

## EXPERIMENT 1

### MICROSCOPY OF LIVING MICROBES

Many students taking microbiology for the first time feel that they are going to have a hard time with the microscope. This lab as an experiment is intended to get you used to your microscope. In microbiology, you know you are dealing with such minute forms that it is essential to use good microscope technique, especially proper illumination, to observe microorganisms clearly. In this experiment we will attempt to duplicate the sort of observations Antonie van Leeuwenhoek made with his simple microscopes more than three hundred years ago. He also had a hard time. But, he persisted and in the 1670's he was the first to see microbes and more importantly he recognized that they were alive! Thus, we will observe representatives of all microbes: bacteria, algae, fungi and protozoa. We, however, have a definite advantage: the modern microscope.

Leeuwenhoek could only tell that microbes were alive when he saw them move. Petri plates, bacteriological media, agar, etc., were not invented and used until the 1880's more than one hundred and fifty years after Leeuwenhoek's observations. Besides observing motility we can test viability (whether organism are alive) by observing their growth on nutrient medium. The medium used in this experiment will be Brain-Heart Infusion Agar a nutrient rich medium solidified with agar.

#### Materials

1. Pond water with big clumps of algae (green stuff) and other suspended material (brown stuff).
2. Hay Infusion....3 or more days before the lab suspend roughly chopped up grass in a beaker with tap water, try to include some soil or lake sediment.
3. Yeast suspension....Make a light suspension of bakers yeast in tap water just before the lab period.
4. Slides, square cover slips and Pasteur pipettes.
5. BHI agar plates.

#### Procedure

##### *Pond Water and Hay Infusion*

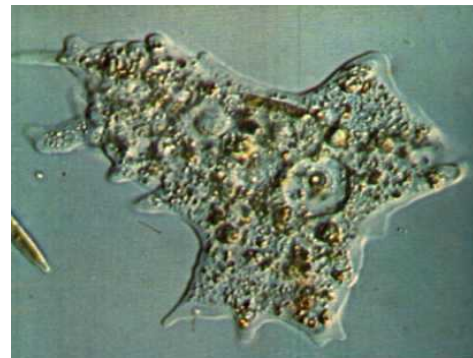
1. Take a clean glass slide and place a drop of pond water **that contain visible clumps** of algae or suspended material. You will observe very little or nothing if you have only clear water. Gently place a cover slip over the material and flatten with your finger if necessary. Make sure the amount of liquid on the slide does not run out from the cover slip. 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. You see the function of here of the 4X and 10X lenses - 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 see them 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).
4. 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?

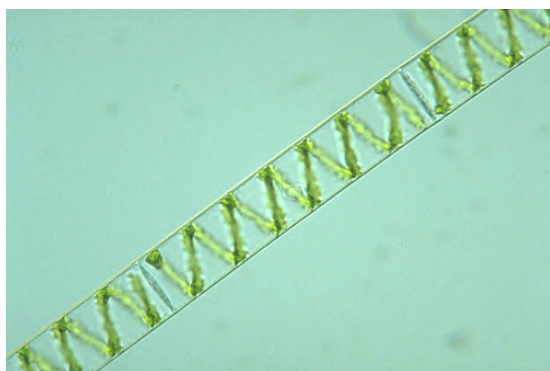
Some algae (Figure 1., 2. and 3.) and protozoa (Figure 4., 5. and 6.) that you will be able to see:



**Figure 1.** Different species of Diatoms sp.



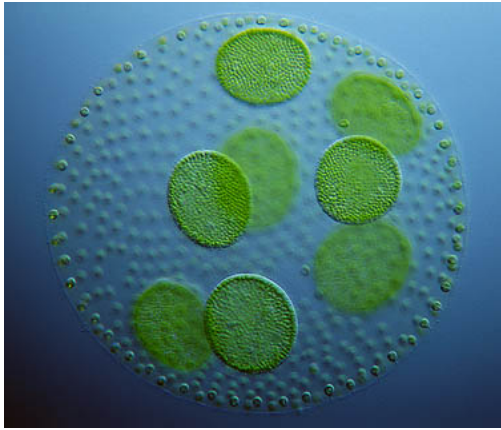
**Figure 4.** Unicellular protozoan Amoeba



**Figure 2.** Filamentous green alga Spirogyra sp.



**Figure 5.** Flagellated protist Euglena sp.



**Figure 3.** Colonial green alga *Volvox* sp.



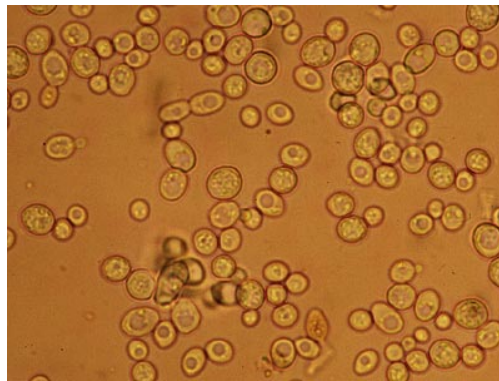
**Figure 6.** Ciliate protozoan *Stentor* sp.

