

Name: _____

Prelab: The Plankton of Boston Harbor

1. (6pts) Create a dichotomous key which will identify the 5 items pictured below using only yes/no questions. For example, if you had a metal paper clip, a metal spoon and a pair of plastic sunglasses, you could use the question, "is this item made of metal?" "If the answer is yes, go to question 2; if the answer is no, it is a pair of plastic sunglasses."



Questions

1)

2)

3)

4)

Question 2. (4pts) If you are looking at an organism in the Nikon compound microscope through the 10X lens and the organism measures $\frac{1}{4}$ the length of the field size, approximately how long is the organism in millimeters and in microns?

The Plankton of Boston Harbor

Objectives:

To think about how organisms are classified

To learn how to use a dichotomous key

To view plankton from the seawater around our campus using microscopes

To classify the plankton we find into groups and to create a dichotomous key

Classification

Scientists try to understand life on Earth by classifying the millions of organisms they have identified into groups. They group organisms by their similarities and they try to show which organisms evolved from other organisms. Similarities in organisms can be in external structures, such as having 2 sets of wings, or they can be in internal structures, such as a 3-chambered heart, or organisms can share molecular similarity in their DNA and RNA sequences. The science of classifying organisms is called **taxonomy**; it is not a static field but one that is constantly changing to encompass the new discoveries and new arguments of how to group organisms made by scientists.

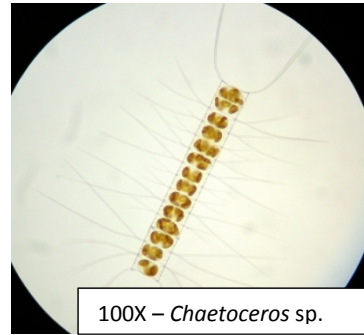
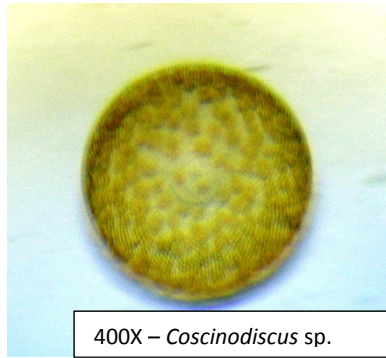
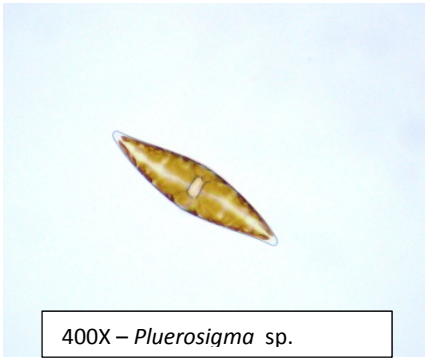
Protists

The protists are a diverse group of small, mostly single-celled organisms that have undergone many taxonomic changes recently. Formerly, the protists were classified in their own kingdom, but now are listed in many different kingdoms of the domain Eukarya. Single-celled organisms may seem simple because they are small but each cell must carry out the survival and reproductive functions of its species. It must obtain or make nutrition, regulate its interior environment, expel wastes, possibly defend itself against predation, and at some point, reproduce. Protists use their cellular organelles and contractile vacuoles to perform these functions.

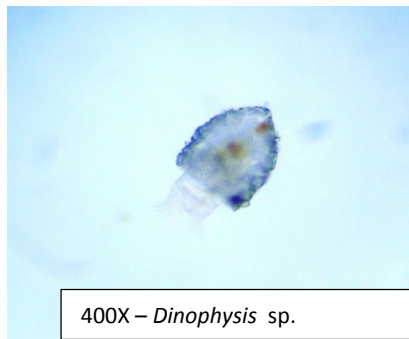
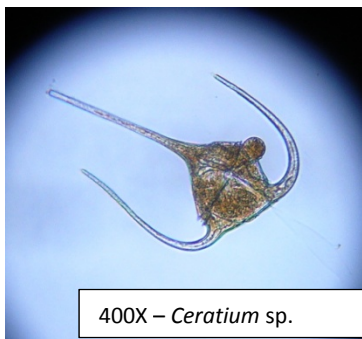
Plankton

