EXPERIMENT 1

Microscopy of Living Microbes

INTRODUCTION

This exercise is intended for you to get familiar and comfortable with using a microscope as well as identifying common microbial groups. Thus, we will observe representatives of all microbes including bacteria, algae, and protozoa. The sizes of the microorganisms viewed today generally range between 1-50 µm and are too tiny to view with the unaided eye and require a light microscope for proper viewing (Figure 1).

Organisms and microorganisms alike are classified taxonomically using the taxonomic hierarchy: Kingdom, Phylum, Class, Order, Family, Genus, and Species. The Kingdom Bacteria hosts phyla of **prokaryotic** microorganisms including bacteria and their photosynthetic counterparts, the cyanobacteria. These organisms are prokaryotes and do not contain a nucleus and membrane-bound organelles.

Conversely, **eukaryotic** microorganisms including algae, diatoms, amoebas, ciliates, and flagellates belong to the Kingdom Protista. The kingdom Protista is made up of organisms that are either animal-like (Protozoa) or plant-like (Algae). These protists all have a nucleus and membrane-bound organelles, such as mitochondria and plastids, within the cells.

To facilitate the characterization of microorganisms, it is sometimes useful to rely on the **pigments** (or color), shape, and arrangement of cells of the microorganisms. Generally, cyanobacteria, green algae, and diatoms can be *single-celled, colonial, or filamentous* (Figures 2, 3, 4). Cyanobacteria are often blue-green or cyan colored or even reddish brown because of their relative concentrations of pigments phycocyanin (blue), phycoerythrin (red), and chlorophyll a (green) (Figure 2). Green algae tend to commonly be colored green to greenish-yellow depending on their chlorophyll a and b,

xanthophylls (yellow), and beta carotene (orange) (Figure 3). Diatoms are organisms encased in a silica shell that resembles a “glass house” (Figure 4). Although diatoms also contain chlorophylls (a and c) and beta-carotenes, they often appear brown due to their high levels of fucoxanthin (a brown pigment).

In terms of **motility**, the ability to move or self-propel, some species within the cyanobacteria, green algae, and diatom groups are capable of motion. Green algae are often found with flagella to help with motility such as the case with the euglanoid *Phacus* sp. (Figure 5b). Cyanobacteria and diatoms do not have appendages that allow movement but instead often use extracellular mucilage to *glide* and grow on surfaces. Amoeba, an amorphous protozoan, moves by means of using a cytoplasmic extension called a **pseudopod** in order to crawl (Figure 5a).

The hallmark of motility is often found in the separate groups of ciliated and flagellated microorganisms. Ciliates are heterotrophic microorganisms that feed on other organisms and contain **cilia**, or hair-like protrusions surrounding the cell in order to move or feed. A good example of a ciliate includes the *Paramecium* and *Stentor* genus (Figure 5 c, d). Another common group of microorganisms include the flagellates that use a **flagella**, or whip-like extension to move through the environment. Flagella are not restricted to a certain group of microorganisms but rather found throughout many different groups of protists including green algae, euglenoids, diatoms, brown algae, and even fungi.

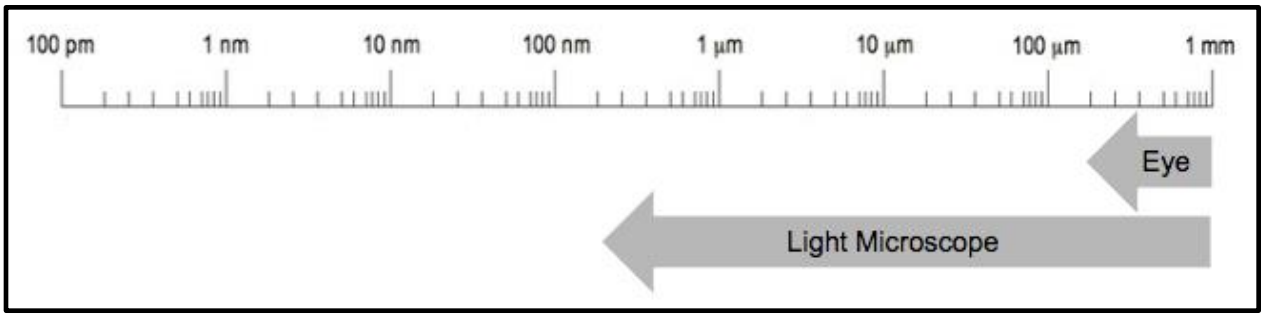


Figure 1. Approximation of size measurements that can be discerned by the unaided human eye and the light microscope.

