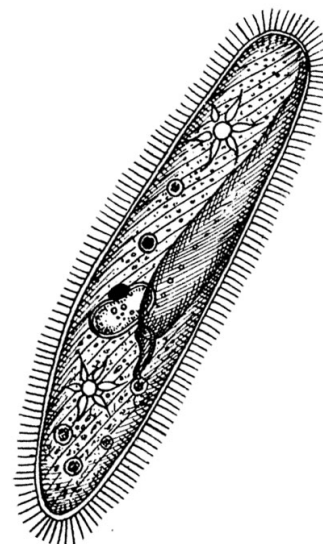


Microscopy and the Diversity of Microorganisms

Today we will learn how to use one of the most important tools a biologist has, the microscope. We will use the microscope to study organisms throughout the semester. All life on Earth depends on the actions of organisms that are too tiny to see without a microscope. These microorganisms are responsible for generating oxygen, releasing nutrients and minerals from dead plants and animals, and serving as the food source at the base of many food chains.

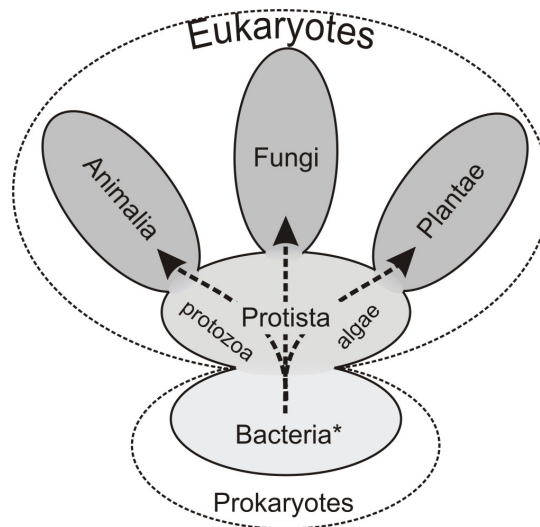


The objectives of this lab exercise are for you to learn to:

- correctly operate a compound microscope, know its basic parts, and be able to put it away properly.
- differentiate between microscopic organisms at the Kingdom level (Bacteria, Protista, Fungi, Animalia).
- make detailed critical observations of microorganisms that reveal the distinguishing properties of each.

Evolutionary relationships

As illustrated in the diagram to the right, biologists classify all life forms into five Kingdoms; unicellular microorganism are found in the **Bacteria***, **Protista**, and **Fungi** kingdoms. Bacteria are believed to be the most primitive cells and the ancestors of all life on earth. The single-celled members of the Protista Kingdom were the first to evolve a complex cytoplasm with a nucleus, and later evolved into multicellular plants (Kingdom **Plantae**) and animals (Kingdom **Animalia**), or fungi (Kingdom **Fungi**)



Prokaryotes vs Eukaryotes

Organisms are subdivided into two groups based upon the structure of the cytoplasm of the cells.

- **Prokaryotes:** simple cytoplasm with few internal structures (No internal organelles, such as a nucleus or mitochondria). Cells are very small. All Bacteria are prokaryotic.
- **Eukaryotes:** more complex cytoplasm with lots of internal organelles. Cells are generally much larger than prokaryotes. All life forms, except bacteria, are eukaryotic.

* In this lab manual we are combining all prokaryotes (Bacteria and Archaea) into a single kingdom called 'Bacteria'. The textbook includes more information about the differences between these two groups.

I. Characteristics of five groups of microorganisms

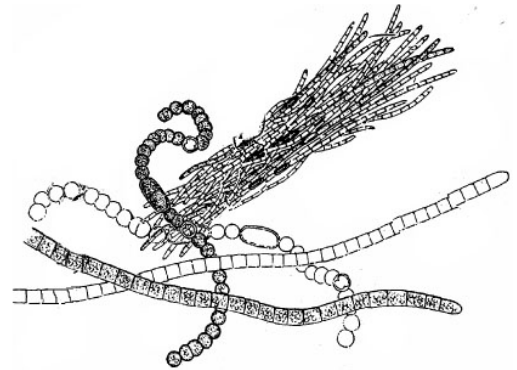
A. Bacteria: Kingdom Bacteria

What are some key characteristics of bacteria?

