells.
4. To prepare a smear of material taken from the gingival surface of the teeth and gum.
2. To stain the smear so as to make the epithelial and bacterial cells easily seen.
3. To determine cellular structures within human epithelial cells and the different morphologies of the bacteria associated with these cells.
4. Examine prepared slides of selected microorganisms, noting the cellular morphology of each.

Laboratory exercises:

A. Parts and operation of the compound light microscope

If necessary, obtain a microscope from the cart or storage area in the laboratory. **Always pick the scope up with two hands, one on the arm and the other supporting the base.**

Examine figure 1 and identify the various parts of the microscope. Your instructor will point out each major part and its function as well as the concept of **resolving power or resolution** of a light microscope.

The microscope is a very useful and necessary tool in biology and must be used carefully and correctly. The following steps are provided to assist you:

1. Begin your observations with the low power objective.

2. Initially use the coarse adjustment knob to bring the image into focus, then the fine adjustment knob to sharpen the image.

3. Always focus slowly and carefully. It may help to move the slide slightly during the focusing procedure.

4. When using the low power objective, the iris diaphragm should be nearly closed so that good contrast is achieved. More light with be needed with higher magnifications therefore you will need to change the opening of the iris diaphragm.

5. When the image of the specimen has been brought into sharp focus with a low power objective, to increase magnification the turret is rotated to the next highest objective lens. Use only the fine adjustment knob after you have changed to the next highest objective -the optical path is parfocal and only a slight adjustment is needed.

6. Keep the microscope, lenses, and stage clean at all times. If you have used the oil immersion objective, be sure to clean all oil from the objective when finished. Use only lens paper and the cleaning solution provided on the ocular and objective lenses.

7. Return the lowest power objective to the operating position when finished with the microscope and return the microscope to its proper storage area.

B. Observation of pond water and specific algae and protozoans from culture

Materials:

Fresh pond water
Cultures of algae and protozoans
Glass microscope slides and cover slips
Pasteur pipettes
Lens paper and cleaning solution

Procedure(s):

1. Using a Pasteur pipette, transfer a small drop of the appropriate sample material to a clean microscope slide. Obtain a glass cover slip, handle the cover slip by its edges (note: they are very thin, be sure you have only 1) and place it over the drop of sample material as demonstrated by the instructor.

2. Place the slide on the microscope stage using the slide holder, and begin your observations with the low power objective, either the 4X or 10X. Remember to adjust the iris diaphragm to allow only a limited amount of light to pass through the specimen

3. Once you have some cells in focus, scan the slide noting the different cellular morphologies present in the sample. Note specifically what the total magnification is at this point.

4. Increase the magnification by switching to the next highest-powered objective. Again scan the slide and note the increase in the size of the image and the cellular detail at the higher magnification.

5. Using a total magnification of 400X, observe and record/draw a minimum of one pond water sample, and two algal and two protozoan samples. Draw representative cells (organisms) from each of the samples in the area provided on the result page.

C. 0bservation of human epithelial cells and associated bacteria

Materials:

Glass slides
Pasteur pipettes
Flat -toothpicks
Methylene blue or crystal violet staining solution
Prepared -stained slides of known bacterial species

Procedure(s):

1.Oral specimen – Using a Pasteur pipette add a small drop of water to a clean microscope slide. Then gently scrape the tooth-gum line of your mouth with the flat end of a toothpick. Mix the collected material in the drop of water on the slide, spreading it out on the slide to the size of a nickel or quarter. This is referred to as a smear. Set the slide aside to **air dry**.

2. When the smear is completely dry, heat fix the smear as instructed by your instructor. After the smear has been heat fixed, you must stain the smear or more correctly, stain the cells within the smear. This will make it easier to see them and cellular structures.

3. Place the slide with the smear side up -always remember which side has the smear on it -on the staining rack over the table sink. Add 2 -4 drops of staining solution (dye) to cover the smear. Allow the stain/dye to remain on the smear for 60 seconds. Gently rinse the smear with water and let it air dry or blot it dry.

4. When the slide has dried, place it on the microscope stage within the slide holder, and begin your observations using the l0X low power objective.

5. You will need to finish your observations using the 40X high dry objective, and the l00X oil immersion objective. Follow the procedure given by your instructor for using the oil immersion objective.

6. Look for your gum cells -epithelial cells. Identify the nucleus and other cell structures. Now look for the bacteria. The bacteria will be found on the surface of your cells as well as in the surrounding medium. Draw representative epithelial cells and the bacteria in the section provided on the results page.

