

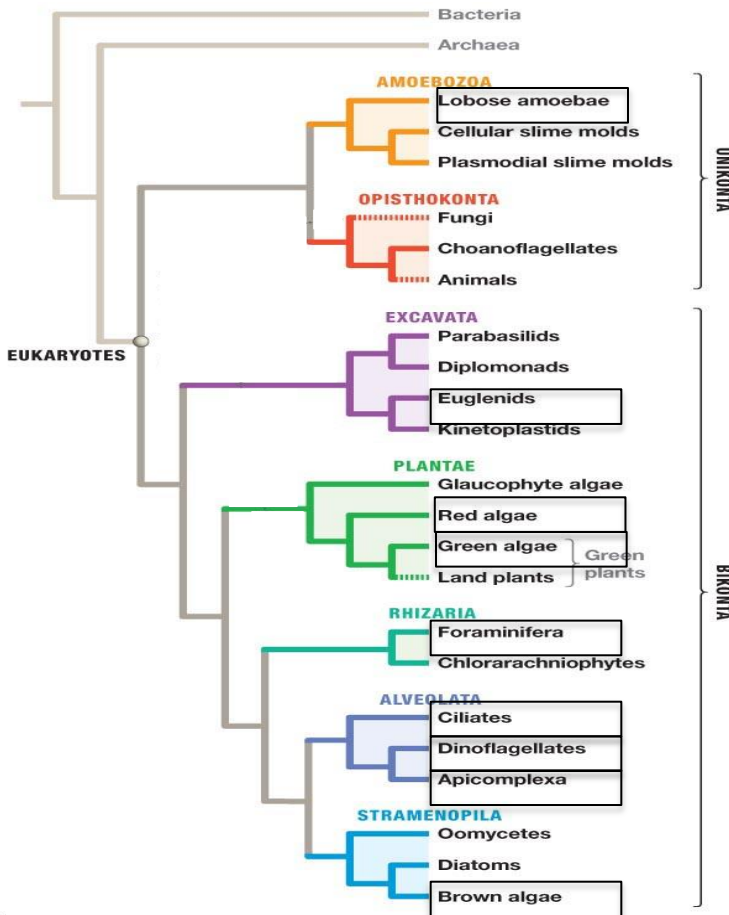
Protist and Microbe Diversity

Introduction

Today, you will explore some of the diversity found in the Bacteria, Archaea and Protist groups. As you know from lecture, these 3 groups are very different and fall into different domains on the tree of life.

Bacteria and Archaea have no nuclear envelope and grouped together as prokaryotes. Both domains are characterized by incredible morphologic and metabolic diversity. You will explore some of that diversity today.

Protists were historically recognized as members of the kingdom Protista. However, this grouping was to some extent out of convenience as the protists have little in common with one another other than their subcellular structure. In fact, Protista is a paraphyletic group. Protists are simple eukaryotes. They can be either unicellular or multicellular but do not form complex tissues like those found in higher eukaryotes such as animals and land plants. Despite their comparatively simple structure, protists are incredibly diverse. In fact *animals and land plants exhibit only a fraction of the diversity found in protists*. In lab today, we will focus on the diversity of methods that protists use to obtain food, diversity in movement and highlight the morphological diversity in structures for support and structure.



To the left is a phylogenetic tree of the groups that include organisms typically/historically called protists. The black boxes indicate organisms that you will observe today in lab. Note that several groups within the Plantae were historically placed with the protist group. We will study two of those groups today (red and green algae) because they have similar morphology and ecological function to other protist groups.

The purpose of today's lab is to get a small glimpse of microbial and protist diversity by examining a combination of prepared and live specimens.