Plankton are mostly protists and small organisms that live in water and move with the currents of the water; they cannot propel themselves through water the way fish can. Plankton are the base of the food web in aquatic habitats; phytoplankton are plant-like and photosynthesize, and zooplankton are animal-like heterotrophs and larval stages of higher aquatic organisms such as sea worms and snails. In our samples of plankton from Boston Harbor, we often see diatoms and dinoflagellates as phytoplankton. Copepods, larval forms of sea worms, mollusks and arthropods; hydra and worms are most of the zooplankton we see here. When we take our 50 micron mesh net to the dock to sweep it through the seawater for our plankton sample, we collect non-living items in the sample as well.

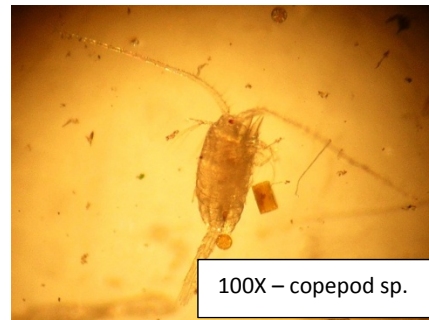
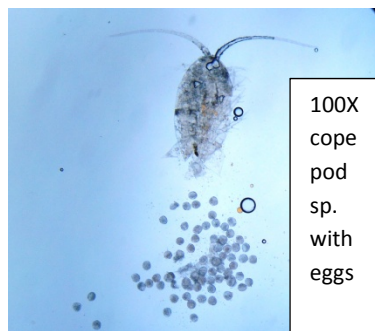
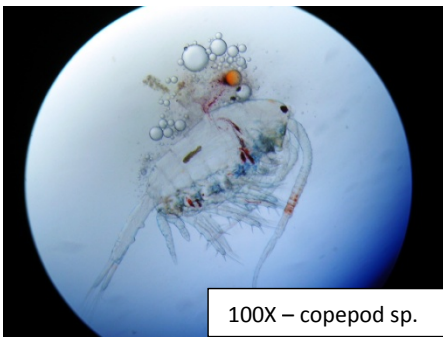
We find fecal pellets, pollen grains, decaying tissues and molts of arthropod exoskeletons; you may find some of these in your sample too.



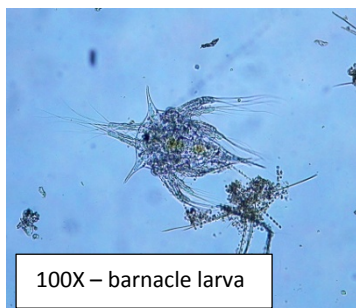
Diatoms – common unicellular phytoplankton, cell walls made of silica, usually bilaterally symmetrical



Dinoflagellates – unicellular, some photosynthesize, most have 2 flagella for locomotion, cause ‘red tide’



Copepods – common zooplankton, fairly large 1-2 mm, with a set of long antenna, transparent



Larval forms of higher invertebrates

Dichotomous Keys

A dichotomous key is a means of identifying organisms (or anything) by using an ordered list of yes/no questions. Answering the questions correctly and in sequence will allow you to identify the organisms. The questions are usually related to observable exterior traits seen with the naked eye, such as shape, color, number of legs etc. Creating a dichotomous key forces you to work backwards and separate the collection of organisms into groups and to formulate questions that can be responded to with a yes or no for each organism. For example, if you wanted to make a dichotomous key to identify 4 types of trees: white pine, red maple, white oak and white birch, you could create these yes/no questions. Other questions and ways to group these trees are possible.

- 1) Is the tree evergreen or not? If yes, it is a white pine; if no, go to question 2.
- 2) Does the tree have white bark? If yes, it is a white birch; if no, go to question 3.
- 3) Does the tree have leaves coming to sharp points? If yes, it is a red maple; if no, it is a white oak.

