Laboratory 9
Visualizing Chemotaxis in *Tetrahymena*

I. Introduction

Cell migration is an essential process for both eukaryotic and prokaryotic cells. In both multicellular and unicellular organisms, cell migration must often be directional. For example, in many multicellular organisms, sperm cells must migrate towards the egg during fertilization and cells of the immune system must migrate towards sites of infection. Single-celled organisms migrate towards nutrient-rich regions of their environment and must migrate away from toxins in the environment. Chemotaxis refers to the directional migration of cells towards or away from a gradient of chemical signals. Cells will migrate towards chemical signals called chemoattractants and migrate away from chemical signals called chemorepellents. The purpose of this week's project is to explore the process of chemotaxis using the ciliated protist *Tetrahymena* as a model system. This microscopic organism is typically found in aquatic habitats and responds to chemical signals to find nutrients and avoid harmful environmental conditions.

As part of this week's laboratory session, you will:

- study *Tetrahymena* by light microscopy using a set protocol and work to determine the best methods of microscopy to study this organism
- Visualize the process of chemotaxis
- Determine whether a food source attracts or repels *Tetrahymena* cells

II. Pre-Lab Preparation

Read the Introduction, Background Information, and Experimental Procedures for this experiment. Also, please read the sections in your Cell Biology textbook which deal with endocytosis, phagocytosis, and cilia. You might also find it helpful to look at your General Biology textbook for more information about protists. Review the material in **Laboratory 2 (Introduction to Microscopy)** on light microscopy. After preparing for the lab, you should be able to answer the following questions.

A. What organisms are usually included in the Kingdom Protista?

B. What is the difference between a cilium and a flagellum?

C. In what ways are these structures similar?

D. Are *Tetrahymena* unicellular or multicellular?

E. What is the purpose of fixing the *Tetrahymena* before examining them microscopically?

F. Which fixative will be used in these experiments?

G. What is the definition of chemotaxis?

H. What is the difference between a chemoattractant and a chemorepellent?

I. What is in the inner and outer chambers during a two-chamber chemotaxis assay?

III. Background Information

A. Biology of *Tetrahymena*

Tetrahymena is a unicellular eukaryotic microorganism that has been used for studies of genetics and cell biology since the 1950s. Although it is naturally found in aquatic habitats like lakes and streams, it can be easily grown in the laboratory. The commonly-used laboratory strains were originally designated *Tetrahymena pyriformis*, but the organism is now called *Tetrahymena thermophila*. *T. thermophila* is a relatively-large actively-motile organism (Figure 9.1). The typical cell is about 50 μm long and about 20 μm wide, with a somewhat pointed anterior end and a more rounded posterior end. There are multiple bands of cilia on the organism's surface, which are involved both in movement and the acquisition of food. *Tetrahymena* finds nutrients by a process of chemotaxis during which it migrates in response to chemical cues. *Tetrahymena* obtains nutrients by a process of phagocytosis: the cilia on the surface sweep bacteria and other small particles into the buccal apparatus, where they are then taken into the cells in membrane vacuoles. We will examine phagocytosis in Lab 10 next week.

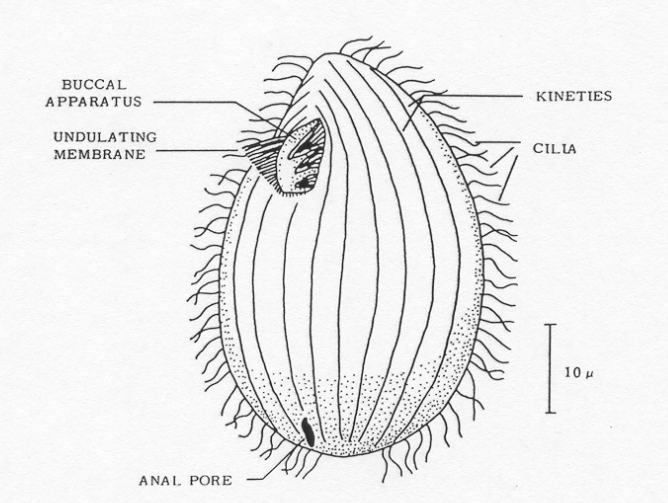


Figure 9.1. External features of *Tetrahymena*.