D. Examination of selected preparations of known bacteria and yeast

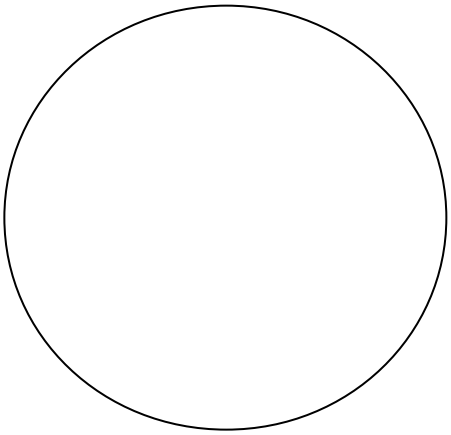
Materials: Prepared -stained slides of known bacterial species

1. Demonstration slides of known bacteria -While you are waiting for your slides to dry, you may begin your observations of the prepared slides of known bacteria. At each microscope you will find the name (Genus species) of the microbe and associated information about the microbe. Examine the cellular morphology of each known microbe on demonstration, paying attention to shape of the cells and any visible internal structures. For each slide record you observations with a quick sketch in the section provided in the results page.

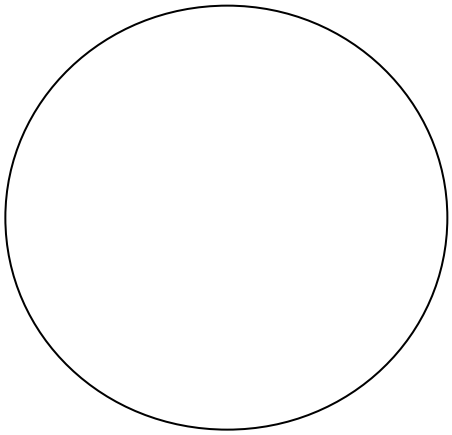
Name _____

Observations of Algae and Protozoans

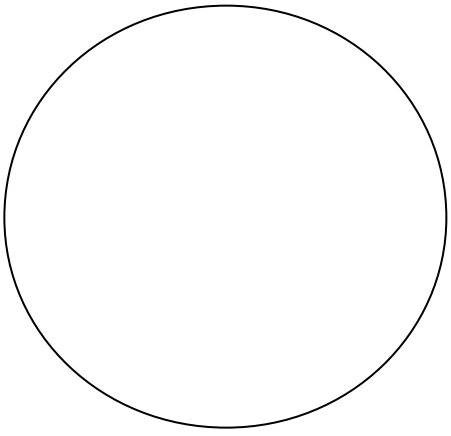
Once you have finished scanning your preparation and can tell a living microorganism from debris or non-living material, draw a representation of the observed organisms in the spaces provided below. Be sure to note the total magnification you were using.