MATERIALS

1. **Pond water**
2. **Yeast suspension**
3. **Microscope Slides**
4. **Pasteur Pipette**
5. **Coverslips**
6. **Unknown sample**

PROCEDURES

Part I. Yeast Cell Suspension Analysis

1. Prepare a wet mount using the yeast sample and a coverslip. First, observe the sample using the 10X objective, and then the 40X. Refer to Appendix Figure 6.

Part II. Pond Water Analysis

1. Take a clean glass slide and place a drop of pond water that contains visible clumps of algae or suspended material. You will observe very little or nothing if you have only clear water. Gently place a coverslip over the material and flatten with your finger if necessary. Make sure the amount of liquid on the slide does not run out from the coverslip. Wipe up any excess liquid with a Kimwipe or towel.

2. Observe first with the 4X lens to center visible clumps and get the microscope in focus. Switch to the 10X lens and attempt to distinguish the various forms of microbes: algae and protozoa. Here you can see how the 4X and 10X lenses work- the 4X gets you in the right area of the slide, and the 10X gets you closer. Some large cells require only a 10X objective to be seen clearly.

3. When you have located an interesting cell, switch to the 40X lens and examine it in close detail.

4. Diagram at least **two** algae and **two** protozoa at the **highest** magnification that shows the fine detail of the cell and its organelles.

5. Most likely you will see cell walls, internal organelles such as vacuoles, chloroplasts, and perhaps nuclei, and flagella or cilia. Attempt to tell

the difference between the cyanobacteria (blue green bacteria that are quite large, but possess no internal organelles - the chlorophyll seems evenly distributed in the cell) and eukaryotic algae (has chloroplasts).

6. Attempt to discern eubacteria. Bacteria are the size of mitochondria, an internal organelle of a eukaryote. They are free in the water and some may be attached to algal filaments. You will need to use the fine adjustment: going up and down to see bacteria pop into focus and then out. They should appear clear when in the plane of focus and dark as they move out of the plane of focus. You should be able to see rods, cocci and filaments. Which forms are non-motile? Motile?

Part III. Unknown Sample Analysis

1. Prepare a wet mount of the unknown sample given with a coverslip. Using the 4X objective, locate an area of observation and then switch to the 10X objective, and eventually to the 100X objective for your recorded observations.

Procedures for Next Lab

MATERIALS

1. **Nutrient Agar plate**
2. **Labeling marker**

PROCEDURES

1. Begin by labeling the back of the nutrient agar petri dish with the proper information.

2. Pick one lab member to gently touch the agar surface with their naked finger and perform a primary streak onto the plate using their finger.

