

THE MICROSCOPE

The microscope is microbiology's first and most powerful analytical tool. With the microscope scientists study the world at a level hidden from the human eye. The field of microbiology grew out of the early microscopic observations of scientists such as Antoni van Leeuwenhoek and Robert Hooke. Except for the invention of the microscope, the microbiological basis of disease, even the very existence of microorganisms, may not have been discovered. Improvements in the design and construction of microscopes stimulated many important advances in many fields of biology. Even in this age of advanced biochemical and molecular analysis, the microscope still has central importance in research and clinical laboratories.

This exercise is designed to give you experience in the proper use of a compound microscope. "Proper use" refers to the correct adjustment of the microscope lens and illumination systems, and to the care of this very expensive piece of equipment. Although you may have had previous exposure to microscopy, you are probably unfamiliar with the use of a 100x power **oil immersion lens**, which is very important in the study of bacteria

Most of the lab exercises that you will be performing this semester will be dedicated to studying bacteria, which are classified as **prokaryotic** based on their cell structure. However, many microbiologists study **eukaryotic** microorganisms, such as fungi, algae and protozoa, and many of these organisms are also important agents of disease. Thus, a fundamental skill of all microbiologists is an ability to recognize and distinguish these different types of microorganisms.

One of the most fundamental characteristics of microorganisms is their small size. The most common unit of measure in the field of microbiology is the micrometer (μM ; 10^{-6} meter), although size range of different microorganisms varies widely. So in this exercise you will also learn how to measure the size of microorganism to reinforce the expected sizes for different types of microbes.

Summary of exercise

1. You will learn the various parts of a compound microscope and the principles of obtaining good image quality.
2. You will examine permanently mounted and living specimens of algae, fungi, protozoa and bacteria to learn the distinguishing characteristics of each group of microorganisms.
3. You will learn how to measure the size of microorganisms.

I. USING THE MICROSCOPE

1. Parts of the microscope

Figure 1 presents a drawing of a typical compound microscope with spaces provided for you to write the appropriate name of each part. The parts of the microscope will be reviewed during the lab period. If you are unable to identify any of these parts on the microscope present at your laboratory work station, ask for assistance from the instructor.

2. Controlling the quality of the image

To obtain the best quality image with a microscope, you need to understand certain theoretical and practical aspects of light microscopy. The quality of a microscopic image is determined by optical parameters that include magnification, resolution and contrast. A proper balance to these parameters is essential to obtain good image quality and to reduce eye strain during microscopic observation.

Magnification. The apparent increase in size of an object viewed through a microscope is called "magnification." The **total magnification** of a microscope can be calculated as the **product** of the magnifications of the **objective** lens and the **ocular lens**, or **eyepiece**. The total magnification of a microscope can be varied by rotating into place different objective lenses. The common objective lenses are called **scanning** (4x), **low** (10x), **high dry** (40x), and **oil** (100x). Oculars commonly have magnifications of 10x. Thus, the total magnification of a 10x ocular and a 40x high dry lens would be 400x.

Resolution. Magnification is critical to the ability to see small objects; however, magnification is useless without sufficient "resolution." Resolution refers to the relative clarity of the image. The resolving power is given by the **numerical aperture (NA)** printed on the lens (Figure 1) --higher NA ratings indicate greater resolution.

Immersion oil must be used with an **oil immersion (100x) lens**. A drop of **immersion oil** is placed between the objective lens and the microscope slide when an oil immersion lens is used. Immersion oil has the same refractive index as glass, and its presence eliminates the air/glass interfaces that otherwise are present. **Note: immersion oil can damage other lenses.**

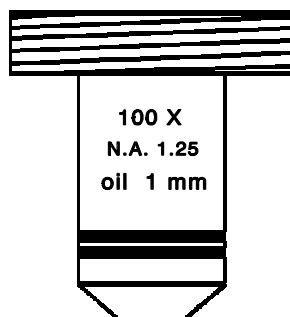


Figure 1. Markings on a typical microscope lens

Meaning

100 X = magnification

N.A. = numerical aperture

colored bands for quick visual identification of lenses.
(Red or black usually signify an oil immersion lens.)

Working distance is the distance between the slide and the objective lens when the specimen is in focus. The objective lenses of most modern compound microscopes are **parfocal**; this means that when a specimen is in sharp focus under one objective lens, a different objective can be rotated into place without hitting the slide. Because of the very small working distance of the high dry (40x) and oil immersion (100x) lenses, these lens should **NEVER** be adjusted with the coarse focus adjustment knob.

