## Nedwidek Revised LABORATORY EXERCISES #4

## PART I: OBSERVING LIVING PROTISTS: "LET'S PLAY LEEUWENHOEK"

### INTRODUCTION

Today you will have the opportunity to observe cells which are actually individual organisms-- unicellular or colonized Eukaryotes. These organisms, which are members of the kingdom Protista, come in many shapes and sizes and exhibit diverse behaviors. As a student scientist, you will practice your observational and microscope skills as you investigate the activities of these creatures. The 17th-century observation Anton van Leeuwenhoek is most known for is his identification of these tiny creatures that he called "animalcules" using a simple compound light microscope he built himself. This lab should give you a sense of what he witnessed in his studies.

### STUDENT OBJECTIVES

1. Prepare and observe wet-mount slides of mixed protists.
2. Improve microscope technique.
3. Improve observation skills.

### PRE-LAB QUESTIONS: to be transferred to report!

You will be working with **Paramecia, Euglenoids**, and, hopefully, **Amoebae**, in this lab. Use your textbook, internet searches and the links provided below to complete the following questions.

1. All of the organisms listed above belong to the kingdom Protista. For **EACH** of the organisms listed above address the following points:
    a) To what phylum does each belong and what are the general characteristics of that phylum?
    b) What is the primary mechanism of locomotion for each of these organisms?
    c) Specify a genus and species for each, and how it accomplishes nutrition.
2. Why would a Euglena most likely be found near the surface of the pond? Where would a paramecium or blepharisma be found?
3. Congo red is called a "pH indicator". What does this mean?
4. Paramecia have specialized structures called trichocysts. What is their function in the cell? Why are the trichocysts launched by a paramecium?
5. Be sure to come to class with your diagram sheet prepared.

### HELPFUL RESOURCES

Below are web sites with common protists—many more than we will have in the culture at lab. It is recommended that you read about some of them. Below are also recommendations for searches in youtube or google to see videos of these organisms. **<u>You can go to Google Images to print out labeled diagrams if you wish. I will also send attachments of diagrams and a key for Protists for you.</u>**

<u>CILIATA</u> (Have patience and use the highest magnification to see cilia move!)

***Paramecium caudatum*– read article, especially about physiology and gathering food. Also click on trichocyst and macronucleus, contracting vacuole -See Google Videos – search "Paramecium feeding" and "symbiosis *Paramecium bursaria* "
http://en.wikipedia.org/wiki/Paramecium

***Blepharisma* –Large and moves fast, PINK and a voracious eater – large vacuoles
http://www.zoology.ubc.ca/courses/bio332/Labs/CiliateProject/blepharisma/bleph1web.html

***Stentor* – great dark GREEN or green-greyish color, trumpet shape when "relaxed", barrel shaped when scrunched, watch for the cilia at the edge of the trumpet.
http://en.wikipedia.org/wiki/Stentor_(protozoa)

***Vorticella* – small, long stalk that curls up like a Slinky, look for cilia at edge of bell.
http://en.wikipedia.org/wiki/Vorticella

***Spirostomum* –BIG, look for chain-like nucleus, NOT a worm
http://www.microscopy-uk.org.uk/mag/indexmag.html?http://www.microscopy-uk.org.uk/mag/artoct98/spiro.html

FLAGELLATES

*Peranema* – flagellum in the front!
http://www.microscope-microscope.org/applications/pond-critters/protozoans/mastigophora/peranema.htm

***Euglena* – has chloroplasts but also locomotes! very small
http://www.fcps.edu/islandcreekes/ecology/euglena.htm
http://en.wikipedia.org/wiki/Euglenid

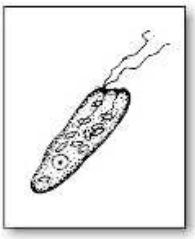
SARCODINA (These are hard to maintain, so I hope we can manage to grow them.)