3. Cover and incubate the plates upside down at 30°C for 48 hours.

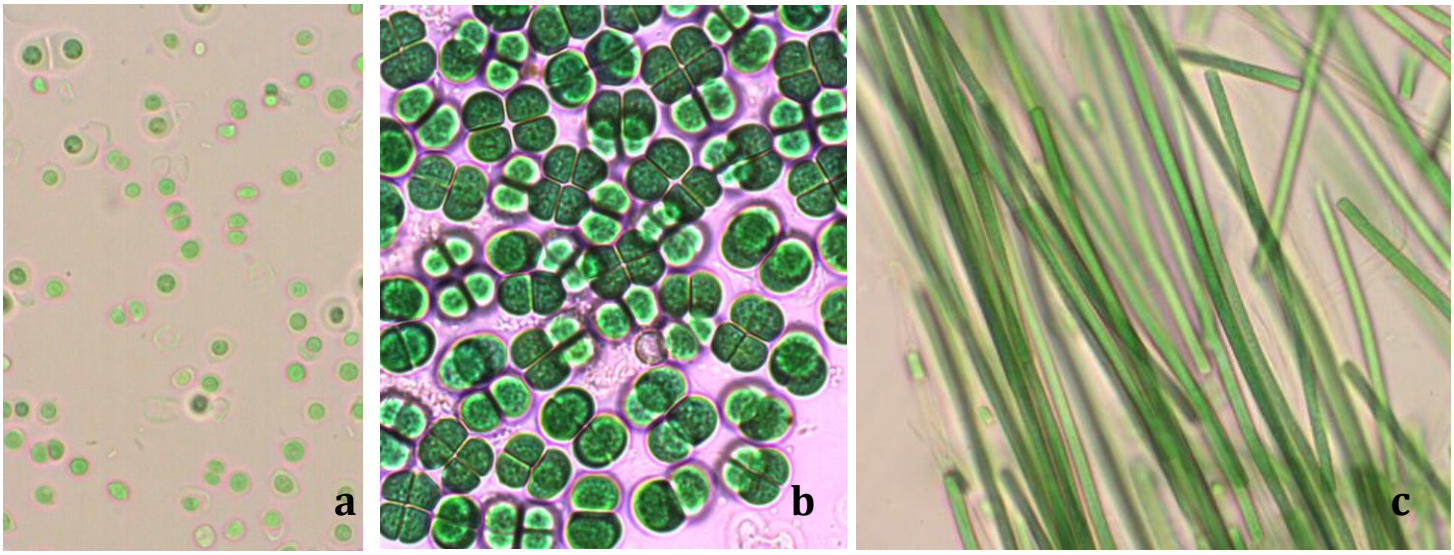


Figure 2. Cyanobacteria: a) Unicellular, b) Colonial, c) Filamentous

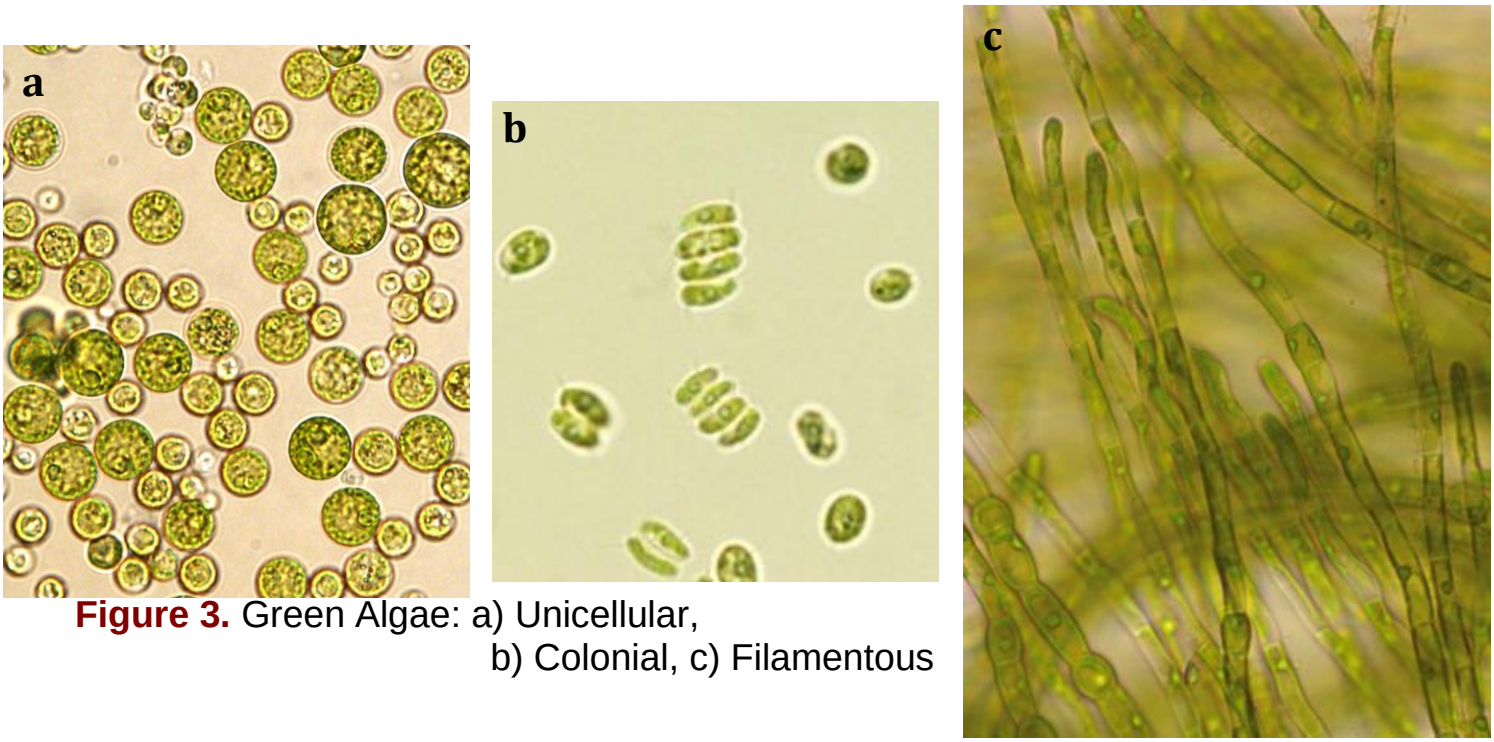