Contrast and brightness. Contrast and brightness, adjusted using the **substage diaphragm**, must be balanced to obtain the best quality image. **Brightness** is the amount of light striking the specimen. Unfortunately, too much brightness decreases the contrast. **Contrast** is the difference in intensity between an object and its surroundings. (Seeing a polar bear on a snow field is difficult because there is little contrast.) As a rule, as magnification is increased, brightness also needs to be increased by adjusting the substage diaphragm. However, increasing brightness too much decreases the contrast, and makes viewing more difficult.

3. Procedure for using the microscope

Initial steps

1. Check to make sure that the condenser lens is raised to the highest position.
2. Always begin to locate a specimen by using the low power objective. Using the coarse focus adjustment knob, raise the stage to the position closest to the low power objective lens. The stage should stop before hitting the lens, but be careful nonetheless.
3. The substage diaphragm should be adjusted next to give the minimum comfortable brightness. Under low power, high contrast is usually more important than good resolution.
4. Place the microscope slide within the slide holder of the mechanical stage, and roughly center the object using the mechanical stage adjustment knobs. This is the logical time to make sure that the slide is right-side up. Much time and frustration have been expended, and damage to oil lenses incurred, due to this very simple mistake.
5. Bring the specimen into focus by **SLOWLY** racking the objective lens **UPWARD** away from the slide using the coarse focus knob.
6. Final adjustments should be made using the fine focus adjustment knob and then the substage diaphragm.

Using the high-dry objective:

1. Make sure the object to be viewed is centered in the field of view.
2. **While watching from the side**, rotate the high-dry objective into viewing position.
3. Focus using the fine focus adjustment knob (the objectives are parfocal). **Never use the coarse focus adjustment knob with high-dry and oil lenses.**
4. Adjust illumination with the substage diaphragm.

Using the oil objective:

1. Make sure that the object is centered in the field.
2. Rotate the objective lens carrier so that it is positioned between the high-dry and oil immersion lenses.
3. Place a drop of immersion oil over the area of the slide to be examined.
4. **While watching from the side**, rotate the oil immersion lens into viewing position.
5. Adjust the illumination and fine focus as required. **Never use the coarse focus adjustment knob with high-dry and oil lenses.**

Oil need not be wiped off the oil immersion lens between specimens, but **MUST** be removed thoroughly using lens paper at the end of the period.

4. Putting away the microscope

1. Rotate the low power (10x) objective into position.
2. Remove the slide.
3. Clean the microscope surfaces free of water and dirt.
4. Clean the oil immersion lens free of immersion oil. Only clean the other lenses if they are dirty, and then only using lens paper.
5. Recenter the mechanical stage.
6. Place the microscope back in the cabinet and lock.

5. Cleaning microscope lenses

Dirt or smudges on different lenses can be located by following the following steps:

1. Rotate the eyepiece. If the dirt is on the ocular, it will rotate also.
2. Rotate in a different objective lens. If the distortion disappears, then the objective lens is dirty.
3. Move the slide. Does the distortion move?
4. Check the surfaces of the condenser lens and the microscope slide.

Utmost care must be used when cleaning lenses. You should ask for the instructor's help before doing so. Use only the cleaning fluid and "lens paper" provided in the lab for this purpose since the lens glass is relatively soft and is easily scratched. 'Kim wipes' are not lens paper and should never be used to clean a lens.

II. CALIBRATING AN OCULAR SCALE

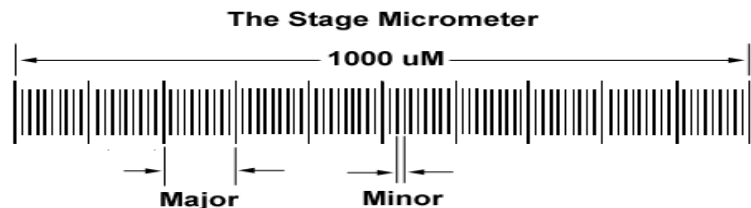
Measurements of microorganisms can be made using a circular glass disk, called an **ocular scale** on which is etched a small ruler, that is placed inside the eyepiece housing. However, **an ocular scale must be calibrated for each objective lens since the apparent distance spanned by the lines will depend upon the magnification of the specimen.** The rulings of the ocular scale are calibrated using a **stage micrometer**, a small ruler etched upon a microscope slide. Ocular scales and stage micrometers are very expensive, so handle them very carefully.

Objective: To calibrate the ocular scale for the 10X, 40X and oil immersion lenses of your microscope and record the calibration values in Table 2.

1. Examine the stage micrometer and ocular scale

A. The Stage micrometer.

Place the stage micrometer on your microscope and focus on it with the 10X objective lens. The stage micrometer is essentially a very small ruler, 1.0 mm (1000 μm) in length. Notice that the stage micrometer scale is divided into 100 small units.