Introduction to Microscopy

Always treat the microscope with great care. Make certain that you do not touch any part of the lens system with anything abrasive (such as a slide or dirty water) or greasy (such as even the cleanest fingers). Never clean a lens with anything except clean lens paper! If the view gets foggy, and lens paper will not clean it, call your TA.

Compound Microscope – magnification of 40X – 400X

(1) Structure of the Compound Microscope

It is very important that you familiarize yourself with the parts of the microscope and their function. Your first task is to locate all of the parts named in the diagram on the next page. Place the microscope so that it is at right angles to you.

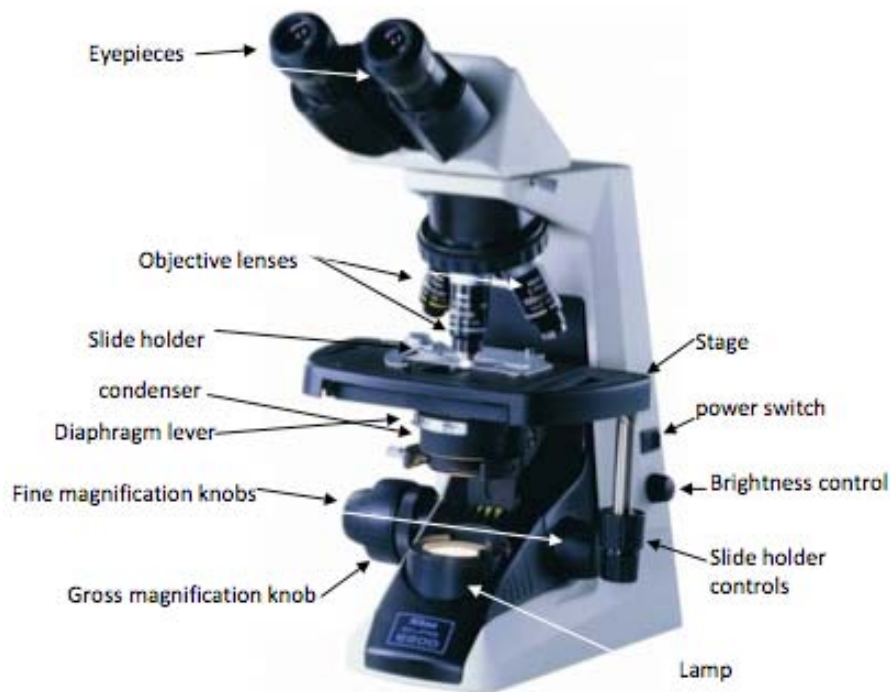
In addition to the stand (arm & base) and a movable stage by which the object can be positioned and focused for viewing, the microscope consists of the following sub-units:

- A. The system involved in illuminating the object to be viewed, i.e., light, diaphragm and condenser.
- B. The lens system - eyepiece, body tube and objective lens which magnify the object.

A. The System of Illumination. Keeping the microscope in the same position: (1) plug it in, (2) turn on the light and (3) move the diaphragm lever as far to the left as possible. Place a clean slide on the stage over the condenser and put a piece of white paper about 25 mm square on top of the slide. Now slide the condenser knob and move the condenser up and down while observing the light on the piece of paper (do not look through the microscope but continue to look at the paper with your naked eye). Note that you see a fairly intense small circle of light when the condenser is at its uppermost position and that this circle gets larger and more diffuse as one lowers the condenser. For

most work with the 4X, 10X and 40X objectives it is best to have the condenser near the top of its travel.

Put your eye at table level and look up at the bottom of the condenser. Now move the diaphragm lever and observe what happens. This is an iris diaphragm. Why do you suppose it is called this? Look at the piece of paper again while opening and closing the diaphragm. The diaphragm serves to regulate the amount of light passing through the condenser. It also serves to cut down stray light. Later when you look through the microscope you will see that the diaphragm can be kept partly closed without cutting down on the light passing through the lens (i.e., only light beyond the field of the lens is being blocked). Further closing of the diaphragm will cause less light to enter the lens and decrease the resolving power of the lens while increasing contrast in the viewed object (Resolving power is how well specimen detail is preserved. Contrast is the ability to see particular detail against its background.) Control of the light entering the microscope is very important.