Figure 3. Green Algae: a) Unicellular, b) Colonial, c) Filamentous

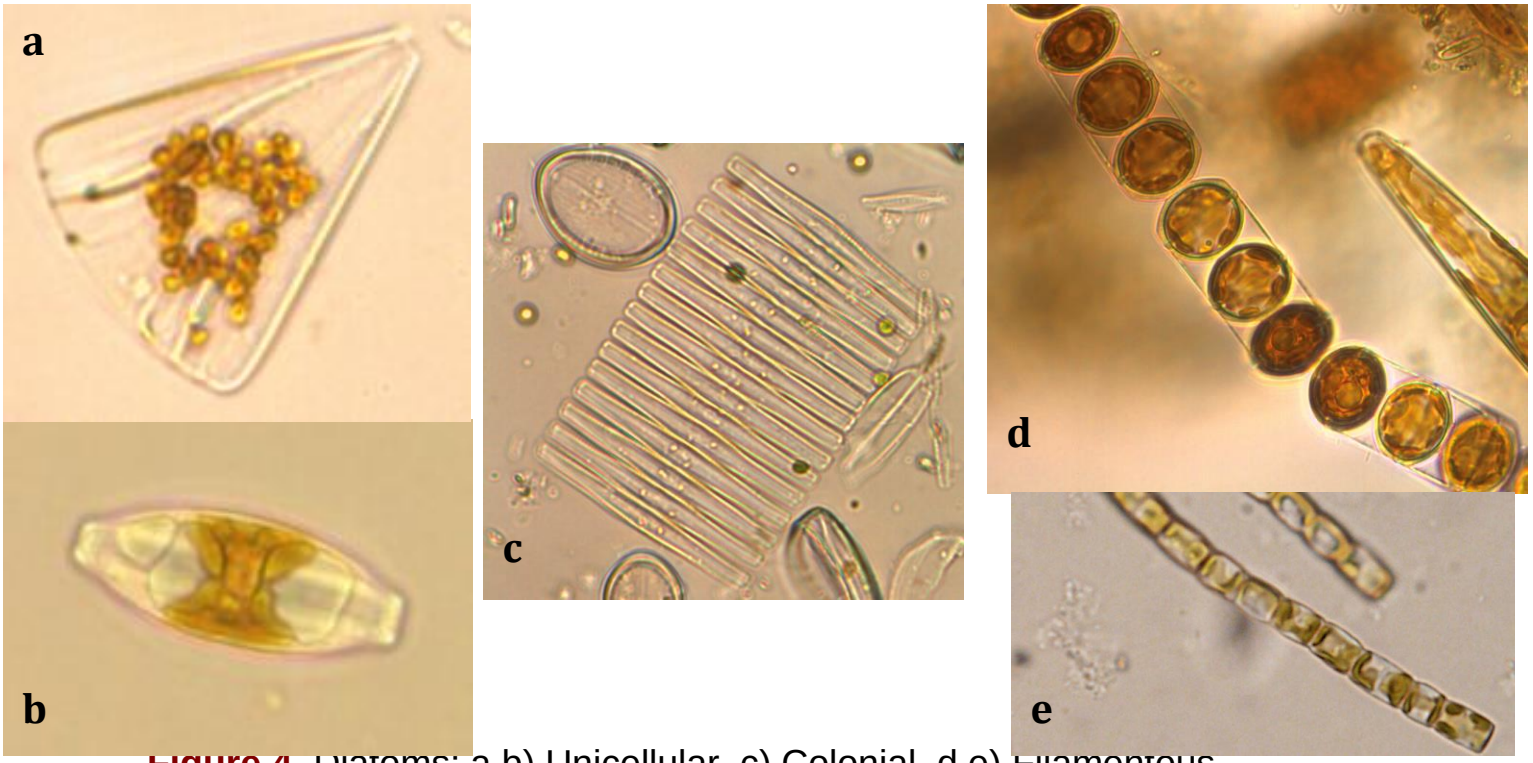


Figure 4. Diatoms: a,b) Unicellular, c) Colonial, d,e) Filamentous