What is the distance between each of the small units? $1000 \mu\text{m} \div 100 = \underline{\hspace{2cm}} \mu\text{m}$

With the scale centered in the field of view, rotate the high-dry objective into place and refocus. What has happened to the image of the scale? Under this magnification:

What is the distance between each of the small units? $1000 \mu\text{m} \div 100 = \underline{\hspace{2cm}} \mu\text{m}$

The answer to both sets of questions are same; why? **Key concept:** The distance between the units of the stage micrometer is always the same despite changes in magnification.

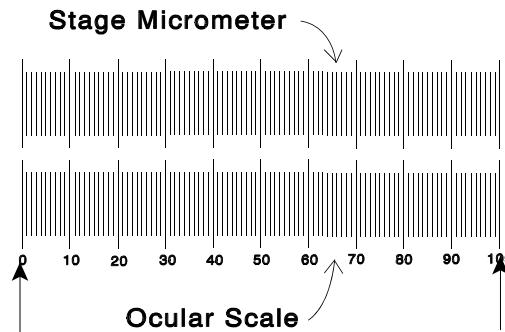
B. The ocular scale

Now place the ocular scale in the eye piece housing as demonstrated by the instructor during the lab period. Identify the ocular scale by slowly rotating the eyepiece. Which scale moves? The ocular scale also resembles a small ruler with 100 divisions. If the scale appears backwards when viewed through the microscope, remove it and flip it over. As noted above, **an ocular scale must be calibrated for each objective lens since the apparent distance spanned by the lines will depend upon the magnification of the specimen.**

2. Calibrate the ocular scale under low power (10x)

To calibrate the ocular scale under low power, align the two scales approximately as shown below.

Scales as viewed through 10x objective



Under the 10X objective, lines of the ocular scale align exactly with those of the stage micrometer at the "0" and "100" positions.

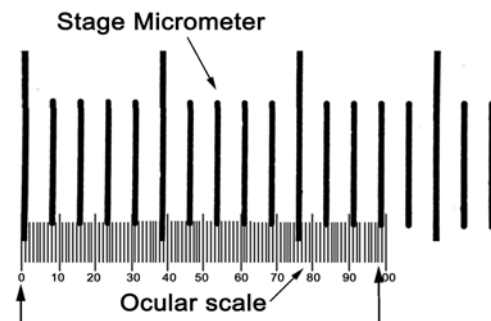
Calibrate the ocular scale for the 10X objective by following the following steps:

- Between the "0" and "100" positions, there are _____ divisions on the ocular scale.
- Between these same two positions there are _____ units on the stage micrometer?
- Since each unit of the stage micrometer = 10 μm , the distance along the stage micrometer equals: (b) $\times 10\mu\text{m} =$ _____ μM . [Why do we multiply by 10?]
- Thus, under 10X, the distance between each division of the ocular scale equals:
(c) \div (a) = _____ μM . Write this number in Table 2.

3. Calibrate the ocular scale under high-dry (40x)

Now rotate the high-dry objective into place, and focus. What has happened to the relative sizes of the ocular and stage scales?

Scales as viewed under higher magnification



To calibrate the 40X objective, the same procedure is followed; the scales are aligned at the '0' lines, and then we find the position furthest to the right where the scales are also aligned. In the above diagram, the 2 scales also overlap exactly at line 13 of the stage micrometer and line 98 of the ocular scale. **For your microscope, different lines will overlap.**

To calibrate your ocular scale under the 40X objective, position the stage micrometer so that the "0" lines of the two scales are aligned as shown above, and then find the position furthest to the right where the scales are also aligned.

For your microscope, the two scales align at the "0" and the _____ positions.

- Between these positions, there are _____ divisions on the ocular scale.
- Between these same two positions there are _____ units on the stage micrometer?
- Since each unit of the stage micrometer = 10 μM , the distance along the stage micrometer equals: (b) x 10 μm = _____ μM .
- Thus, under 40X the distance between each division of the ocular scale equals:
(c) \div (a) = _____ μM . Write this number in Table 2.

4. Calibrate the ocular scale under oil immersion (100x)

To calibrate your ocular scale under the 100X objective, follow the same procedure as described above for the 40X objective: First position the stage micrometer so that the "0" lines of the two scales are aligned as shown above, and then find the position furthest to the right where the scales are also aligned. Because of the magnification, you will need to align the lines of the ocular scale with either the right or left edge of the lines on the stage micrometer.

For your microscope, the two scales align at the "0" and the _____ positions.

- Between these positions, there are _____ divisions on the ocular scale.
- Between these same two positions there are _____ units on the stage micrometer?
- Since each unit of the stage micrometer = 10 μM , the distance along the stage micrometer equals: (b) x 10 μm = _____ μM .
- Thus, under 100X, the distance between each division of the ocular scale equals:
(c) \div (a) = _____ μM . Write this number in Table 2.