One of the unusual features of *T. thermophila* is that it has two nuclei. The smaller micronucleus contains two sets of chromosomes (it is diploid) and is capable of undergoing both mitosis and meiosis. The micronucleus is largely inert in terms of gene expression, and so serves primarily as the "germ line" reservoir of genetic information. The large macronucleus contains many copies of a subset of the complete genome which has been formed by recombination. The macronucleus is very active in terms of transcription and so serves as the basis of "somatic" cell function (Figure 9.2).

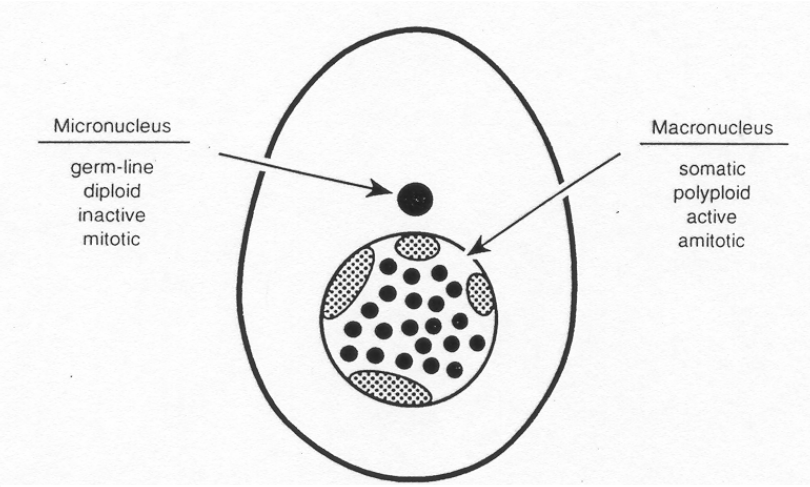


Figure 9.2. Schematic diagram of *Tetrahymena* and its nuclei.

T. thermophila also contains all the other organelles characteristic of a eukaryotic cell. The plasma membrane is composed of proteins and lipids and separates the cytoplasm from the external environment. Extensions of the plasma membrane called ciliary membranes surround each of the cilia. Just underneath the plasma membrane are the cortical alveoli, which are flattened sacs of membranes that may serve to store calcium ions. Within the cytoplasm there is an extensive endomembrane system consisting of the rough and smooth endoplasmic reticulum and the Golgi apparatus. *T. thermophila* is a strict aerobe that makes ATP by a process of cellular respiration. Mitochondria are distributed throughout the cytoplasm, although they tend to be concentrated near the surface. The cytoplasm also contains numerous peroxisomes, which contain catalases and enzymes involved in fatty acid oxidation. Like many other aquatic protists, *Tetrahymena* contains a contractile vacuole that allows excess water to be removed from the cells (Figure 9.3)

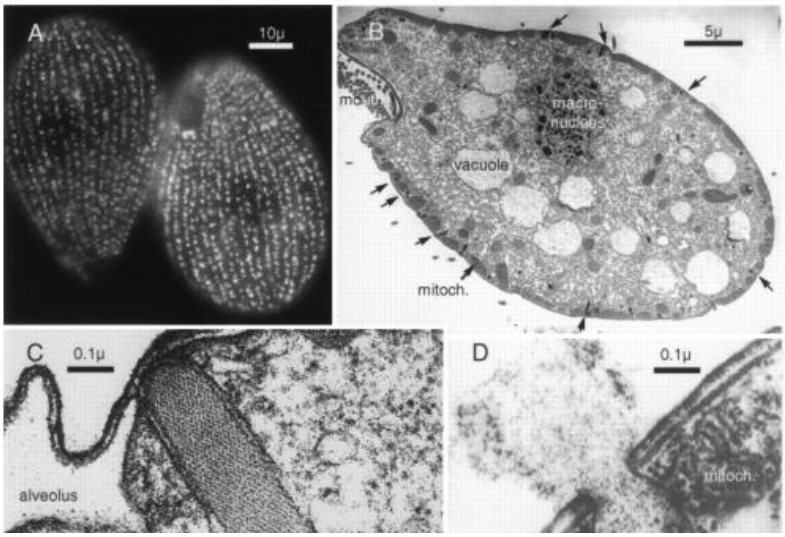


Figure 9.3 Electron micrographs of some of the structures found in a typical *Tetrahymena* cell.