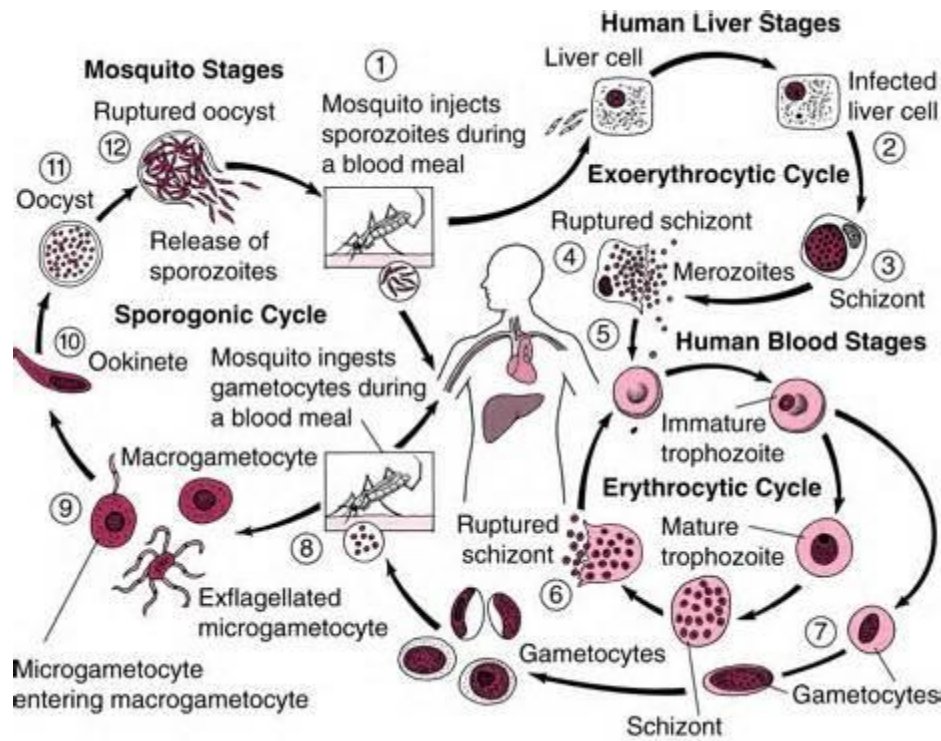
You will be using the microscope today. Remember to use good microscope technique!!

Modes of Nutrition, how Eukaryotes (primarily Protists) obtain food.

Protists have a diversity of ways to obtain the energy needed to grow and reproduce. Protists are able to obtain food by: 1) ingesting other organisms, 2) by absorbing nutritive materials from the environment or other organisms, 3) through photosynthesis, or 4) through a combination of the aforementioned mechanisms (called mixotrophy).

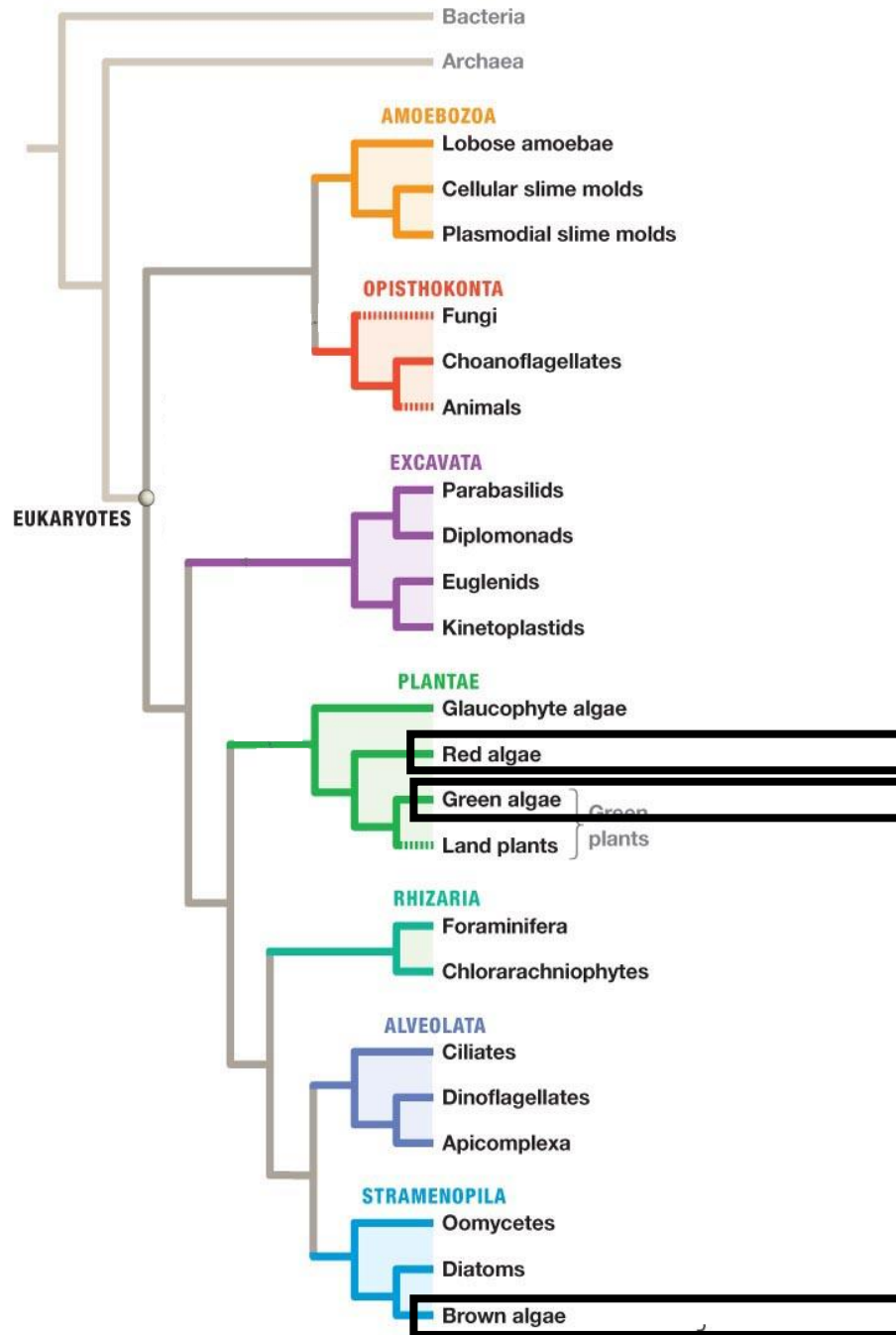
Absorption. The absorptive lifestyle is present in many groups of organisms, all three Domains contain organisms with an absorptive lifestyle. Absorptive protists make their living by absorbing materials from the environment (which includes absorbing materials directly from other organisms, either alive or dead).

