Lab 4 Pre-Lab **Name**_____

Microbial Diversity & Microscopy

Protozoa

1) Using Campbell, draw a rough sketch and briefly describe the following organisms:

Amoeba

Paramecium

Vorticella

Spirogyra

Diatoms

2) Using the information in your text book, draw a phylogenetic tree that includes the following organisms. You do not need to include information about when the last common ancestor existed. Do include the names of the kingdoms and phyla. Please draw it on the back of this sheet.

humans

fish

c-ferns (they are regular ferns)

Paramecium

Volvox

Vorticella

3) Which two organisms from question 2 are the most closely-related?

Microbial Diversity & Microscopy

Objectives:

To become familiar with the proper use of a compound microscope in order to comparatively study and measure cells.
To learn how to make a wet mount of a living culture and observe with a microscope.
To observe eukaryotic unicellular organisms (protozoa) and their subcellular components.
To look at some organisms on the border between plants and animals and to contrast them with organisms of other domains.

Introduction:

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 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 need to learn to use a microscope, 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. This material will be on the **Lab Practical** so take good notes.

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 I: How a Microscope Works

To see small things like cells one must learn to use a microscope. 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 (as it probably will sometime during the semester), and lens paper will not clean it, call your laboratory instructor.

A. First, familiarize yourself with the parts of the microscope and their function. Locate the main parts named in the diagram. These include the stand (arm and base), the light, the condenser lens with its diaphragm, the movable stage or the non-movable stage with slide clips, the objective lenses, the nosepiece, the body tube, and the ocular lens.

B. Plug in the light cord, turn on the light, and then move the diaphragm lever as far to the left (closed) 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 move the condenser up and down while observing the light on the piece of paper (do not look through the microscope yet, just 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 l0X and 40X objectives it is best to have the condenser near the top of its travel.

C. 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 close two points can be and still be distinct. Contrast is the distinction of a particular detail against its background.)

D. Light passes through the condenser, through the object which is placed on the slide, and into the lens system. The lens system consists of: (l) an objective lens - the revolving nosepiece of your microscope has at least two of these, (2) a body tube - in your microscope the body tube has prisms in it to allow the tube to be inclined and (3) the eyepiece (ocular) lens. Basically, the objective lens magnifies the object and forms an image in the tube which is further magnified by the eyepiece lens. The objective lens is the most important (and most expensive) part of the microscope and

the quality of a microscope is largely a question of the quality of its objective lens. The ones in your microscope are very good indeed and deserve care. The l0X objective (low power) has a working distance (the distance from lens to object when the object is in focus) of about 4 mm. The 40X objective (high power) has a working distance of about l mm.

E. Move the stage down well clear of the objective lenses by turning the coarse adjustment knob. Now rotate the nosepiece and notice that each lens clicks into the proper position. Move the l0X objective into position. Next move the stage up until the objective lens is about 4 mm from the slide. Notice while doing so that the knob you are turning is both a coarse and fine adjustment and that extreme movement of the knob moves the stage rapidly, but immediately after you reverse the direction of movement, the stage moves almost imperceptibly for a short distance. This fine adjustment allows precise focusing. The compound microscope has a very limited depth of field. It is necessary to continually focus up and down to get an impression of depth.

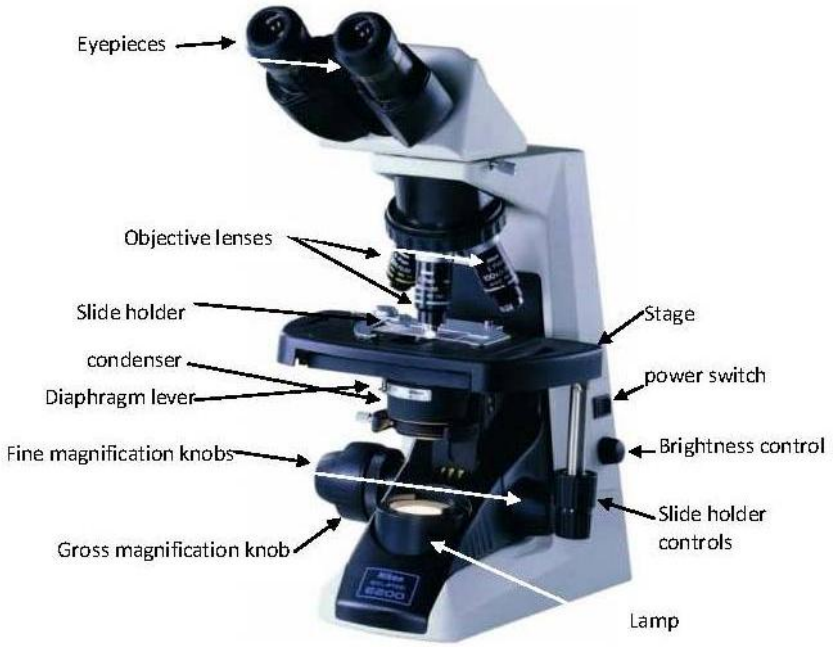


Diagram of a typical bright-field compound microscope. Though various styles exist, all compound microscope have the same basic components and they are labeled in the picture above.

