LAB EXERCISE: Microscopy and the Cell

Laboratory Objectives

After completing this lab topic, you should be able to:

1. Identify the parts of compound and stereoscopic microscopes and be proficient in their correct use in biological studies.
2. Describe procedures used in preparing materials for light microscopy.
3. Describe features of specific cells and determine characteristics shared by all cells studied.
4. Discuss the evolutionary significance of increasing complexity from unicellular to multicellular organization and provide examples from the lab.

Introduction

According to cell theory, the cell is the fundamental biological unit, the smallest and simplest biological structure possessing all the characteristics of the living condition. All living organisms are composed of one or more cells, and every activity taking place in a living organism is ultimately related to metabolic activities in cells. Thus, understanding the processes of life necessitates an understanding of the structure and function of the cell.

Given the fundamental role played by cells in the organization of life, one can readily understand why the study of cells is essential to the study of life. Cells, however, are below the limit of resolution of the human eye. We cannot study them without using a microscope. The microscope has probably contributed more than any other instrument to the development of biology as a science. Two types of microscopes are named according to the source of illumination used: light microscopes and electron microscopes. We will be using light microscopes exclusively in our study of cells, and we will view electron micrographs of cell structures not visible with the light microscope.

The microscope is designed to make objects visible that are too difficult or too small to see with the unaided eye. There are many different kinds of light microscopes, including phase-contrast, darkfield, polarizing, and UV. These differ primarily in the source and manner in which light is passed through the specimen to be viewed.

Place the microscope on your desk with the arm of the microscope nearest you. With the aid of the illustration, locate the various parts of the instrument. Learn to understand the function of each part. You should also become familiar with the terms frequently used in microscopy, some of which are described in the following.

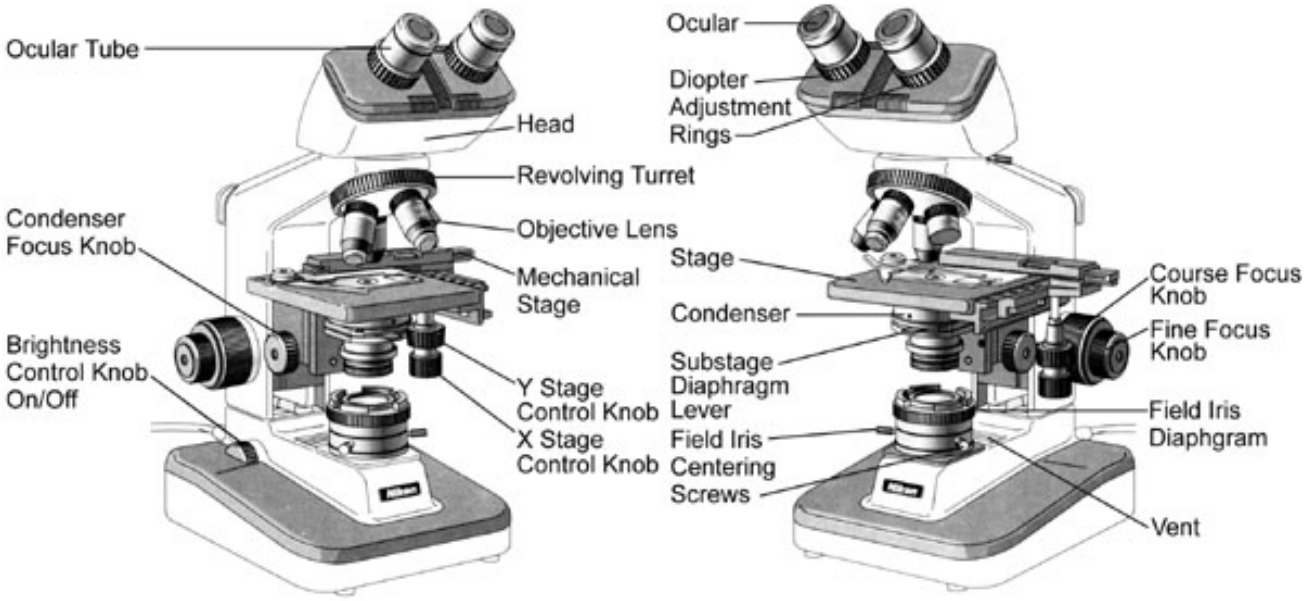

Figure 1. Compound light microscope.