B. Chemotaxis

Chemotaxis refers to movement of an organism in response to a chemical gradient. During this chemosensory behavior, cells detect changes in the concentration of the chemical and alter their mobility accordingly. Cells can be attracted (positive chemotaxis) or repelled (negative chemotaxis) by a variety of chemical signals (Figure 9.4).Cells move towards chemicals called chemoattractants, which include amino acids, peptides and/or proteins that may signal the presence of food in the natural environment. Alternatively, cells migrate away from chemorepellents which are chemical signals that may signal the presence of toxins or other unfavorable environmental conditions. In multi-cellular organisms, chemotaxis is critical to early development. For example, it allows sperm cells to move towards an egg during fertilization. Single-celled organisms such as bacteria and *Tetrahymena* are known to find food through chemotaxis. *Tetrahymena* is strongly attracted to proteins and amino acids, particularly hydrophobic amino acids.

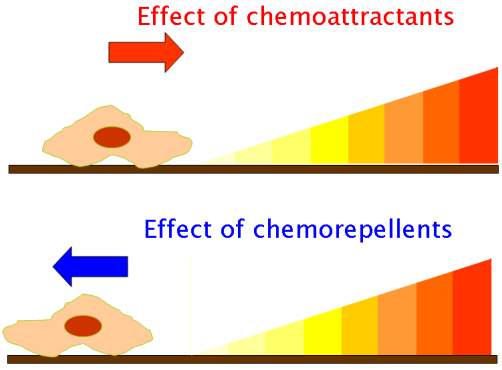


Figure 9.4. Effects of chemoattractants and chemorepellents on the direction of cell migration

Chemotaxis by moving cells can be analyzed using a two-chamber capillary assay (Figure 9.5). In the capillary assay, an outer chamber contains mobile cells while an inner chamber contains a chemical to be tested. If the chemical being tested is a chemoattractant, the cells will migrate into the inner chamber. If it is a chemorepellent, the cells will remain in the outer chamber. The number of cells within the inner chamber after 10-60 minutes can be used to determine whether the chemical is stimulating positive or negative chemotaxis.

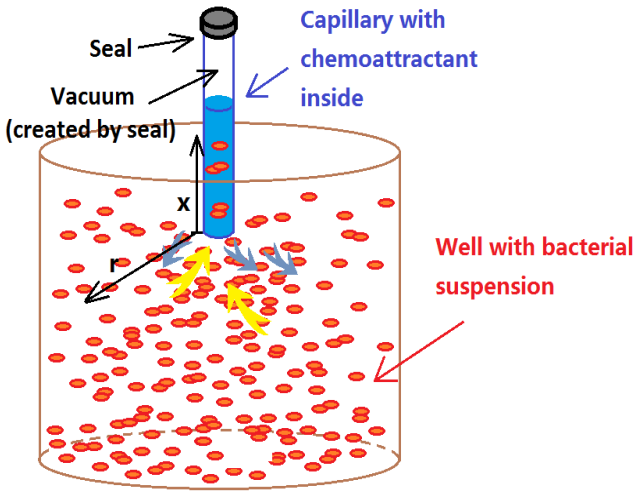


Figure 9.5. Example of a two-chamber capillary assay. A capillary tube forms the inner chamber and contains a chemical to be tested for its ability to induce a chemotactic response. The capillary tube is placed in an outer chamber containing migratory cells. If the chemical is a chemoattractant as shown, the cells will migrate into the inner chamber.