Plasmodium (Alveolata - Apicomplexa), is a protist parasite of humans that causes malaria and lives an absorptive lifestyle. Look at the prepared slide of *Plasmodium* in blood (the red stained cells are Red Blood Cells and the Purple stained cells either inside the RBCs or free are the Plasmodium). View the slide at 400X. Locate several examples of the protist amongst the many healthy red blood cells present and determine, using the lifecycle below, what stages you have observed. Sketch and label examples of the Plasmodium stages you observed along with a few blood cells adjacent.



Photosynthesis. Representatives of all three Domains (Bacteria, Archaea, Eukarya) can photosynthesize. For most people, land plants are the most familiar example of an organism that can capture energy from the sun to manufacture organic compounds. Within the Eukarya, photosynthetic organisms are found in many of the major lineages (Excavata, Plantae, Rhizaria, Alveolata, Stramenopila). As you know, most Protists are unicellular, but most of the representatives we are looking at in this exercise are multicellular.

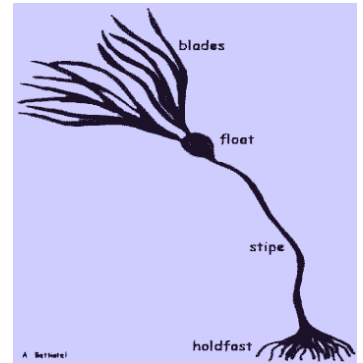
Lineages containing multicellular algae to be viewed today.



Look at the macrophytic algae samples in the hood, they include: *Laminaria*, *Chondrus* and *Ulva*.

I. The brown alga, *Laminaria*, (Stramenopila – Brown Algae) contains a brown pigment known as fucoxanthin. Brown algae are multicellular and develop differentiated tissues.

- Sketch, label and describe the overall appearance in your lab notebook.
- Locate and label the holdfast, stipe and blade using the guide.
- This organism is sessile (stationary) in the adult stage (which is the stage before you). In other life stages it is motile. Which stage to you think is motile? Why?



II. *Ulva* (Plantae – Green Algae) was formerly considered a protist. *Ulva* is commonly called sea lettuce, it is likely you have seen it on a beach. *Ulva* is multicellular and green due to its photosynthetic pigments, which are similar to land plants. Don't confuse *Ulva* with the Land Plants, which are much more structurally complex.

- Sketch, label and describe the overall appearance in your lab notebook.
- Locate and label the holdfast, stipe and blade using the guide. Is *Ulva* missing any of these parts?
- This organism is sessile (stationary) in the adult stage (which is the stage before you). In other life stages it is motile. Which stage to you think is motile? Why?