F. Only when the object is in focus under low power should you go up to higher power. Move the object to the center of the field of view, and then rotate the 40X objective into place. You may need to adjust the light strength, condenser height and the diaphragm opening. When you adjust the focus, use only small fine movements, or you run the risk of hitting the coverslip with the lens and damaging both.

Part II. Observing Prokaryotic Life

Sampling & Inoculation Procedure:

1. 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.

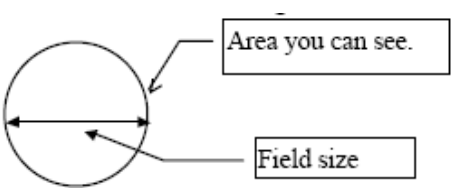
2. Look at prepared slides and images of several prokaryotes and Archaea. Draw what you see, note the size of the organisms. What structures are visible? Do you see nuclei?

Part II. Observing Unicellular Eukaryotic Life: Protists

1. You will be given prepared slides as well as live samples 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 using the prepared slides and then make a wet mount of the live cultures using the directions to follow. Draw, label and measure (see #2) the following organisms and the organelles you can find.

Amoeba proteus	*Euglena sp.*	*Paramecium caudatum*	
Vorticella sp.	*Volvox globator*	*Spirogyra sp.*	Diatoms

2. How big is it? You can use the microscope to measure the size of the cells you are looking at. For each magnification, the table below gives the diameter of the field of view (field size). For the microscopes we use:

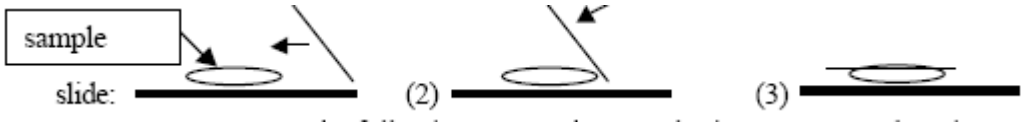


Magnification shown on objective lens	Actual magnification		Field size (millimeters)	Field size (microns (μm))
3.5x	35x	⇒	5.1	5100
10x	100x	⇒	1.8	1800
40x	400x	⇒	0.45	450

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

3. **Preparing a Wet Mount 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:

(a) put a drop of sample on the slide
(b) while holding the coverslip at an angle, slide the edge of the coverslip to the edge of the drop
(c) slowly let the coverslip fall flat:



(d) Place the wet mount on the (dry) microscope stage, and use the slide clamp to hold it. Move the slide holder until an edge of the coverslip is right over the center of the condenser.
(e) Start with the low power (10X) objective, and focus up and down until you see the edge of the coverslip. This puts you in the right focal plane. Adjust the

light strength, the condenser height, and the condenser diaphragm for good contrast.
(f) Move the slide so the drop is under the objective and look for cells. When you find one, put its image in the center of the field of view, and only then rotate the high power (40X) objective into place. Do not use the 100X lens for most purposes because it requires special immersion oil between the lens and the object.

4. **Looking at a mixture of organisms in pond water or a plankton tow from Boston Harbor.** Take a drop of water from the pond or sea water samples and place it on a clean slide with a cover slip. Quickly scan the slide with low power to find an observable living organism, ideally unicellular. Look at it under all magnifications you have on your microscope. Try to determine what type of cell it is by its components and its behavior. Draw your cell or multicellular organism; label all parts you can and determine its size.

Points & Tips to Remember:

To slow down fast-moving protozoa, as you set up the wet-mount:

Take a clean slide & make a small ring about 1/2 inch in diameter of "Proto-slow" – viscous methyl cellulose that slows protozoa because it is thick and difficult for them to swim through. Drop a drop of the protozoa in the middle of the ring. Put on the coverslip and observe. The protozoa will gradually slow down as the proto-slow reaches them.

Compound Microscopes are good for looking at small things. Adjust the magnification by changing the objective lens, grasp the ring above them to rotate, not the objectives themselves. Be sure it clicks solidly when changing magnification or you won't see anything. Helpful quick microscope tips:

1. Use the condenser and diaphragm correctly, too much light removes your image.
2. Do all preliminary focusing under low power.
3. Do not move the stage upward when first getting the object in focus (i.e. beware of smashing slide and lens together).

4. Try to use the microscope with both eyes open - it will seem hard at first, but is easier in the long run.
5. Use the fine adjustment constantly to keep things in focus.
6. Use lens paper to clean the lenses occasionally, you will find that the microscope works best when clean. Do not use any other paper to clean it, only lens paper.
7. You should look at pictures in your text book and online to familiarize yourself with what you will be looking at.

References:

Campbell et al., 1999. pp 502-517,520-543, 583.

Van De Graaff, K. M., and J. L. Crawley, 1996. A Photographic Atlas for the Biology Laboratory. Morton, Englewood CO. pp 24-44.

Brocks JJ, Logan GA, Buick R, Summons RE, 1999. Science 285:1033. Archean molecular fossils and the early rise of eukaryotes.