The number of cells from the inner chamber can be determined numerous ways. Cells can be counted using a flow cytometer, a technology that electrically senses cells as they pass through at least one laser. The number of cells can also be estimated by a plate count during which cells are spread onto an appropriate nutrient plate and the number of colonies that form are counted. Recall that you did a plate count to estimate the number of yeast cells in Lab 7. Finally, cells can be counted using a special slide called a hemocytometer (Figure 9.6). A drop of the cell suspension is placed on top of a grid that is etched into the surface of the hemocytometer. The grid of the hemocytometer contains 25 squares of known volumes. Cells are counted in 5 of the 25 squares and the resulting number can be used to calculate the concentration of the cell suspension. Please read the introductory material for Lab 6 for a more detailed review on hemocytometer use.

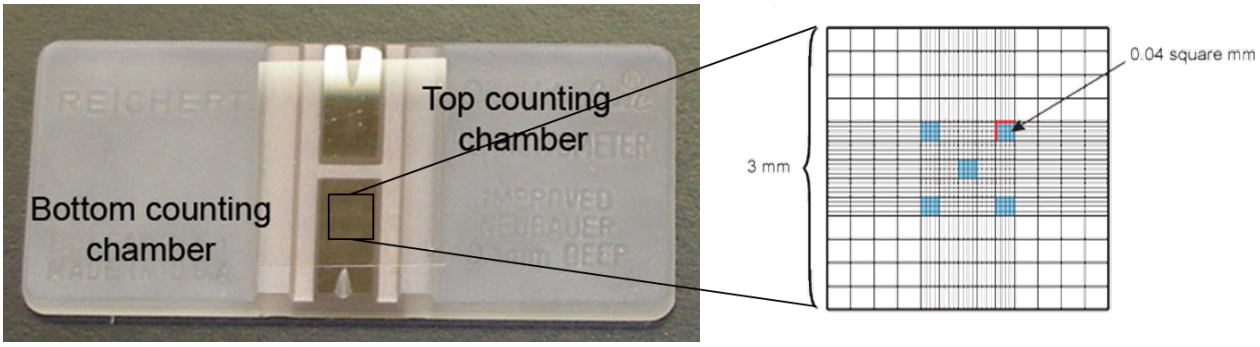


Figure 9.6. Hemocytometer slide and grid. Blue squares on the right indicate represent the 5 squares that are typically counted.

IV. Experimental Procedures

The following is a flow chart for this laboratory session:

Visualization of *Tetrahymena* by Light Microscopy (Section IVA)
↓
Preparation of food extracts (Section IVB)
↓
Preparation of *Tetrahymena* cells for chemotaxis assay (Section IVC)
↓
Measurement of chemotaxis in *Tetrahymena* (Section IVD)
↓
Analysis of Chemotaxis Data (Section IVE)

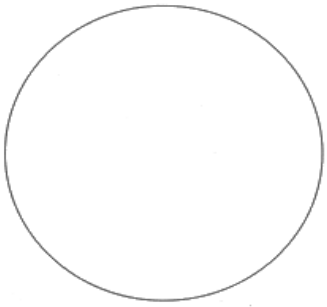
A. Visualization of *Tetrahymena* by Light Microscopy

The objective of this part of the lab is to learn to recognize *Tetrahymena* and to describe its general morphology and motility. You will use the same Leica DME microscopes you used in **Laboratory 2 (Introduction to Microscopy)** so review the material from that experiment.

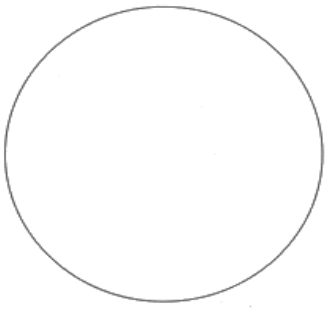
1. An actively-growing culture of *Tetrahymena* in 2% peptone broth will be available. With a P-100 micropipetter and a small tip, place a 20 µl drop of this culture on a clean microscope slide and cover it with a coverslip.