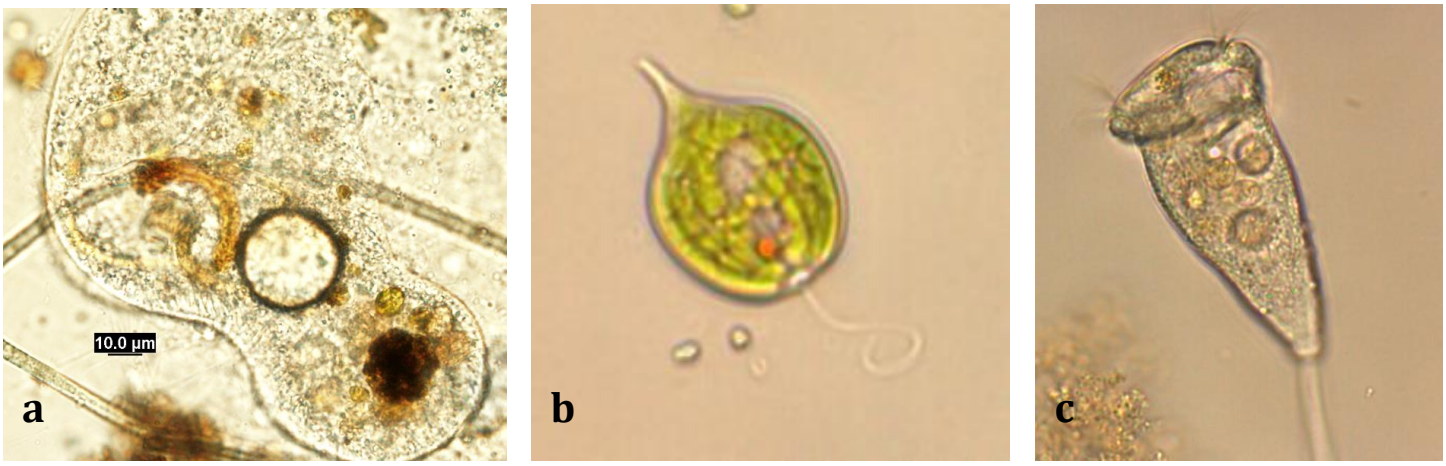
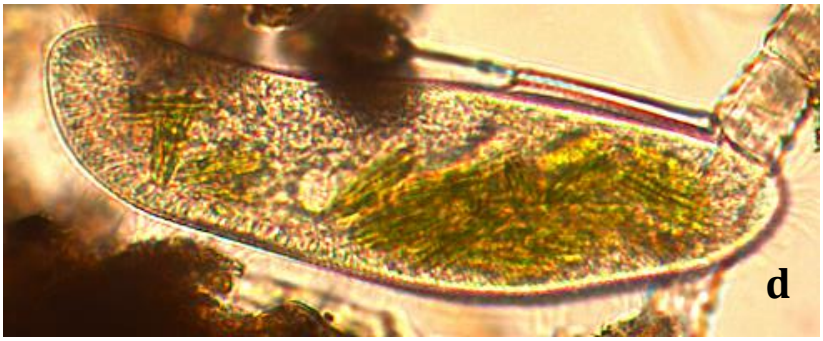


Figure 5. Common Protists

a) Amoeba
b) Flagellate (*Phacus* sp.)
c) Ciliate (*Stentor* sp.)
d) Ciliate (*Paramecium* sp.)