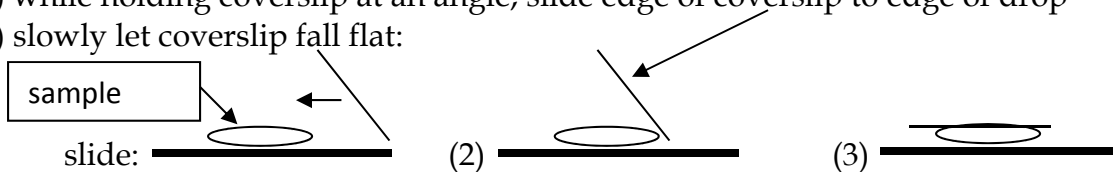


The Nikon compound microscope

Preparing A Slide and Making Observations.

Your lab instructor will show you how to make a slide. The great art here is to avoid air bubbles when you lower the coverslip! A useful trick for this is to:

- (1) put drop of sample on slide
- (2) while holding coverslip at an angle, slide edge of coverslip to edge of drop
- (3) slowly let coverslip fall flat:

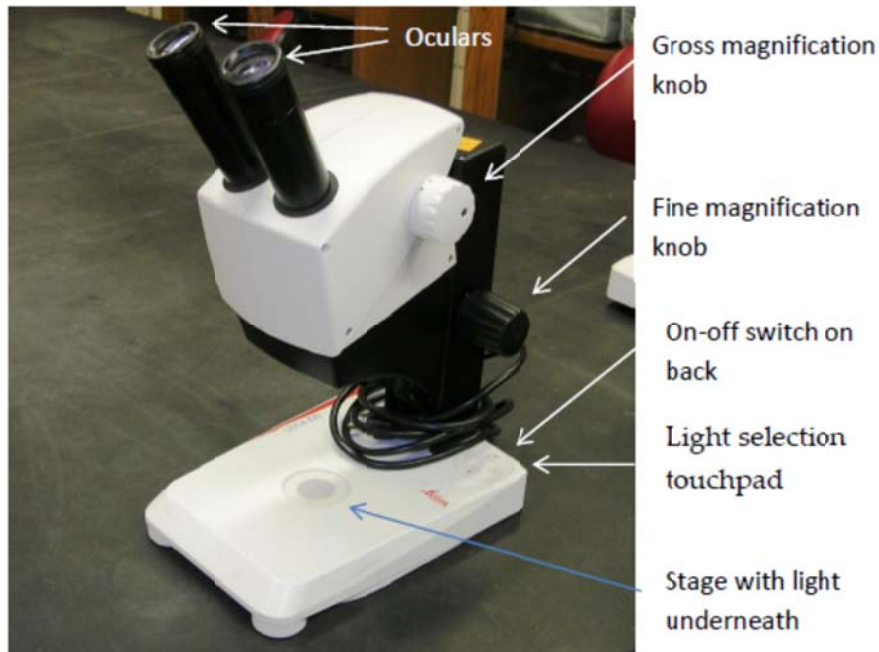


POINTS TO REMEMBER: The following are worth remembering as you use the microscope.

- 1) Do all preliminary focusing under low power. Find a corner of the cover slip and focus on that first. When you have your sample in focus, change the lens to increase the magnification.
- 2) Use the fine adjustment constantly to keep things in focus.
- 3) Use lens paper to clean the lenses occasionally, you will find that the microscope works best when clean.
- 4) Control the amount of light entering the microscope - the contrast at which you view the specimen will control how much detail you view.
- 5) Keep an eye on the stage while using the image focusing knob so that you don't put the objective through the slide.
- 6) Become familiar with how the microscope works before trying to view a specimen.