### ***Teeth Scraping***

1. Prepare a drop of tap water on a slide with a small bit of material scraped off between your teeth with a clean toothpick. Make sure there is visible material in the drop.
2. Place on your microscope and observe. What types of bacteria are in your mouth? It is common to also see squamous epithelial cells (cheek cells).

### ***Yeast Suspension***

1. Prepare a standard wet mount of the yeast suspension (Figure 7). Observe first with the 10X lens and then the 40X lens. What sorts of cellular material are evident? What type of cells are yeasts? Do they have organelles? Is it possible to name them?



**Figure 7.** Budding yeast cells

### ***Growth of Microbes on BHI-Agar***

1. On half of the plate, place one drop of crystal clear pond water (not containing observable clumps) on the plate.
2. On the other side of the plate touch your finger on the agar surface. Do not push hard, but enough to leave a slight impression, can you see it?
3. As a group, expose a plate of laboratory air - with the top off for 30 minutes.
4. Cover all plates and incubate at 30°C for 48 hours. Observe the resultant growth.
5. Save this plate for the next experiment, the Gram stain.

### **QUESTIONS**

1. What is Brownian motion and why is it generally seen when bacteria are observed in liquid media? Some bacteria, perhaps many in your preparation did not display Brownian motion. Why is that?
2. How can Brownian motion be distinguished from motility?
3. What are flagella? What is the difference between bacterial flagella and eukaryotic flagella?
4. In wet mount preparations, is it possible to see eukaryotic flagella? prokaryotic flagella?
5. Does crystal clear pond water contain living bacteria? What about air? Your finger?

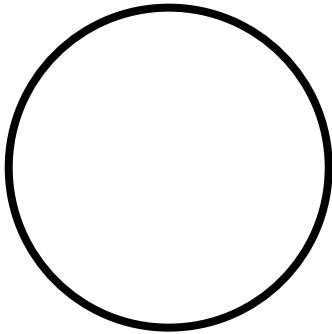
#### **HINTS**

1. Always make sure the specimen is on the top side of the slide.
2. Specimen should be directly over the light hole in the stage of the microscope.

**DATA:** Record your data on the next two pages.

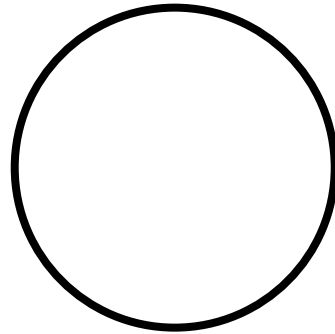
**Name:**

**Pond Water-Alga 1**



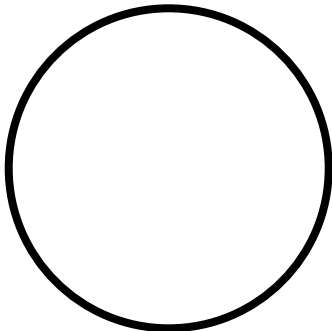
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**Pond Water-Alga 2**



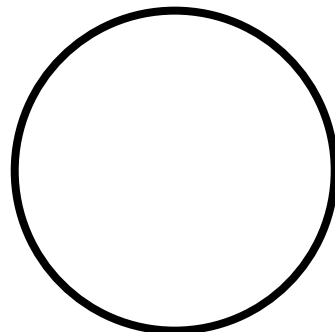
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**Pond Water-Protozoa 1**



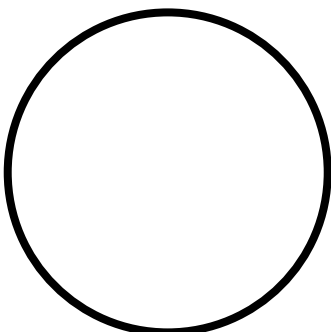
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**Pond Water-Protozoa 2**

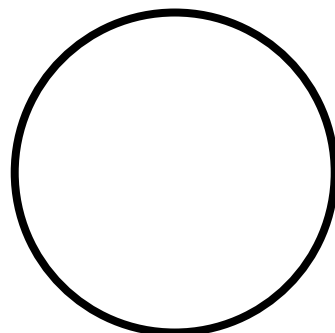


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**Hay Infusion-Alga**



**Hay Infusion-Protozoa**

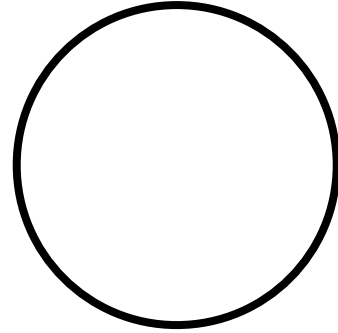
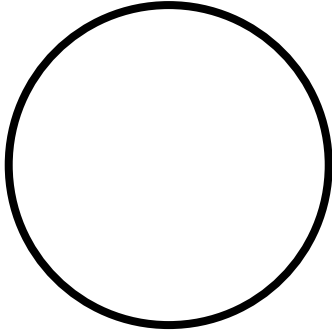


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**Yeast suspension**

**Tooth Scraping**



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**Conclusions:**