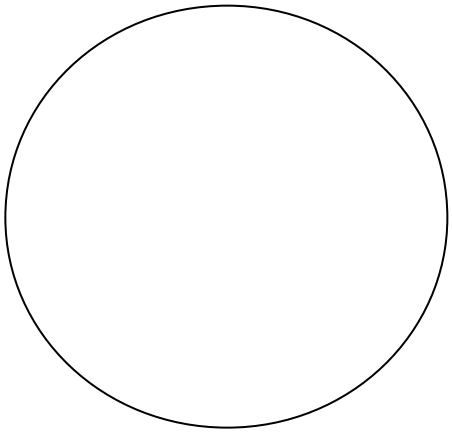
POND WATER SAMPLE
Mag. = _____



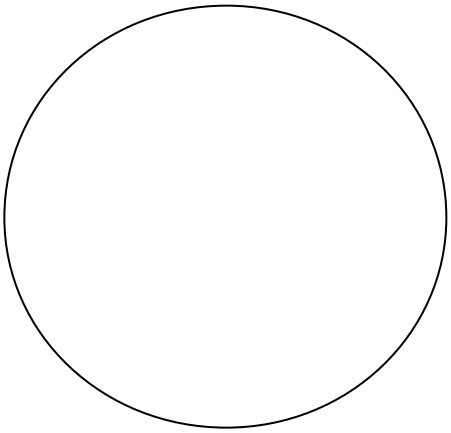
ALGAE SAMPLE
Mag. = _____



ALGAE SAMPLE
Mag. = _____



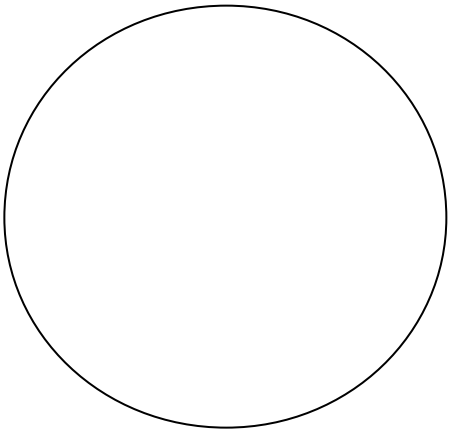
PROTOZOAN SAMPLE
Mag. = _____



PROTOZOAN SAMPLE
Mag. = _____

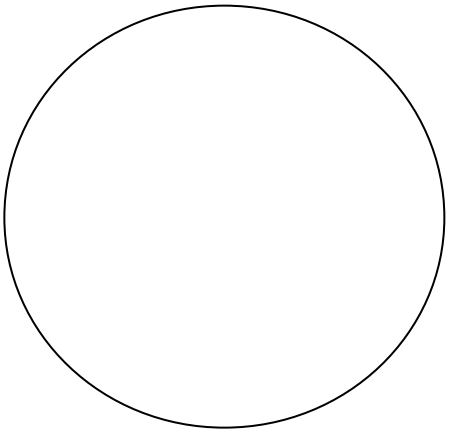
HUMAN EPITHELIAL CELLS AND ASSOCIATED BACTERIA

Once you have found one of your epithelial cells using the 40X objective (total magnification = 400X) draw a representation of this cell or cells in the space provided below. Label as many of the different cellular structures that you can find and identify.



GUM OR EPITHELIAL CELL
Mag. = 400X

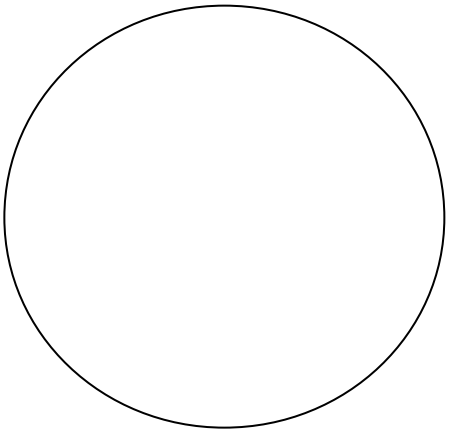
Now – without changing the position of the slide – increase the magnification to a total of 1000X by placing a drop of oil on the slide and moving the oil-immersion lens into place. Again, draw a picture that represents your gum – epithelial cell at this magnification. Label all cellular structures that you can identify.



GUM OR EPITHELIAL CELL
Mag. = 1000X

Now look for the bacteria that are associated with your cells. You may have to examine another area of your smear. The bacteria will appear as small round dots (cocci) or rod shaped (bacilli) on the surface of your cells or near by. They may also be in the form of a micro-colony.

Draw a representative picture of your cells and the associated bacteria.



GUM OR EPITHELIAL CELLS WITH BACTERIA
Mag. = 1000X

Examine the cellular morphology of the bacteria carefully – refer to Figure 2.

Which type of bacterium, based on cellular morphology, is most common? _____

Demonstration slides of specific bacteria, fungi, and protozoa

Table 1. Observed morphology of specific bacteria at a magnification of 1000X

Genus species	Cell Shape	Cell Arrangement	Drawing/Observation
Bacillus subtilis			
Staphylococcus areus			
Streptococcus pyogenes			
Micrococcus letus			
Spirillum volutans			

Table 2. The natural habitat, disease, and mode of transmission for selected bacteria.

Genus Species	Natural Habitat	Disease	Mode of Transmission
Staphylococcus areus			
Streptococcus pyogenes			

Table 3. Observed morphology of specific protozoa at a magnification of 400x.

Genus species	Cell shape – drawing/observation
Giardia lamblia	
Trichomonas vaginalis	
Plasmodium vivax	

Table 4. The natural habitat, disease, and mode of transmission for selected protozoa observed in Table 3.

Genus Species	Natural Habitat	Disease	Transmission
Giardia lamblia			
Trichomonas vaginalis			
Plasmodium vivax			

Table 5. Observed morphology of select fungi at a magnification of 400x.

Genus species	Cell shape – drawing/observation
Candida albicans	

Table 6. The natural habitat, disease, and mode of transmission for selected protozoa observed in Table 5.

Genus Species	Natural Habitat	Disease	Transmission
Candida albicans			