- Small cell size
- **Prokaryotic cell structure**
- Lack internal organelles
- Common shapes are spheres (cocci) and rods (bacilli)
- Some form long filaments
- Some are photosynthetic (called 'cyanobacteria')

Examples: (both of these are photosynthetic cyanobacteria, and tend to have a bluish-green color)

- *Oscillatoria*: occurs as long filaments, but the individual cells are very hard to discern.
- *Anabaena*: tends to occur in short strands of small spherical cells



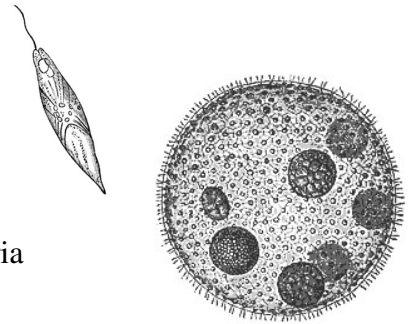
B. Algae: Kingdom Protista

What are some key characteristics of algae?

- **Photosynthetic** and often green due to presence of **chlorophyll**
- Large, **eukaryotic cell structure**
- Internal organelles, including nuclei, chloroplasts and mitochondria
- Can occur as single cells, filaments, or cell colonies

Examples:

- *Euglena*: is an example of a single-celled alga, that is motile by use of thin, hairlike flagella.
- *Spirogyra*: occurs as a long filament of cylindrical cells linked end-to-end. The chloroplast in *Spirogyra* has a fascinating spiral shape. Look for the faint cell walls that separate individual cells of the filament.
- *Volvox*: one of the most beautiful of algae, it occurs as a **colony** of cells arranged in a large hollow ball. The cells possess hair-like "flagella" that beat in unison to propel the colony through the water. As shown in the diagram above, small green *Volvox* cells make up the shell of a hollow sphere, and newly forming 'daughter colonies' appear as dark green cluster within it.



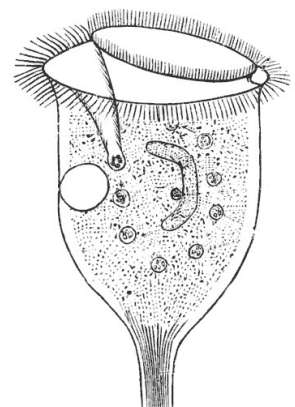
C. Protozoa: Kingdom Protista

What are some key characteristics of protozoa?

- **Heterotrophic** (not green)
- **Eukaryotic cell structure**
- Almost always unicellular
- Some motile using numerous **cilia** (similar to but smaller than flagella)

Examples:

- *Paramecium*: a very large motile protozoan
- *Vorticella*: vase-shaped cell with a long stalk
- *Amoeba*: cells lack a defined shape and move by flowing of cytoplasm



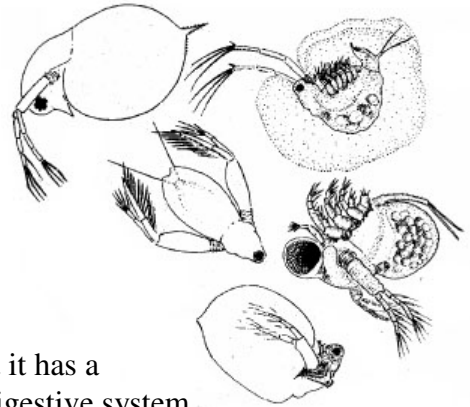
D. Microscopic animals: Kingdom Animalia

What are some key characteristics of microscopic animals?

- **Heterotrophic** (not photosynthetic)
- Multicellular, with tissues, organs and appendages of specific functions.
- Can be as small as single celled protozoa, or just visible to the unaided eye.

Examples:

- **Rotifers:** are no larger than many types of protozoa. Note that it has a distinct mouth opening and a clearly discernable internal digestive system.
- **Daphnia:** related to crustaceans such as crabs and lobsters (notice the hard shell covering much of the body). When examined under the microscope (4x or 10x objective) the remarkable structural complexity of these animals can be seen. The body possesses appendages that aid in swimming and gathering food.