Carefully remove the stage micrometer, wipe it clean of oil, and place it back into its protective case.

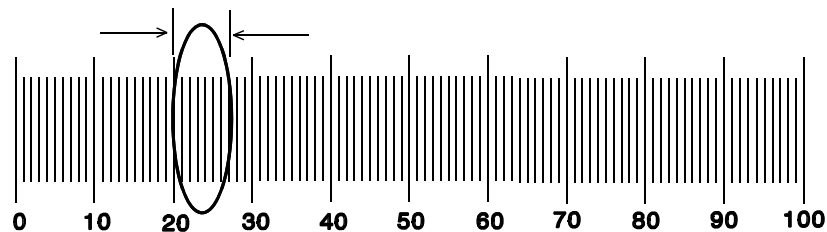
III. Studying the characteristics of microorganisms

1. How to prepare a wet mount

1. Place a small sample of the microorganism culture in the center of a clean microscope slide. If the sample is dry (e.g., a fungal culture) then place on the slide a drop of distilled water in which the sample can be suspended.
2. Add a cover slide over the specimen. Do not place so much liquid that the cover slide "sloshes" around over the microscope slide.
3. Make observations under low and high dry objectives, but **do not attempt** to use the oil immersion lens with these wet mounts.

2. How to measure the size of microorganisms

The size of microorganisms can be measured by using the calibrated ocular scale. With the ocular scale in place, focus on the organism to be measured, and position it under the scale, as shown below.



The ocular scale can be rotated as necessary to align it with the dimension of the organism that you wish to measure. In the above figure, the diameter of the cell, measured under high dry, is 7 units. If the calibrated distance between each ruling of the ocular scale under the high-dry lens is $5 \mu\text{M}$, the diameter of this cell equals $5 \mu\text{M} \times 7 = 35 \mu\text{M}$.

3. Identify and measure the sizes of the different types of microorganisms as described below. Record your results in tables 3 - 7.

Supplies

mixed culture of algae and cyanobacteria
mixed culture of protozoa
permanently mounted bacteria

plates of *Rhizopus*
Yeast suspension

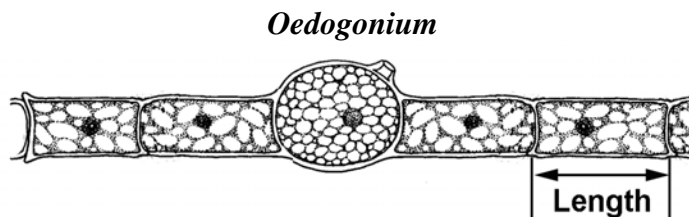
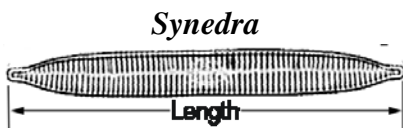
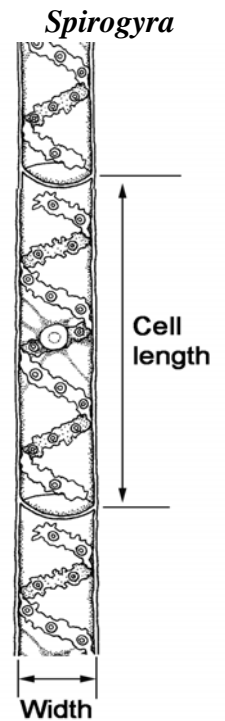
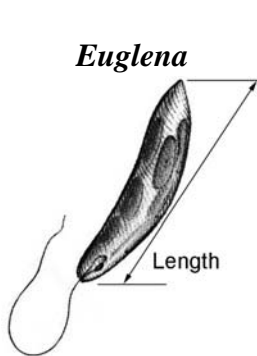
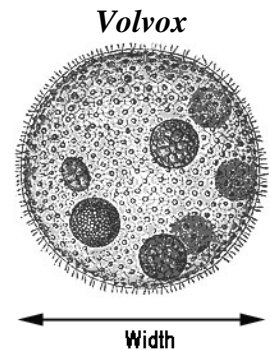
A. Algae & Diatoms: Kingdom Protista (use 10X & 40X objectives)

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

Find and make measurements of the following organisms (listed approximately from largest to smallest).

- **Volvox**: a colony of cells arranged in a large hollow ball. Newly forming 'daughter colonies' appear as dark green clusters within.
- **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.
- **Oedogonium**: like *Spirogyra*, a filament of cylindrical cells but with normal shaped chloroplasts. Look for enlarged egg and sperm producing cells.
- **Scenedesmus**: Cells (typically four, but can be fewer or more) arranged as a flat plate with spines extending from outer corners.
- **Euglena**: is an example of a single-celled alga, that is motile by use of thin, hairlike flagella.
- **Diatoms**: such as *Synedra* and *Cyclotella*, are known for the intricate structure of their highly siliceous cell wall.