Microscope parts and their functions

Eyepiece – Topmost series of lenses through which a specimen is viewed.
Body Tube – Holds nosepiece at one end and the eyepiece at the opposite, conducts light rays.
Nosepiece – Revolving device; holds objectives for interchange.
Low Power Objective – 10 X lens holder; shortest lens holder which should always be in position at the beginning of a lab and at the finish of the lab.
High Power Objective – 43 X lens holder.
Oil Immersion Objective – 97 X lens holder; used with oil in a special technique.
Stage – Holds and supports microscope slides.
Arm – Supports upper parts and provides carrying handle.
Coarse Adjustment Knob – Knob used to move the body tube for approximate focus (use only with low power objective.)
Fine Adjustment Knob – Knob used to move the body tube for minute adjustment in focusing.
Inclination Joint – Permits tilting of parts attached to arm; should be used only with permission of the instructor.
Pillar – Supports microscope arm and its attached parts.
Base – Bottom portion that supports the microscope; provides carrying support.
Condenser – Concentrates and directs light through specimen.
Iris Diaphragm – Controls area of illumination through the condenser.

To determine magnification:
If the eyepiece is marked 10 X and the objective in position is marked 10 X, the specimen is magnified 100 times (10 X 10). If the eyepiece is 10 X and the objective in position is 43 X, then the specimen is magnified 430 times (10 X 43 = 430).

Working distance:
The distance between the specimen and the objective lens is the working distance. This distance decreases as the magnification is increased. Therefore, the greater a specimen is magnified, the greater is the chance for the specimen and the lens of the objective coming into contact. This requires that the student exercise care when the high power objective or the oil immersion objective is in position. On a commercially prepared slide this could mean the difference between a broken slide and one that is kept in good condition.

Stains:
Stain are used to color so that a specimen being viewed with light coming through it will appear more distinct. Obviously, a specimen which is naturally colored will not need any additional staining. The most commonly used stain in this laboratory text is diluted iodine. Iodine is a very useful stain because it is easy to prepare, stores for long periods without deterioration, and it colors the specimens which will be used in these exercises. However, iodine is toxic and will kill most forms of life. There is no danger to the student in these procedures ordinarily. Some care is necessary in handling the stain because it will not wash out of many types of clothing.
VITAL STAINS are also used to stain and color certain specimens in a natural state. The advantage of this type of stain is that it either does not kill or kills slowly so that one may observe the living organism. In most instances the vital stain is no more harmful than food coloring.

Methyl cellulose or protoslo:
Methyl cellulose is a harmless material which is used to slow some fast-moving microscopic forms.

EXERCISE 1. Basic Microscope Techniques

Materials

clear ruler
coverslips
prepared slides: letter and crossed thread
Kimwipes®
lens paper
dropper bottle with distilled water
blank slides

Introduction

In this exercise, you will learn to use the microscope to examine a recognizable object, a slide of the letter *e*. Practice adjusting your microscope to become proficient in locating a specimen, focusing clearly, and adjusting the light for the best contrast.

Procedure

1. Clean microscope lenses.

Each time you use the microscope, you should begin by cleaning the lenses. Using lens paper moistened with a drop of distilled water, wipe the ocular, objective, and condenser lenses. Wipe them again with a piece of dry lens paper.

***Use only lens paper on microscope lenses. Do not use Kimwipes®, tissues, or other papers.**

2. Adjust the focus on your microscope:

a. Plug your microscope into the outlet.

b. Turn on the light. Adjust the light intensity to mid-range if your microscope has that feature.

c. Rotate the 4 X objective into position using the revolving nosepiece ring, not the objective itself.

d. Take the letter slide and wipe it with a Kimwipe® tissue.

Each time you study a prepared slide, you should first wipe it clean. Place the letter slide on the stage, and center it over the stage opening.

***Slides should be placed on and removed from the stage only when the 4 X objective is in place. Removing a slide when the higher objectives are in position may scratch the lenses**.

e. Look through the ocular and bring the letter into rough focus by slowly focusing upward using the coarse adjustment.

f. For binocular microscopes, looking through the oculars, move the oculars until you see only one image of the letter *e*. In this position, the oculars should be aligned with your pupils. In the margin of your lab manual, make a note of the **interpupillary distance** on the scale between the oculars. Each new lab day, before you begin to use the microscope, set this distance.

g. Raise the condenser to its highest position, and fully close the iris diaphragm.

h. Looking through the ocular, slowly lower the condenser just until the graininess disappears. Slowly open the iris diaphragm just until the entire field of view is illuminated. This is the correct position for both the condenser and the iris diaphragm.

i. Rotate the 10 X objective into position.

j. Look through the ocular and slowly focus upward with the coarse adjustment knob until the image is in rough focus. Sharpen the focus using the fine adjustment knob.

***Do not turn the fine adjustment knob more than two revolutions in either direction. If the image does not come into focus, return the 10 X and refocus using the coarse adjustment**.

k. You can increase or decrease the contrast by adjusting the iris diaphragm
opening. Note that the maximum amount of light provides little contrast. Adjust the aperture until the image is sharp.

l. Move the slide slowly to the right. In what direction does the image in the ocular move?

m. Is the image in the ocular inverted relative to the specimen on the stage?

n. Center the specimen in the field of view; then rotate the 40 X objective into position while watching from the side. *If it appears that the objective will hit the slide, stop and ask for assistance.*

***Most of the microscopes have parfocal lenses, which means that little refocusing is required when moving from one lens to another. If your scope is not parfocal, ask your instructor for assistance.**

o. After the 40 X objective is in place, focus using the fine adjustment knob.