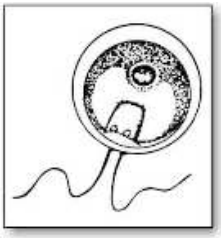
*Amoeba proteus* - http://microbewiki.kenyon.edu/index.php/Amoeba_proteus

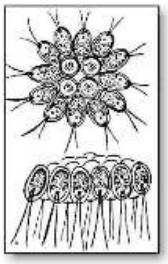
*Pelomyxa* – Large amoeba; formerly called *Chaos chaos*; multinucleate http://www.microscope-microscope.org/applications/pond-critters/protozoans/sarcodina/chaos-chaos.htm

**MATERIALS:**
Slides, coverslips, methyl cellulose, toothpicks, mixed protist cultures, paper towels.

**PROCEDURE**
1. Make a wet-mount slide of a mixed protist culture by using the following technique:
   a. Clean the slide and coverslip with a small piece of paper towelling. NO SMUDGES, PLEASE.
   b. Hold the dropper and squeeze out all the air bubbles (not all the air though) before you put it into the culture.
   c. Gently lower the dropper into the fluid and draw up no more than 3 cm of the fluid. **DO NOT DIG INTO THE AGAR AT THE BOTTOM OF THE CULTURE DISH.**
   d. Gently expel 1 drop onto the slide.
   e. Place the coverslip on the slide at an angle, wet the edge with the liquid on the slide and lower it. If there are bubbles, use the tip of your pencil to gently force them out.
   f. Observe the slide under LOW power. If the protists are moving too fast, you can make another slide. Wipe off the slide with paper towel. Place **1-2 drops** of methyl cellulose (Detain) on the slide and **1-2 drops** culture fluid. Stir gently with a toothpick and place a coverslip on top. You may have better success without the Detain, though.
2. Observe the Protista under low and then high power. **Draw in pencil two organisms on your prepared diagram sheet, clearly labeling the overall magnification underneath each (for example, 40X) and their estimated sizes in micrometers (µm). In addition label the cell membrane and the nucleus for each with a line pointing to the structure.**
3. Label any other cellular structures that you can observe. Make sketches which capture their activities, such as their way of moving or eating.


Chilomonas


Chlamydomonas


Gonium


spirostromum

**Pond Critters:** These line drawings should be use as models for your own drawings.

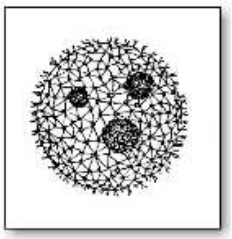
From [www.microscope-microscope.org](www.microscope-microscope.org)