B. Protozoa: Kingdom Protista (use 10X & 40X objectives)

What are some key characteristics of protozoa?

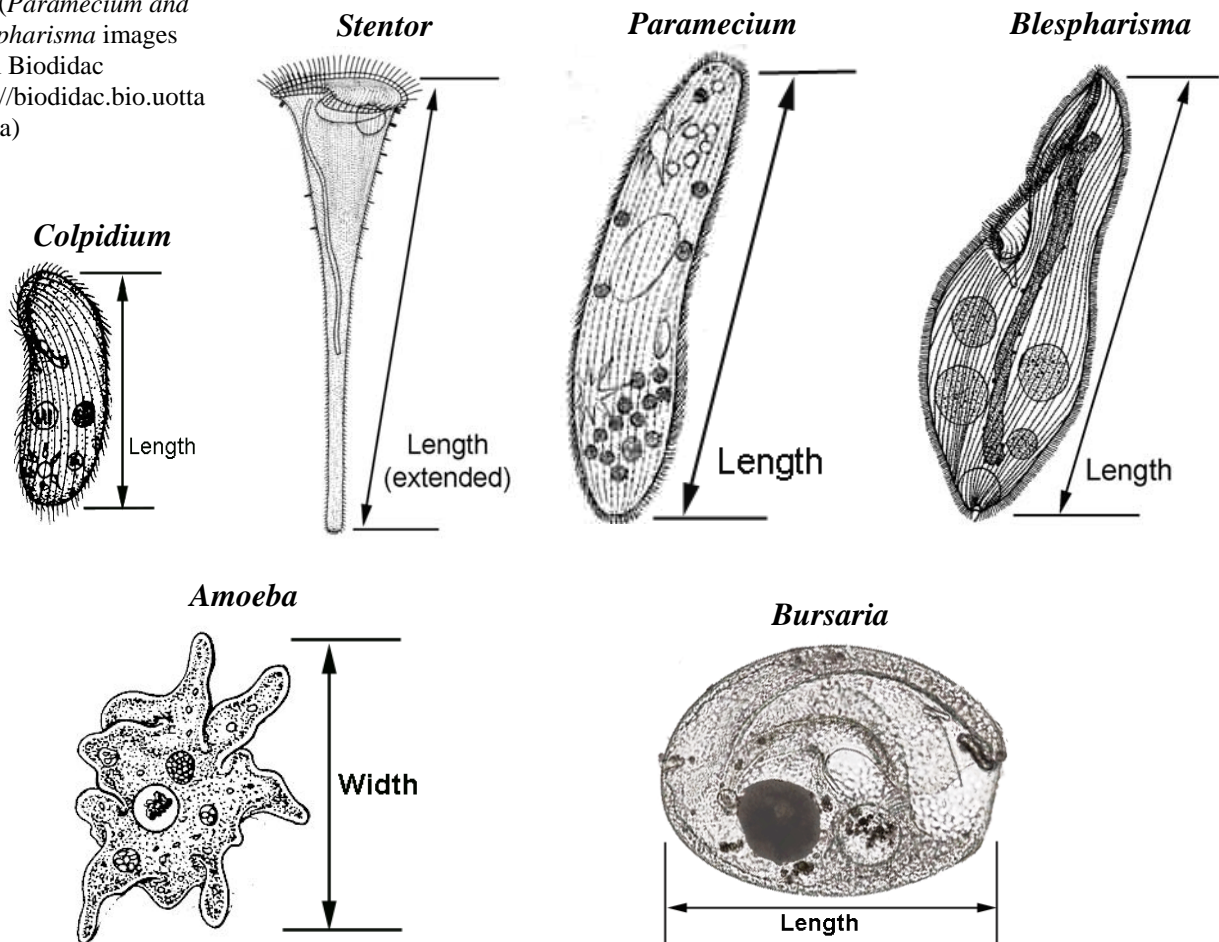
- **Heterotrophic** (not green)
- **Eukaryotic** cell structure
- Almost always unicellular
- Some motile using numerous cilia or a few flagella

Examples:

- Large ciliated protozoans, such as
 - *Blespharisma* has a reddish tint and a one end that is distinctly more narrow and elongated, whereas...
 - *Paramecium* lacks coloration and is more symmetrical, and...
 - *Bursaria* is the most rounded in shape, but with a flattened end.
- *Colpidium* is a relatively small protozoan, and may be very abundant.
- *Vorticella and Stentor*: vase-shaped cells with a long stalks, often attached to a surface. *Vorticella* has a distinct cup-shaped cell body attached to a retractable stalk.
- *Amoeba*: cells lack a defined shape and move by flowing of cytoplasm into extensions called pseudopods