III. This multicellular species, *Chondrus crispus* (Plantae- Red Algae), was formerly considered a protist and can be found growing along the Atlantic Coasts in both Europe and North America. Its common name is Irish moss. You may not realize it but you have probably eaten this red algae many times (or at least the polysaccharides derived from it) or maybe even applied it to your face. This polysaccharide is often used to stabilize ice cream, make jelly, thicken soups, milkshakes and marshmallows, and make beer and wine. What is this polysaccharide called? You can answer this in the lab or do a quick internet search after you leave.

- Sketch, label and describe the overall appearance in your lab notebook.
- Locate and label the holdfast, stipe and blade using the guide.
- This organism is sessile (stationary) in the adult stage (which is the stage before you). In other life stages it is motile. Which stage to you think is motile? Why?

Unicellular Algae. Most photosynthetic Protists are unicellular. Several of the lineages within the protists have representatives that appear roughly similar in structure to and serve a similar ecological role (there are also bacteria that are similar in ecological role, called cyanobacteria).

Different lineages have different photosynthetic pigments which can often be used to identify the group they belong to.

Look at *Chlamydomonas* (Plantae – Green Algae) in the container using your eyes alone (no magnification). The organisms pigmenting the water are unicellular and have the ability to actively propel themselves within the water column.

What color green are they?

What other organisms that you have looked at today under the microscope are photosynthetic?

Ingestion. Because of their increased body size (over the prokaryotes), directly ingesting other organisms was a novel form of obtaining nutrients that first appeared in the Protista, this feeding method allowed them to feed on the smaller Bacteria, Archaea and other Protists. Organisms such as *Amoeba* (Amoebozoa – Lobose Amoebae), *Stentor* (Alveolata - Ciliates), and *Paramecium* (Alveolata - Ciliates) ingest other organisms as way to obtain food. Most animals, although multicellular and not protists, also have an ingestive lifestyle.

Make three preps, one each of live *Paramecium caudatum* and *Amoeba* and *Stentor*. There are instructions for making an *Amoeba* prep in the motility section. There may be *Paramecium* in your *Amoeba* prep but they are much smaller! Observe and sketch the protists in your notebook.

How do the protists feed?

Do they use specialized structures to do so?



This is *Amoeba*!



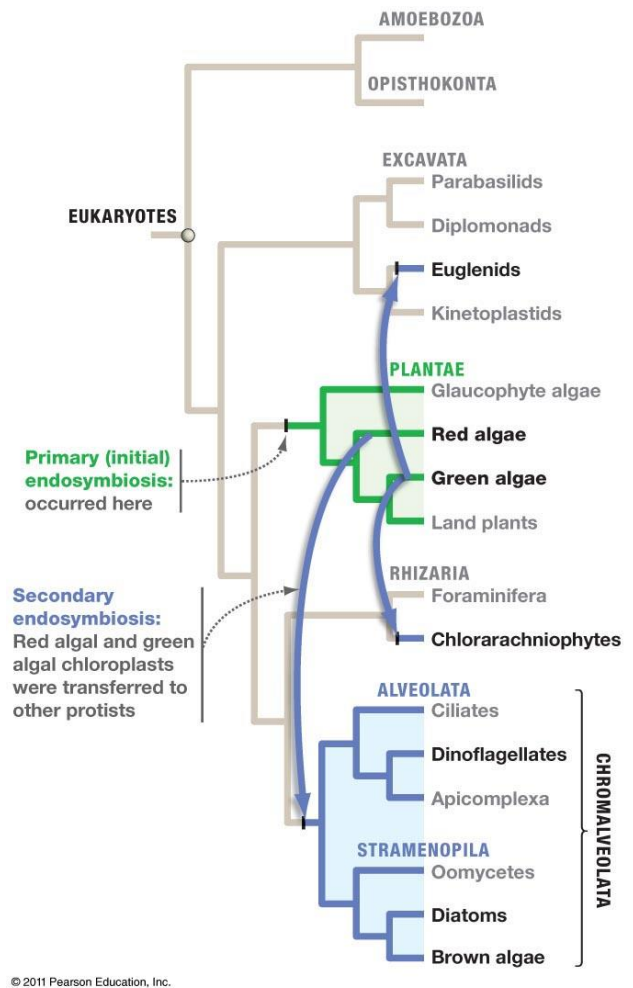
This is a *Paramecium*!



This is *Stentor*!

Mixotrophs. Mixotrophs are organisms that use two or more of the modes of nutrition to satisfy their needs. Within the protists there are many examples of organisms that use multiple modes of nutrition to obtain food. For example, species of *Euglena* (Excavata - Euglenids) can photosynthesize and also ingest other organisms.