Dissection Microscope – magnification of 13X – 56X

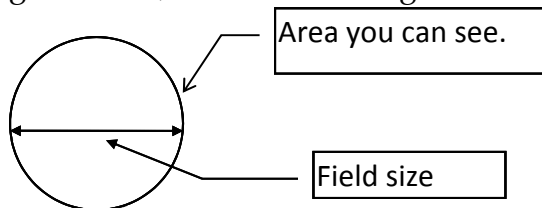
Larger, thicker objects can be viewed in the dissection microscopes. Sample containers can be put directly on the stage of the dissection microscope.



Leica EZ 4 Dissection Microscope

How big is it?

You can use the microscope to measure the approximate size of the objects you are looking at. Given the magnification, the table below gives the diameter of the field of view. See diagram:



Once you know that, you can estimate the size of what you're seeing. If the field size is 450 μ m and the thing you're looking at is half as wide as the field, then it's about 220 μ m wide.

Magnification shown on objective lens	Actual magnification		Field size (millimeters)	Field size (microns (μ m))
4x	40x	\Rightarrow	5	5000
10x	100x	\Rightarrow	2	2000
40x	400x	\Rightarrow	0.5	500

Nikon compound microscope field view sizes

Magnification shown on knob	Actual magnification		Field size (inches)	Field size (millimeters)
1.3x	13x	\Rightarrow	0.74	18.8
2.0x	20x	\Rightarrow	0.47	12
3.2x	32x	\Rightarrow	0.30	7.5
5.6x	56x	\Rightarrow	0.17	4.3

Leica EZ 4 dissection microscope field view sizes

Part I : Plankton

Lab Procedure

(1) A Size Standard: a Grain of Salt

It is often difficult to get a sense of the size of microscopic objects. As a link to the macroscopic world, you will use one of the smallest things you can see with the naked eye - a grain of salt - as a size standard.

Obtain some salt grains from your TA. Put them on a slide WITHOUT WATER and estimate their size. Pool the class results and record the 'typical' size of a salt grain here:

salt grain size = _____ μ m

(2) Viewing the Boston Harbor Plankton in the Microscopes

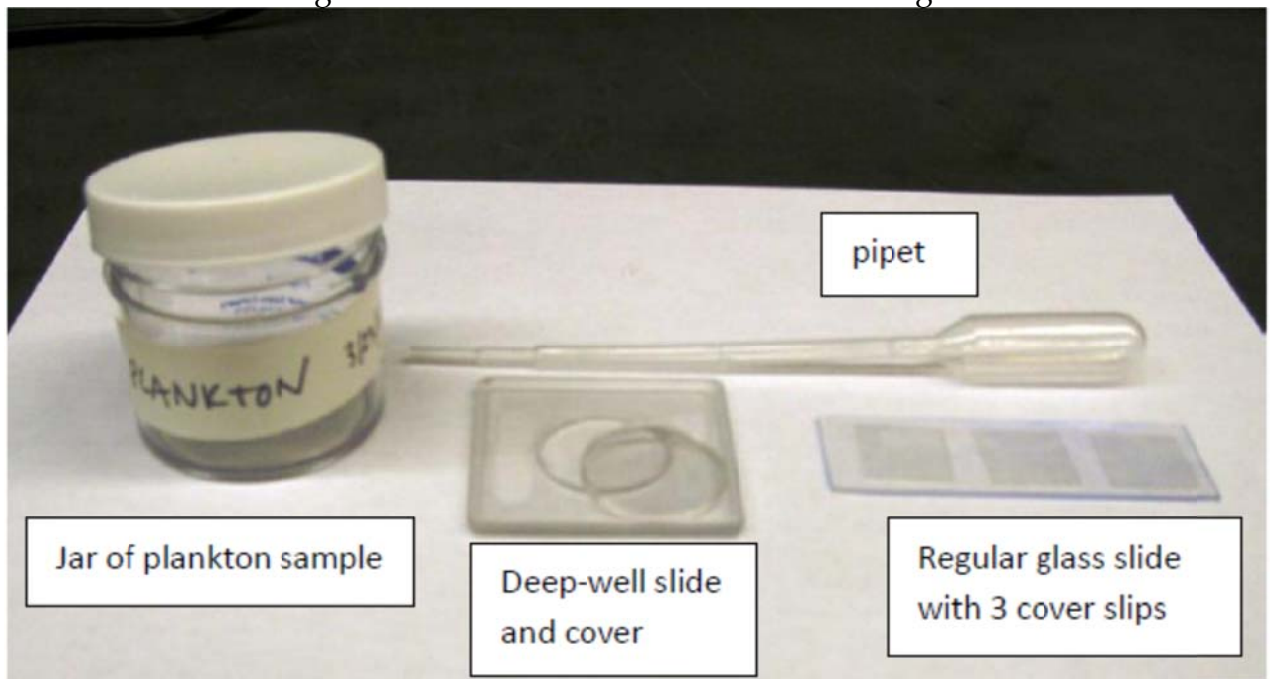
Recently, we sampled plankton from boat docks here at UMass, Boston. You have some of that sample in the plastic container on ice. The temperature of the water off the docks is about 6 $^{\circ}$ C (48 $^{\circ}$ F) this time of year so we wanted to keep the sample close to that temperature.