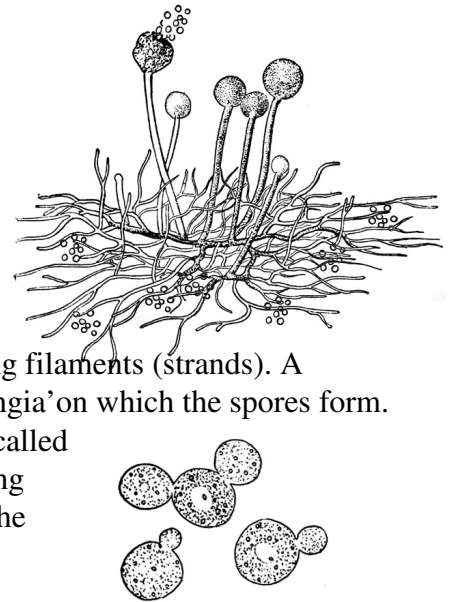
E. Fungi: Kingdom Fungi

What are some key characteristics of fungi?

- **Heterotrophic** (not photosynthetic)
- Occur as long filamentous cells (molds); or spherical cells (yeasts)
- Reproduce with the production of small spherical spores

Examples:

- **Rhizopus:** an example of a mold-type fungus. The cells occur as long filaments (strands). A culture may also contain many spherical spores and stalked-‘sporangia’ on which the spores form.
- **Bakers yeast (*Saccharomyces*):** fungi that form spherical cells are called ‘yeasts’. *Saccharomyces* is the genus used in the baking and brewing industries. They reproduce by forming small cells that bud off of the larger cells.



II. Some basic principles of microscopy

Magnification. Magnification is the apparent increase in the size of an object as viewed through a microscope.

Objective (lens near the specimen) magnifications typically are 4X, 10X and 40X

Ocular (eyepiece) magnification is typically 10x.

Total magnification = (mag. of objective) X (mag. of ocular)

Contrast. Contrast is the difference in intensity between an object and its surroundings. Contrast is adjusted using the **substage diaphragm** (located below the condenser lens). When students have problems locating or focusing on an object, the most common problem is too little contrast due to an improperly adjusted substage diaphragm. In general, the diaphragm should be nearly closed under the scanning (4X) objective, and opened to increase the brightness as you switch to higher power objectives.

Resolution. Resolution is the relative clarity of the microscopic image. The resolution of the image is principally controlled using the **coarse and fine focus knobs**. However, intensity of light, controlled by the **substage diaphragm** (located below the stage), also has a dramatic effect upon the resolution of the image.

Parfocal lenses. The objective lenses on our microscopes are **parfocal**. This means that when a specimen is in sharp focus under one objective lens, another objective lens can be rotated into place without hitting the slide and brought into focus with only minor adjustment of the fine focus knob. **Thus, do not lower the microscope stage when switching between objective lenses.**

Important things to remember when using the microscope

- **Inspect your microscope** before use. Make sure the microscope surfaces are clean of dust and oils.
- Always begin to locate a specimen with the **4x objective** in position with the **substage diaphragm set to the minimum brightness**.
- Set the **light intensity knob** (on the base of the microscope) to position "8".
- Only use the coarse focus knob with the 4X and 10X objectives.
- If you cannot locate the specimen, first try **closing the substage diaphragm** to increase contrast.
- If a specimen is in focus under one objective, switch to another magnification simply by rotating another objective into place. Since these **objectives are parfocal**, only minor adjustment of the focus will be necessary to bring the specimen into sharp focus.
- The substage diaphragm should be adjusted after changing objectives to obtain the best contrast and resolution.
- Never remove the ocular lens from the microscope.

What to do about dirty lenses

A dirty lens will cause distortion of a microscopic image. Dirt or a smudge can occur on the eyepiece, objective, condenser lenses, or the microscope slide itself. It is usually possible to identify the location of the dirt or smudge by following a simple procedure:

1. Rotate the eyepiece. If the dirt is on the ocular, it will also rotate.
2. Change the objective lens. If the distortion disappears, then the objective lens is dirty.
3. If the above steps fail, remove the slide and check the condenser lens and the microscope slide.