Endosymbiosis. In the primary endosymbiosis event that formed the chloroplast, an ancestor of the green algae engulfed and retained a cyanobacterium. Over time the cyanobacteria lost the ability to live outside the host and was reduced structurally, resulting in the organelle the chloroplast. The chloroplast has double membranes around it that indicate its origin in a free living organism. Many Eukaryotic lineages other than green plants contain chloroplasts, but their chloroplasts are surrounded by four membranes. Four membrane chloroplasts are thought to be the result of secondary endosymbiosis of a protist engulfing a green plant (that already had a double membrane chloroplast). Additionally, some protists contain photosynthetic endosymbionts. The endosymbiont is another species that lives inside the protist. This relationship is a mutualism, with the endosymbiont providing the host with organic compounds and the host providing habitat.



Prepare slides of and examine the *Paramecium bursaria* (Alveolata - Ciliates), *Euglena* (Excavata - Euglenids) and *Chlamydomonas* (Plantae - Green Algae). Sketch them in your notebook and describe any unique features. *Euglena* and *Chlamydomonas* have chloroplasts, whereas the *Paramecium* you are viewing contains an endosymbiont green alga called *Chlorella*.

- How many membranes do the chloroplasts of *Chlamydomonas* have? Why?
- How many membranes do the chloroplasts of *Euglena* have? Why?
- How many membranes do the chloroplasts of *Chlorella* within *Paramecium* have? Why?

Phototrophic and Chemotrophic Bacteria - Winogradsky column.

Today, we have been looking mostly at Protists, but here we will focus on Bacteria. A Winogradsky column is a microcosm of a pond or marsh ecosystem, in which a variety of microbes with diverse metabolic activity grow. We made this column from salt marsh mud collected in Fairfield, to which carbon and sulfur sources were added. After sitting in the sun for 4-6 weeks, the growth of different groups of microbes can be seen. Observe the Winogradsky column and compare it to the diagram.

Identify the green and purple sulfur bacteria. These pigmented bacteria have bacteriochlorophyll, and photosynthesize using hydrogen sulfide (H_2S) as an electron donor, instead of water.

Notice the gradients of oxygen and sulfur that form in the column (see diagram).

These phototrophic sulfur bacteria grow in lakes or marshes where oxygen is _____ (low/high) and sulfur is _____ (low/high).

Can you see any evidence of chemotrophic bacteria in the column?

Modes of motility, how Eukaryotes (primarily Protists) move.

Many protists are motile which allows them to actively move to obtain food. Some use specialized structures to swim (e.g., cilia or flagella) whereas others use various methods to slide/crawl along a surface. Other protists are sessile (they do not move).

For each of the below, draw the basic shape of the cell and describe the way it moves. In your description, discuss the relative speed of each movement and which mode of motility the organism uses. We have included pictures so that you know what you are looking for but you should observe and sketch the live organism rather than the picture.

a) *Paramecium caudatum* (Alveolata - Ciliates)



Does *Paramecium* actively swim? Using what type of structure? Describe the movement you see.

b) *Euglena* (Excavata - Euglenoid)



Does *Euglena* actively swim? Using what type of structure?

Euglena is primarily photosynthetic. Unlike *Euglena*, many photosynthetic protists do not actively move but float passively on water currents. How would active swimming help *Euglena* obtain food?

c) *Amoeba proteus* or *Pelomyxa carolinensis* (Amoebozoa - Lobose amoebas)

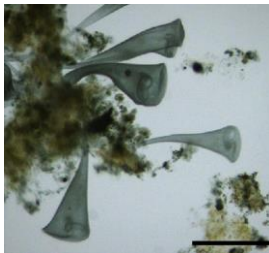
You can see the amoeba on the bottom of the culture dish without using the microscope. Use a dropper to dislodge the amoeba and transfer to a depression well slide. Cover the slide with a cover slip. Observe the amoeba at 40x. Make sure to turn off the light when you aren't directly observing them as they die quickly. Watch the cytoplasmic streaming as the pseudopodia move forward.



How does the lack of a cell wall facilitate movement?

d) *Stentor* (Alveolata - Ciliates)

Stentor is a ciliate with both sessile and swimming forms. Find a *Stentor* (use a dropper to transfer a bit of the greenish material to a slide). Find a sessile form and observe its movement. Make sure you observe the beating of cilia. What is the function of the cilia in this case?



What is the function of the cilia in the sessile form?

Structures for Support and Protection.