2. First examine the cells with bright-field optics using the low power (10X) objective lens. Because the cells are transparent, you may need to reduce the light intensity. Be careful to distinguish the organisms from all of the background debris that may be present in the culture. The cells tend to move relatively quickly so watch for their motion.

3. Now examine the cells with the 20X or 40X objective. When you have found a portion of the slide with clearly visible cells, switch to the dark-field and phase-contrast optics as you did in **Laboratory 2**. Be sure to change the condenser setting for each optical system. **Indicate the total magnification, make a drawing of what you see and write down any observations you may have.**



Dark Field _____X



Phase Contrast _____X

4. Using the ocular micrometer that you calibrated earlier as part of **Laboratory 2**, measure the length and width of 10 cells as seen with the 20X or 40X objective. You can use whichever optical system gives you the clearest image of the cells. Make a new slide if necessary. Calculate the mean values for the length and width and convert the ocular units to an actual number of μm as you did in **Laboratory 2**.

Cell	Length (Ocular Units)	Width (Ocular Units)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
Mean		

Convert the ocular units to micrometers :

Mean length (μm) _____

Mean width (μm) _____

B. Preparation of food extracts

The purpose of this part of the experiment is to prepare an extract of your food sources in a simple buffer solution so that it can be used for the chemotaxis assay.

1. Record the following information for your food source:

What was the food source that you used?

_____

2. Measure 0.1 g of the material you are testing and transfer it to a clean 1.5 mL microcentrifuge tube.

3. Using a P-1000 micropipetter, add 1 mL of 10 mM Tris, pH 7.4 to the food material and vortex for 5-10 seconds to break up any clumps that might be present.

4. Place the tube in a plastic float and boil the sample for 5 minutes.

5. Centrifuge the sample for 5 minutes at max speed. Transfer the supernatant to a clean 1.5 mL microcentrifuge tube. **This is your undilute food extract.**

6. Make 3 serial 1/10 dilutions of your unknown solution in the following way. Add 900 µL of 10 mM Tris, pH 7.4 to each of three 1.5 ml microcentrifuge tubes. Add 100 µL of your undiluted food extract to the first tube. Close the cap and invert several times to mix. Then add 100 µL of the 1/10 dilution to the second tube to make a 1/100 dilution. Again, close the cap of the second tube and invert to mix. Finally add 100 µL of the 1/100 dilution to the third tube to make a 1/1000 dilution. Close the cap and invert to mix. It may be a good idea to vortex these before proceeding to the next step. **You will use the Undilute food extract as well as the 1/10, 1/100 and 1/1000 dilutions for the chemotaxis assay in Section C**.

C. Preparation of *Tetrahymena* cells for chemotaxis assay

The purpose of this part of the experiment is to prepare the Tetrahymena cells for the chemotaxis assay by suspending the cells in a nutrient-free buffer.

1. Add 10 mL of *Tetrahymena* culture to a clean 15 mL centrifuge tube.

2. Centrifuge the cells for 3 minutes at 2500 rpm. We will do this centrifugation step as a class.

3. Decant the supernatant into an appropriate waste container, being careful not to disturb the pellet.

4. Resuspend the pellet (by finger flicking) in 10 mL of 10 mM Tris, pH 7.4. **Do not vortex the cells.** Save resuspended cells for the chemotaxis assay in Section D.

D. Chemotaxis Assay

Chemotaxis is the process where chemical signals stimulate directional movement of cells. The objective of this part of the experiment is to visualize chemotaxis and to determine whether a given food source acts as a chemotactic signal.

1. Obtain two 1.5 ml microcentrifuge tubes and label them P and T. Use a large tip and a P-1000 micropetter to add 1.0 mL of 10 mm Tris, pH 7.4 to the **T** tube and 1.0 mL of peptone broth to the **P** tube.

2. Place two 100 mm capillary tubes into each of the following tubes/solutions: Undilute Food Extract, 1/10 dilution, 1/100 dilution, 1/1000 dilution, P, T. Seal each capillary tube with parafilm on one end.