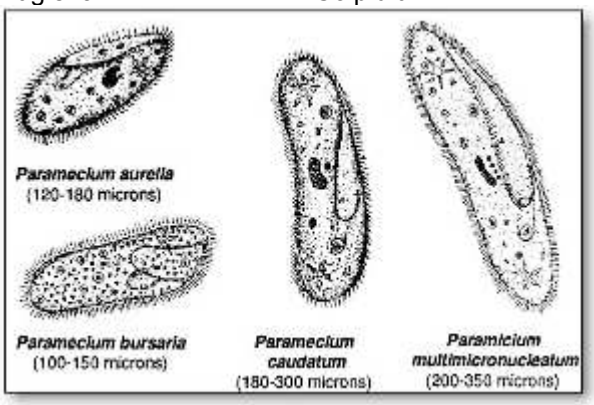
Click onto any protist to find out more


Euglena

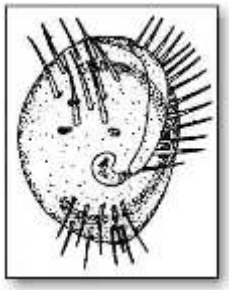

Colpidium

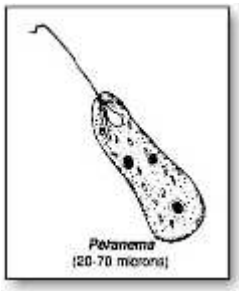

Stentor


Volvox

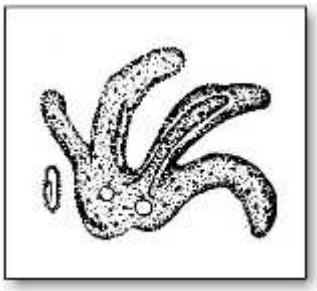


paramecium species

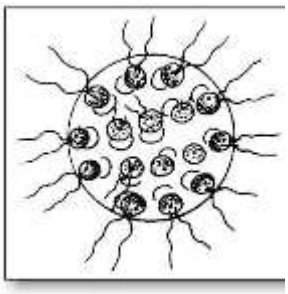

euplotes

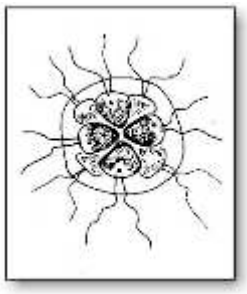


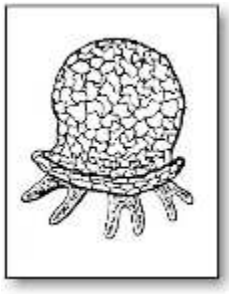
peranema


Pelomyxa,
chaos-chaos

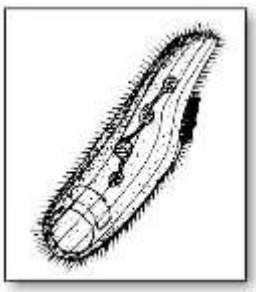

didinium


eudorina

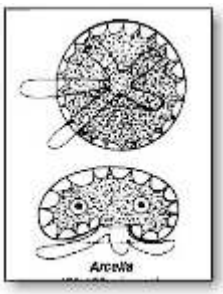

pandorina


difflugia

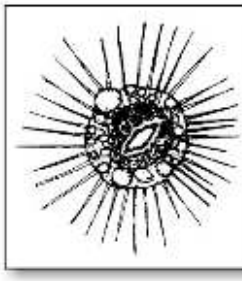

Vorticella

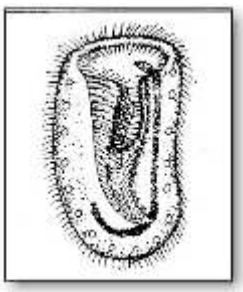

Blepharisma

**ivin**



Arcella
top and side view


Actinosphaerium


Bruseria truncatella

**L**


Amoeba proteus

## PART II: HOW DOES THE STRUCTURE OF A PARAMECIUM ENABLE IT TO FUNCTION IN ITS ENVIRONMENT?

### INTRODUCTION

Paramecia are familiar organisms in the biology laboratory. They are often used as representative Protists. Many of their activities and structures are similar to other members of their Kingdom. Locomotion, feeding, and defense are three behaviors which you will readily observe today. As you make your observations, keep in mind the importance of the relationship between structure and function.

### STUDENT OBJECTIVES

1. Observe the structure of paramecia.
2. Relate structure of paramecia to their functions and behaviors.
3. Improve microscope technique.
4. Improve observation skills.

### MATERIALS

Slides, coverslips, methyl cellulose, toothpicks, congo red yeast, acidified ink, paramecium cultures, paper towels.

### PROCEDURE

1. Clean a slide and a coverslip.
2. **WITHOUT INTRODUCING BUBBLES INTO THE CULTURE**, use the dropper to pick up about 2 ml of fluid from the culture. Place a drop on the slide and return remaining culture back to the culture dish.
3. Add 1 drop of methyl cellulose (Detain) to the slide and mix the drops with a toothpick. Slowly place the coverslip on the culture and try to avoid trapping any air bubbles under the coverslip.
4. Observe some Paramecia under **LOW** power. Move your slide around and observe freely swimming paramecia. You might find some that are feeding on clumps of green food.
5. Observe the way the swimming paramecia move forward. Contrast this with their motions when they are feeding or bumping into material from the culture fluid.

Paramecia

6. Switch to **HIGH** power. Observe the cilia along the paramecium's body and how they help propel it. Note the position of the oral groove, which is surrounded by cilia. The beating contractile vacuole, helpful in getting out excess water from within the cell, may also be evident within the cytoplasm of the cell as an asterisk-like, pulsating structure.
7. **CONGO RED and YEAST** – Take your slide off the microscope stage. Place one drop of the congo red yeast (food) next to one drop of the living ciliate culture. (**Congo red is a pH indicator which means its color will change as the pH changes**). Gently mix with a toothpick, and gently cover with a coverslip.

Focus under **LOW** and then **HIGH** power. Observe the yeast as it is swept up by the paramecium. As it ingests the yeast, food vacuoles are being filled up with the stained yeast. Watch the food vacuoles and observe their movement and any changes in their color over time.

8. **DRAW a sketch of a paramecium under high power showing several yeast-filled food vacuoles.** Label the cell membrane, the nucleus, and the filled vacuoles, indicating magnification underneath.

9. **INDUCTION OF TRICHOCYSTS (time permitting):** Clean and dry the slide and coverslip. Place 1 drop of ciliate culture on the slide. Next to the culture, place 1 drop of acidified blue ink. Gently place the coverslip on the drops **WITHOUT MIXING**. Observe the slide under **LOW** then **HIGH** power. As the two different fluids diffuse into one another, observe how the paramecia react by discharging their trichocysts.

**10. DRAW a sketch of a paramecium below under high power which has discharged its trichocysts.** Label the trichocyst structure and indicate magnification underneath the drawing.