(*Paramecium* and *Blespharisma* images from Biodidac <http://biodidac.bio.uottawa.ca>)



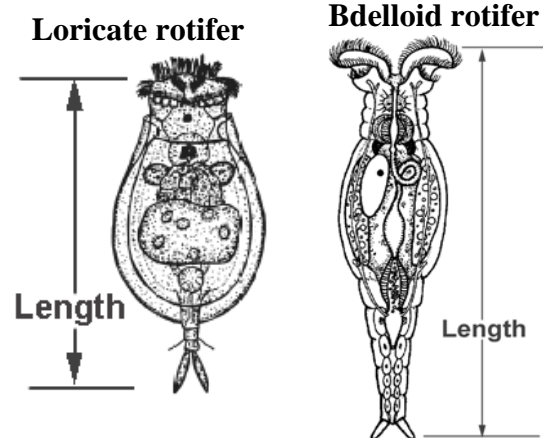
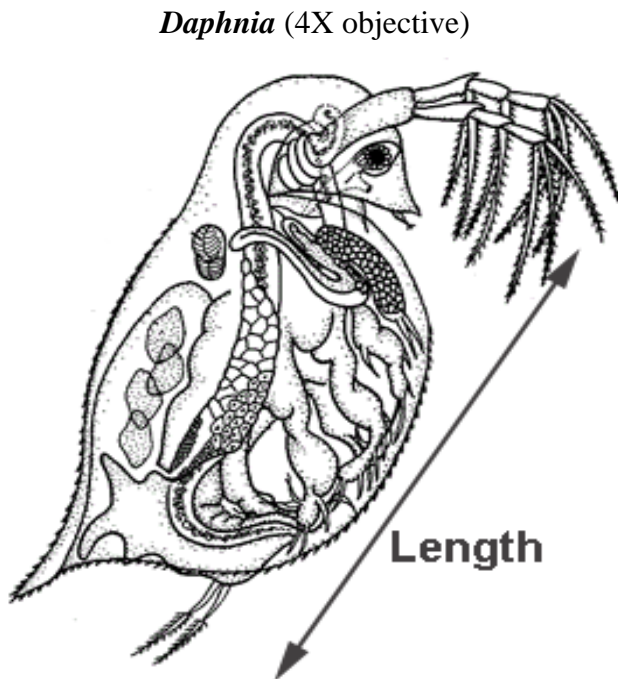
C. Microscopic animals: Kingdom Animalia (use 10X & 40X objectives)

What are some key characteristics of microscopic animals?

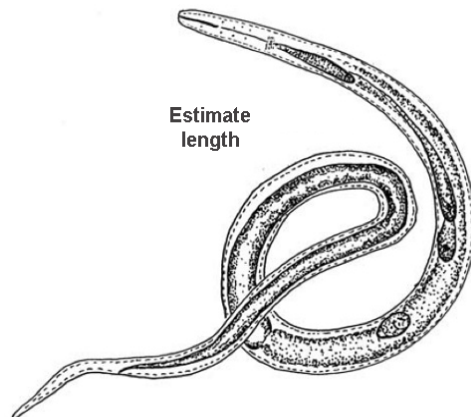
- **Heterotrophic**
- **Multicellular**, with tissues, organs and appendages of specific functions.
- Can be as small as single celled protozoa, or just visible to the unaided eye.

Examples:

- **Rotifers:** are no larger than many types of protozoa. Bdelloid rotifers have a flexible outer layer, which allows them to stretch and retract while the clasping ‘toe’ is attached to debris. Loricated rotifers have a more rigid outer covering, and often have a pair of longer spine-like toes. (Bdelloid Rotifer image is from Biodidac <http://biodidac.bio.uottawa.ca>)
- **Nematode:** are long, worm-like animals that are very common in water and soils. Some are pathogens
- **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.



Nematode (4X or 10X objective)



D. Fungi: Kingdom Fungi (use 10X and 40X objectives)

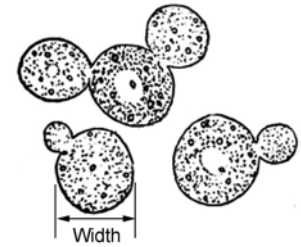
What are some key characteristics of fungi?

- **Heterotrophic** (not photosynthetic)
- Occur as long filamentous cells (**molds**); or spherical cells (**yeasts**)
- Reproduce with the production of small spherical **spores**
- Molds grow as long filaments or **hyphae** (sing. hypha) that often grow into a visible mass called a **mycelium**.