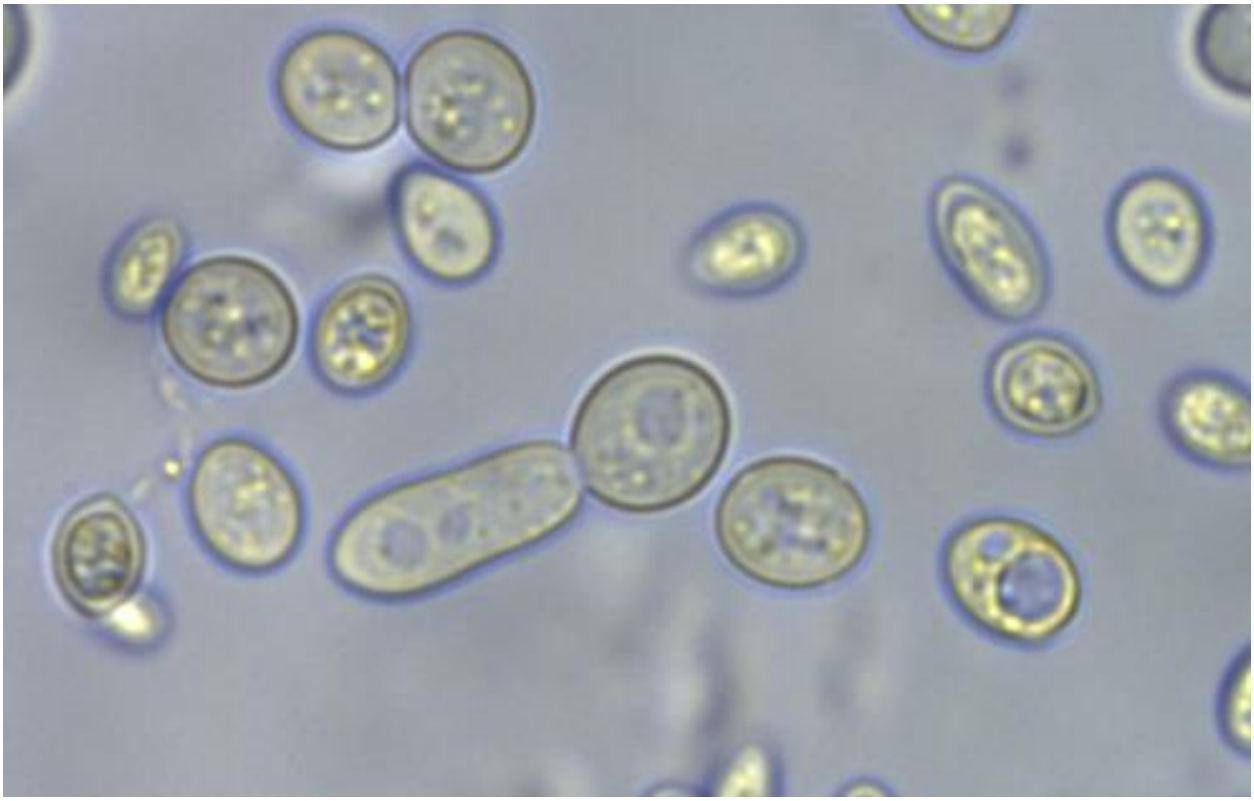


Figure 6. Baker's yeast cells after 48hr incubation. Observe the budding yeast cell and visible nuclei (1000X).

Part I. Microscopic Observations

Directions: Complete the chart below by including sketches/details of your microscopic observations. Include detailed drawings and characteristics. **(5 points)**

Group Name	Sketch	Characteristics	Total Magnification
Yeast			
Cyanobacteria			
Protozoa			
Green Algae			
Unknown			1000X

Part II: Questions
Directions: Answer the following questions using full sentences. **(5 points)**

1. What cellular structures were evident among the **known** microorganisms observed? **(1 point)**

2. From the microorganism observed, do certain structures strictly belong to certain groups? (i.e. diatoms, green algae, cyanobacteria). **(1 point)**

3. Is your **unknown** sample eukaryotic or prokaryotic? What group does your unknown it belongs to? What characteristics led you to this conclusion? **(2 points)**

4. What differences were you able to observe between green algae and cyanobacteria? Between green algae and protozoa? **(1 point)**