3. Obtain 6 13 x 100 mm test tubes and label them 1-6. Place 1 ml of *Tetrahymena* cells (from Part B) into each tube.

4. Place two capillary tubes containing each of your test solutions into a tube of *Tetrahymena* cells (two capillaries tube per tube of cells) according to the following table:

Tube Number	*Tetrahymena* (ml)	Extract in Capillary Tubes
1	1	Peptone Broth
2	1	Tris
3	1	Undilute Extract
4	1	1:10 Dilution
5	1	1:100 Dilution
6	1	1:1000 Dilution

5. Incubate at room temperature, undisturbed, for 20 minutes.

6. Obtain 12 0.65 mL microcentrifuge tubes and label them 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, 5B, 6A, and 6B). Using a P-100 micropipetter and a small tip, add **20 µl** of 0.3% formaldehyde to each tube. Formaldehyde is a fixative, which will kill the cells. Once the cells have been fixed, you can look at them microscopically at a more leisurely pace.

7. Remove capillary tubes from the cell suspensions. Using a P-100 micropipetter and a small tip, push the contents of capillary tubes into the corresponding 0.65 ml tube containing formaldehyde. Carefully mix the cell sample with the formaldehyde by drawing the liquid up and down (gently).

8. With a P-100 micropipetter and a sterile tip, remove 20 µL of the cell suspension. Place the pipet tip in the notch under the coverslip on a hemocytometer and slowly add the liquid to the slide. You will see the liquid move over the surface of the slide. Add just enough liquid to cover the central part of the slide. The coverslip should not float off of the bridges.

9. Place the hemocytometer on the stage of the microscope and move it into position under the 10X objective. Bring the grid into focus using the coarse focusing knob.

10. When you have found the grid, be sure that you understand its layout. Refer back to the Background Information of Lab Exercise 6 (Analysis of the RNA Content of Yeast Cells – Part A) if necessary. Then rotate the 40X objective into position. Adjust the light intensity so that you can see both the grid and the cells clearly.

11. Count the yeast cells in five (5) of the 25 major squares for each sample.Rinse the hemocytometer using an appropriate waste container in between each sample. **Record the data here:**

		Cell Count					
Sample	**Extract**	**Square 1**	**Square 2**	**Square 3**	**Square 4**	**Square 5**	**Average**
1A	Peptone Broth						
1B	Peptone Broth						
2A	Tris						
2B	Tris						
3A	Undilute Extract						
3B	Undilute Extract						
4A	1:10 dilution						
4B	1:10 dilution						
5A	1:100 dilution						
5B	1:100 dilution						
6A	1:1000 dilution						
6B	1:1000 dilution						

E. Analysis of Chemotaxis Data

1. Calculate the cell concentration for each of your replicates using the following formula:

$$\frac{\text{Average \# of Cells}}{0.004\ \mu l} \times \frac{1000\ \mu l}{ml} = \text{Cell concentration (cells/ml)}$$

Record the cell concentration in the following table:

Extract	**Cell Concentration (cells/ml)**	
	Replicate 1	**Replicate 2**
Peptone Broth		
Tris		
Undilute Extract		
1:10 Dilution		
1:100 Dilution		
1:1000 Dilution		

2. Calculate the average cell concentration and standard deviation for each of your samples and record the information in the table above.

3. Calculate the chemotactic response for your samples using the formula below. Note: the peptone broth sample is the positive control for this experiment. All other samples are test samples which will be compared to the control.

$$\frac{\text{Cell Concentration}}{\text{Average Control Cell Concentration}} \times 100 = \text{Chemotactic Response (\%)}$$

Extract	Chemotactic Response (%)		Average	Standard Deviation
	Replicate 1	Replicate 2		
Tris				
Peptone Broth				
Undilute Extract				
1:10 Dilution				
1:100 Dilution				
1:1000 Dilution				

4. Now prepare a graph in which you plot the mean chemotactic response for each test sample. Include error bars of +/- 1 standard deviation above and below each data point. (See Lab 1 if you do not recall how to make these graphs) **What conclusion can you draw from these data? Include a copy of this graph in your lab manual.**