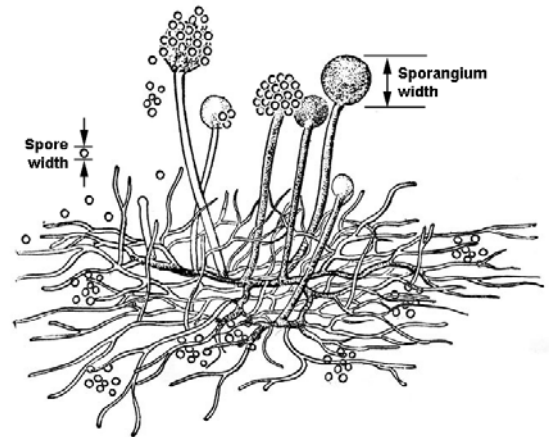
Examples:

- **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



Rhizopus



E. Bacteria: Domain Bacteria (Eubacteria)

What are some key characteristics of bacteria?

- Small cell size
- Prokaryotic cell structure
- Commons shapes are spheres (cocci) and rods (bacilli)
- Some form long filaments
- Cyanobacteria are photosynthetic

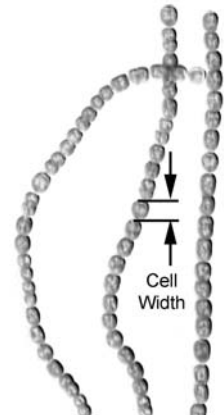
Cyanobacteria:(use 40X objective)

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

Permanently mounted specimens (use oil lense)

- Mixed culture of cocci and bacilli,

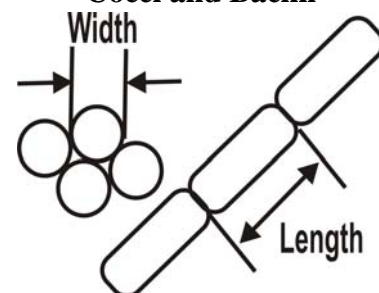
Anabaena



Oscillatoria



Cocci and Bacilli



Names: _____

Figure 2. Parts of a compound microscope. Label the following components of a compound microscope: fine focus adjustment knob, coarse focus adjustment knob, illuminator, illuminator adjustment knob, objective lens, ocular lens, condenser lens, condenser adjustment knob, and substage diaphragm.