Many protists have structures for support and protection. The diversity of mechanisms for support and/or protection is vast and varies across lineages. Unlike methods of obtaining food or motility, in many cases, these structures are synapomorphies that help us identify monophyletic groups within the protists. Make sure you can identify which structures are synapomorphies (use your textbook to help).

Observe the prepared slides. The cells have been preserved such that the support and protection structures are visible. For a and b, sketch each organism – make sure you label and describe the support/protection structures. For c, list the organisms that contain each type of internal structure.

a) Cell walls outside of cell membrane

- *Ceratium* (Alveolata - Dinoflagellates)

b) Hard external structures -

- Diatoms (Stramenopila - Diatoms) (silica)

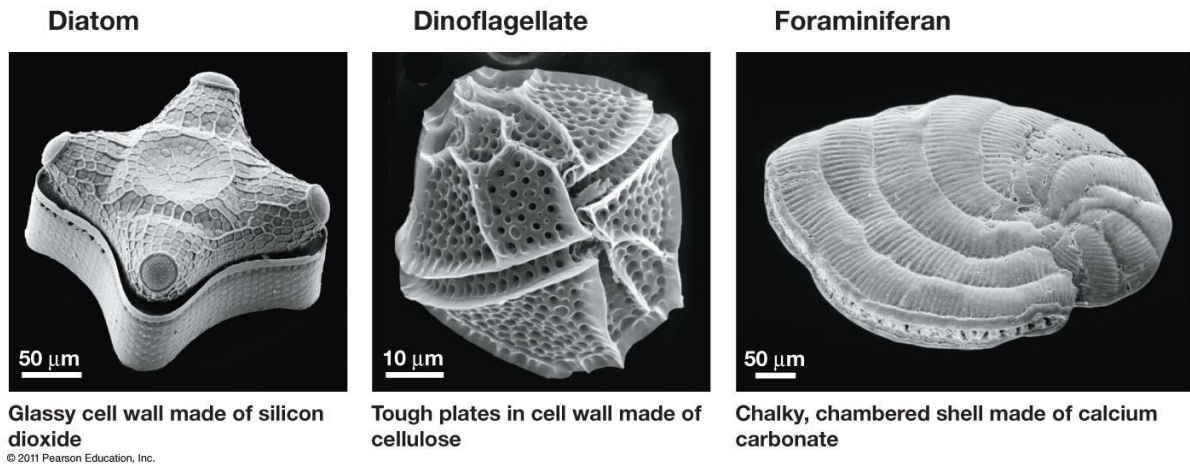
- Foraminifera (Rhizaria - Foraminiferas) (calcium carbonate)

- Radiolarians (Rhizaria - Radiolaria) (silica)

c) Rigid structures within the cell

- alveoli (Alveolata) – which organisms have you seen that contain alveoli?

- unique microtubule structures (Excavata, Parabasalids and Euglenids) – which organisms have you seen that contain these structures?



Synthesis.

There are two activities designed to help you synthesize what you've observed in lab today. Do this portion of the lab after you've completed the rest of the exercises.

a) Comparing protists and bacteria: *Lyngbya* and *Spyrogira*

Although protists and bacteria belong to 2 different Domains, some species may be similar in morphology and ecological function. The 2 species here are both phytoplankton (an ecological not phylogenetic grouping). The name comes from the Greek words *phytos* (plant) and *planktos* (drifter). Phytoplankton are photosynthetic organisms that form the base of the food web in many aquatic systems.

One of the organisms is a protist and the other is a cyanobacteria. Make slides of each of the organisms. Draw the organisms in your notebook paying careful attention to color and internal features.

Which one is a protist? What group do you think it belongs to? How can you tell?

Which one is a cyanobacteria? How can you tell?

b) Table of protists seen in lab.

This table will help you better understand which characteristics are synapomorphies and which are paraphyletic. You may need to use your textbook to complete the table. Make sure you tape or staple the completed table in your lab notebook.

Protists seen in lab	Lineage (group and subgroup) (e.g., Alveolata, Ciliates)	Mode of nutrition (e.g, ingestive, absorptive, photosynthetic, mixotrophic)	Mode of motility	Characteristic features of group
<i>Paramecium</i>				
<i>Ameoba</i> or <i>Pelomyxa</i>				
<i>Euglena</i>				
Diatoms				
<i>Ceratium</i>				
<i>Ulva</i>				
<i>Laminaria</i>				
<i>Chondrus</i>				
<i>Plasmodium</i>				
<i>Stentor</i>				