a. Place the entire container of plankton on the stage of the dissection microscope and see if you can see and identify the larger organisms in the sample. You will only be able to focus on one depth of the sample at a time, for example you could focus on the bottom of the container to see the organisms at that depth, or you could focus on the surface plane to see the organisms there. Moving organisms will be changing their depth constantly and you will have to constantly change your plane of vision to try to keep that organism in focus. Take notes on the appearance of the organisms you find and how they move.

b. Next, take a deep well slide and remove the cover. Use your plastic pipet and transfer some of the plankton to the deep well slide. The deep well slide holds about 20 drops of sample. Put the cover on and view the deep-well slide under the dissection microscope. You should be able to get a better view of organisms here. Try to identify what you see using the phytoplankton and zooplankton ID sheets. Draw the organism or take its picture; estimate the organism's size using the tables above as you did with the grain of salt.

c. You can also take this deep-well slide and put it on the stage of the Nikon compound scope. You can focus this slide under the 4X or the 10X lenses, **but it is too thick to fit under the 40X lens.**

d. Take the cover off the deep-well slide and pipet out an organism you want to look at more closely. Place 1 or 2 drops of the plankton including your organism of interest on a regular, glass, microscope slide and cover with a cover slip. Focus the organism; here you can use the 40X lens. Try to find at least 8 different organisms and list their traits in the following chart.



Jar of plankton and 2 kinds of slides to view the plankton under the microscopes.

(3) Making a Dichotomous Key

Fill in the following chart and use it as a guide in making a dichotomous key which will identify 6 of the organisms you found in your plankton sample.

	organism name	phyto or zoo	size	shape	color	head?	antenna?	eyes?	moving?	single cell/ colony/ filamentous
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

Plankton organism characteristics

Part II: Microbial Diversity: A Review of the Three Domains

All living things are made up of cells, but as life varies greatly so do the cells that make it up.

Prokaryotes (Archaea and Bacteria) are organisms in a group which lack true nuclei and contain few organelles. **Eukaryotes** (Fungi, Plants and Animals) on the other hand have true nuclei, cytoplasm, and a plasma membrane surrounding their cells and contain a variety of other organelles. They also differ in that some are unicellular organisms and others are multicellular. Whether eukaryotic or prokaryotic, a general term for any life form needing magnification in order to be seen is “microbe” and many (but not all) of these are single-celled organisms rather than multicellular.

The average eukaryotic cell is much larger and easier to observe with a microscope than the average prokaryotic cell. You will observe examples of prokaryotes with the microscope and will see how small they are compared to eukaryotic cells, which you will spend much more time observing. Protists are all contained in the Domain Eukarya within different kingdoms and phyla reflecting their great diversity. They are unicellular creatures and some are animal-like and called Protozoa (*Paramecium* and *Amoeba*); others are more like plants (green algae, diatoms); and still others seem to be both plant and animal at the same time (*Euglena*).