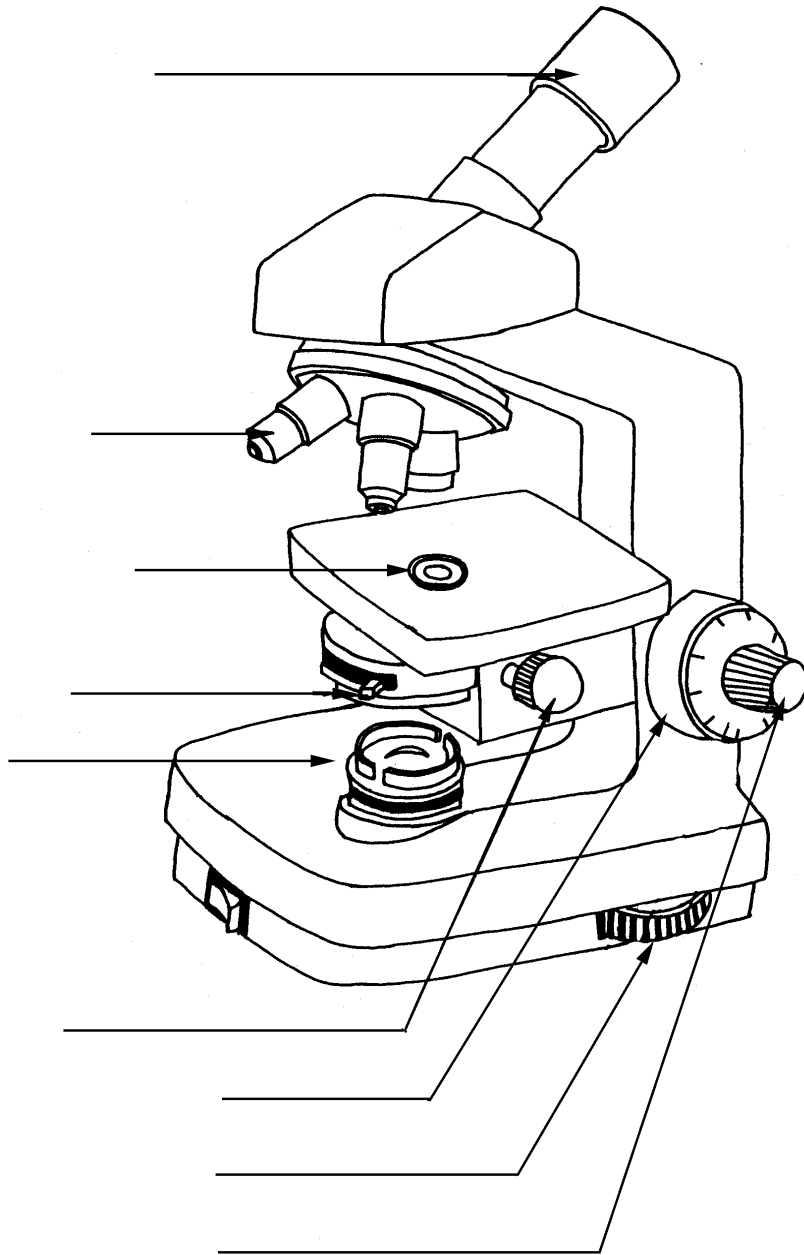


Table 1. Characteristics of the microscope and objective lenses.

Objective	Objective Magnification	Ocular Magnification	Total Magnification
Low Power			
High Dry			
Oil			

Table 2 Calibration of the ocular scale.

	On the ocular scale:	On the stage micrometer:	
	How many units are between the two aligned positions? (a)	How many micrometers are between the two aligned positions? (c)	Calibrated length of each unit of the ocular scale (c) ÷ (a)
Objective lens			
Low power		μM	μM
High-dry		μM	μM
Oil immersion		μM	μM

Complete the following sentences.

- _____ is the difference in brightness between the specimen and the background.
- _____ is the relative clarity of the image.
- _____ is the distance between the slide and the bottom of the objective lens.
- _____ is the apparent increase in size of the specimen.
- _____ means that when a specimen is in sharp focus under one objective lens, a different objective can be rotated into place without hitting the slide.
- Opening the substage diaphragm increases the _____ but decreases the _____.

Observations of Microorganisms

Table 3. Identify and measure at least 3 different species of algae

Organism name	Objective used	Dimension measured	Measurement in ocular scale units (A)	Ocular scale calibration (B)	Calculated dimension (A x B)
<i>Spirogyra</i>		cell length		μM	μM
<i>Spirogyra</i>		cell width		μM	μM
				μM	μM
				μM	μM
				μM	μM
				μM	μM

Table 4. Identify and measure at least 3 different species of protozoa

Organism name	Objective used	Dimension measured	Measurement in ocular scale units (A)	Ocular scale calibration (B)	Calculated dimension (A x B)
				μM	μM
				μM	μM
				μM	μM
				μM	μM

Describe two important characteristics that distinguish algae and protozoa?

- 1.
- 2.

Table 5. Identify and measure 2 species of microscopic animals

Organism name	Objective used	Dimension measured	Measurement in ocular scale units (A)	Ocular scale calibration (B)*	Calculated dimension (A x B)
				μM	μM
				μM	μM
				μM	μM

*Note: the calibration of the ocular scale for 4X objective will be 10X that of the 40X objective.

Describe two important characteristics that distinguish microscopic animals and protozoa?

- 1.

- 2.

Table 6. Record your measurements of Fungi

Organism name	Objective used	Dimension measured	Measurement in ocular scale units (A)	Ocular scale calibration (B)	Calculated dimension (A x B)
<i>Rhizopus</i> sporangium				μM	μM
<i>Rhizopus</i> spore				μM	μM
<i>Rhizopus</i> hypha width				μM	μM
Yeast width				μM	μM

Describe two important characteristics that distinguish fungi and algae?

- 1.

- 2.

Table 7. Record your measurements of Bacteria

Organism name	Objective used	Dimension measured	Measurement in ocular scale units (A)	Ocular scale calibration (B)	Calculated dimension (A x B)
<i>Oscillatoria</i> *	40X			μM	μM
<i>Anabaena</i> *	40X			μM	μM
Bacillus	oil	length		μM	μM
Coccus	oil	width		μM	μM

* Measure these during next week's cytology cytology lab

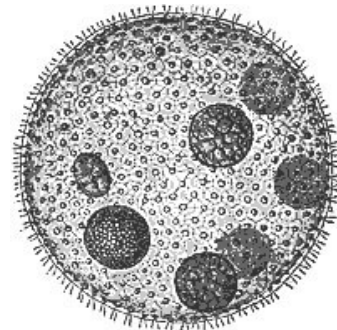
Describe two important characteristics that distinguish fungi and algae?

- 1.

- 2.

In this diagram of *Volvox*, use arrows to identify:

- individual cell
- daughter colony



If the following diagram represents the size of a *Spirogyra* cell, draw next to it the relative size of a cell of *Anabaena*.



Table 4. Comparison of characteristics of microorganisms.

Organism	Cells are eukaryotic or prokaryotic?	Kingdom	Size range	Cells are photosynthetic? Yes, No or Sometimes
Algae				
Protozoa				
Fungi				
Bacteria				
Microscopic animals				