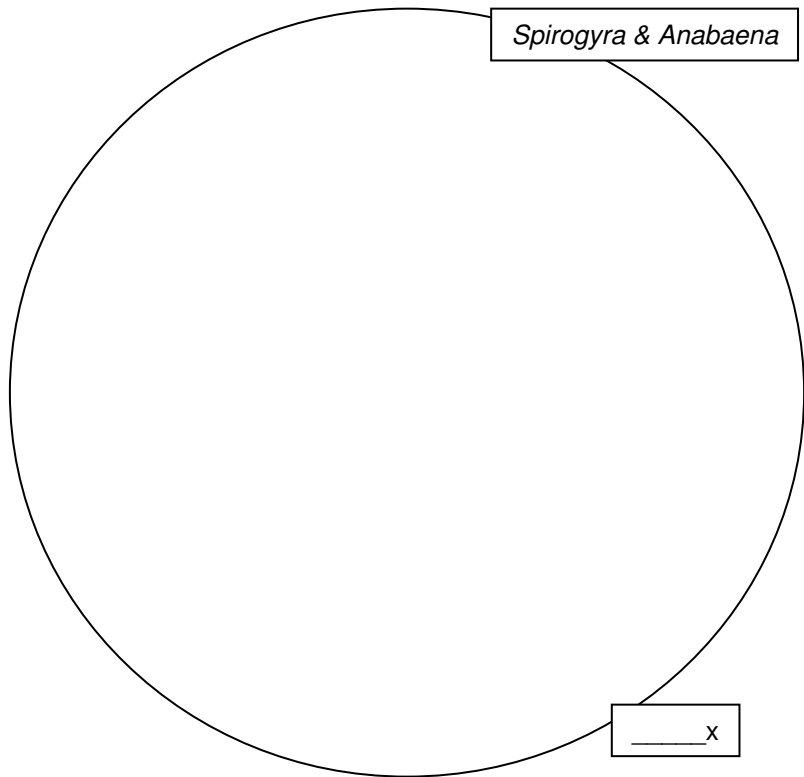
Care must be used when cleaning lenses, and you should ask for the instructor's help before doing so. Only special "lens paper" provided in the lab should be used for this purpose, since the glass of which lenses are made is relatively soft and is easily scratched.

Putting away the microscope

1. Rotate the scanning (4x) objective into position.
2. Remove the slide.
3. Clean the microscope surfaces of any water or dirt.
4. Only clean the lenses if they are known to be dirty, and then only using lens paper.
5. Reposition the slide hold-down clamps on the microscope stage.
6. Place the microscope back in its proper place in the cabinet.

Comparison of the size of bacteria and algae.

Find a location on your slide where both *Spirogyra* and *Anabaena* are visible side-by-side. Viewing under the 40x objective, make a drawing that accurately compares the relative sizes of these organisms. Label an individual cell in each.



B. Observations of protozoa

Make a wet mount of a sample from the mixed culture of protozoa. This mixed culture contains a variety of protozoa as well as rotifers. Find and identify three protozoa indicated by your instructor. Make drawings of the organisms under the magnifications indicated.

<p><i>Paramecium</i></p> <p>100 x</p>	<p>_____</p> <p>____ x</p>	<p>_____</p> <p>____ x</p>
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E. Complete the following tables and questions

Table 1. Characteristics of lenses of your microscope.

Objective	Objective magnification	Ocular magnification	Total magnification
Scanning			
Low		same as above	
High		same as above	

Table 2. Comparison of characteristics of microorganisms.

Organism	Cell structure is eukaryotic or prokaryotic?	Kingdom	Cells are photosynthetic? Yes, No or Sometimes
Algae			
Protozoa			
Bacteria			
Microscopic animals			

- Which of the following is a characteristic of all organisms referred to as "prokaryotic?"
 - are photosynthetic
 - cytoplasm contains organelles
 - have a "filamentous" cell shape
 - cytoplasm lacks internal organelles
 - are visible to the unaided eye
- How do protozoa move?

Match the following terms with the optical property that they describe.

Term

Optical property

- | | |
|--------------------------|---|
| 3. Resolution ____ | A. The distance between the objective lens and the microscope slide. |
| 4. Magnification ____ | B. The apparent increase in size of the object. |
| | C. The actual size of the object on a microscope slide. |
| 5. Working distance ____ | D. The difference in brightness between an object and the background. |
| | E. The relative clarity of a microscopic image. |

6. Why are the objectives of a microscope said to be "parfocal"?

7. Suppose that you began to focus on a microscope slide, but had difficulty locating the specimen. You noticed that the image appeared very bright. Which component of the microscope should you adjust?

8. When you are finished using a microscope during a laboratory exercise, you should turn off the illuminator and rotate the 40x objective into place.

A. True

B. False

9. What property of a microscopic image is described by each of these terms?

a. Magnification:

b. Resolution:

c. Contrast:

10. On the following page, name the indicated structures on the microscope.

A typical compound microscope