Plant cells are often easy to identify in that the typical plant cell, in addition to nuclei, cytoplasm, and a plasma membrane, has a cell wall - a rigid structure made up chiefly of cellulose that surrounds the plasma membrane. Plants also possess chloroplasts - structures within the cell that contain the green pigment chlorophyll. The typical plant cell has much of its volume taken up by a large vacuole containing water, salts, sugars, and other compounds whereas most animal cells are largely filled with cytoplasm.

In this portion of the lab, look at and learn to recognize some representatives of the major microbial groups. These include bacteria, some fungi, and within the protists, protozoa and some algae. To do this you will view demonstrations prepared under microscopes, in order to distinguish the basic cellular structures: cell wall, nucleus, vacuoles, flagella, chloroplasts. As you examine each species, try to determine its method of movement and nutrition, and check out its phylogenetic classification.

Cells				
Cell Parts & Organelles	Prokaryotes	Eukaryotes		
	Bacteria	Fungal	Plant	Animal
true nucleus	no	yes	yes	yes
cell wall	yes	yes	yes	no
cell membrane	yes	yes	yes	yes
chloroplasts	some	no	yes	no
vacuole	no	yes	yes, large	yes, small
flagella	some	no	no	some

Part II A. Observing Prokaryotic Life

Sampling & Inoculation Procedure:

- During this lab, inoculate a bacterial growth plate with a sample from some common environment. You may expose the plate to room air, or dust, or a drop of water from the fish tank. (Do not use human samples because we are not equipped to diagnose possible pathogens). Seal the plate with parafilm, turn it upside down, label it with your name and the date, and what sample was taken. Give it to your instructor to leave on a shelf in the lab room. Next week, you can look at it and describe the different kinds of colonies present, and their relative numbers. You can view the cells under the microscope to see their structure.
- Look at prepared slides and images of several prokaryotes and Archaea. Draw what you see, note the size of the organisms. What structures are visible? Do you see nuclei?
- *Spirogyra* sp. is a type of **cyanobacteria**. Cyanobacteria are the only group of prokaryotes that photosynthesis. Note the green color. This species is the filamentous type. Look at a live culture and prepared slide.

Part II B. Observing Unicellular Eukaryotic Life: Protists

1. There are prepared slides set up of the following protozoa to look at. Names in *italics* are genus and species names. Those in normal type are names of phyla. The abbreviation *sp.* means “*species*”, that is, the genus is known but the exact species is not. Familiarize yourself with the species. Draw, label and estimate a measure the following organisms and the organelles you can find.

Euglena sp.

Paramecium caudatum

Volvox globator

Lab Assignment

You may find it necessary to consult your textbooks for some of the information required.

- Must be typed; handwritten assignments will not be accepted. Hand-drawn and labeled drawings are fine.
- Due next week at the start of the lab session you are currently in. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab assignment. Each person’s assignment must be in his or her own words as much as possible.

- Your lab assignment must contain: (see the next page)

Part I: Protozoa:

For each of the organisms you saw.

- (1) A drawing or photo of the 8 organisms you looked at with size clearly marked. These may or may not be on the plankton identification sheets.
- (2) Sketch a crystal of table salt. Next to it, sketch the outline of each organism you observed **to scale** with the salt grain. Your drawings need not include all details, but should show the relative sizes of the organisms and the salt grain. Note that, since all the sizes you will measure are approximate, your drawings only have to be roughly correct – we will not grade on the precision of your measurements.
- (3) Produce a dichotomous key with at least 5 yes/no questions that will allow someone to identify the 6 organisms of your collection. Many different keys will be possible; we are looking for a key that is reasonable and made from careful observations.

Part II: Eucarya, Archaea and Bacteria

- (4) A representative drawing of one type of protist with size and structures indicated and which domain it belongs in.
- (5) Name an example of microbial life for each of the three domains and give some characteristics of each.