***Never focus with the coarse adjustment knob when you are using the high power objective.**

p. The distance between the specimen and the objective lens is called the **working distance**. Is this distance greater with the 40 X or the 10 X objective?

***Answer the appropriate questions at the end of the microscopy exercise.**

Determine spatial relationships.

The depth of field is the thickness of the specimen that may be seen in focus at one time. Because the depth of focus is very short in the compound microscope, focus up and down to clearly view all planes of a specimen.

a. Rotate the 4 X objective into position. Take a slide of crossed threads, wipe it with a Kimwipe, and place the slide on the stage. Center the slide so that the region where the two threads cross is in the center of the stage opening.

b. Focus upward (move the stage up) with a coarse adjustment until both threads are just out of focus. Slowly focus down using the fine adjustment. Which thread comes into focus first? Is this thread lying under or over the other thread?

c. Rotate the 40 X objective into position and slowly focus up and down, using the fine adjustment. Which thread comes into focus first? Is this thread lying under or over the other thread?

***Answer the appropriate questions at the end of the microscopy exercise.**

EXERCISE 2. The Organization of Cells

In this exercise, you will examine the features common to all eukaryotic cells that are indicative of their common ancestry. However, you will observe that all cells are not the same. Some organisms are **unicellular** (single-celled), with all living functions (respiration, digestion, reproduction, and excretion) handled by that one cell. Others form random, temporary **aggregates**, or clusters, of cells. Clusters composed of a consistent and predictable number of cells are called **colonies**. **Multicellular** organisms have large numbers of cells with specialized structure and function, and no one cell can exist successfully by itself.

Unicellular Organisms

Materials

microscope slides	coverslips
Amoeba culture	*Paramecium* culture

Procedure

1. Prepare wet mounts of both amoeba and paramecium samples.

a. Using a clean pipette (it is important not to interchange pipettes between culture dishes), transfer a drop with several amoebas or paramecia to your microscope slide. To do this, squeeze the pipette bulb *before* you place the tip under the surface of the water. Disturbing the culture as little as possible, pipette a drop of water with debris from the *bottom* of the culture dish.

b. Cover your preparation with a clean coverslip.

c. Under low power on the compound scope, scan the slide to locate an amoeba. Center the specimen in your field of view, then switch to higher powers.

d. Identify the following structures in the amoeba:

Cell membrane is the boundary that separates the organism from its surroundings.
Ectoplasm is the thin, transparent layer of cytoplasm directly beneath the cell membrane.
Endoplasm is the granular cytoplasm containing cell organelles.
The **nucleus** is the grayish, football-shaped body that is somewhat granular in appearance. This organelle, which directs the cellular activities, will often be seen moving within the endoplasm**.**
Contractile vacuoles are clear, spherical vesicles of varying sizes that gradually enlarge as they fill and then empty its contents into the surrounding environment. These vacuoles serve an excretory function for the amoeba.
Food vacuoles are small, dark, irregularly shaped vesicles within the endoplasm. They contain undigested food particles.
Pseudopodia ("false feet") are fingerlike projections of the cytoplasm. They are used for locomotion as well as for trapping and engulfing food in a process called **phagocytosis**.

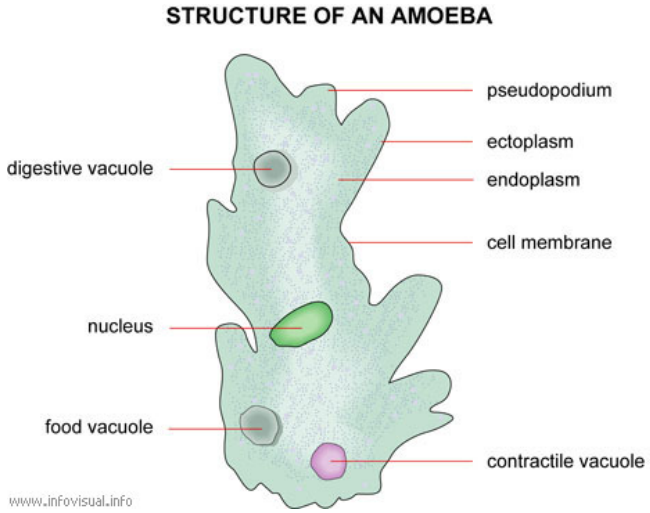

Figure 3. *Amoeba*. An *Amoeba* moves using pseudopodia.

d. Follow the same procedure for *Paramecium* and identify the following structures:

Cilia: minuscule cilia that envelop the paramecium and are used for locomotion.
Contractile vacuole: cavity of the paramecium that is able to contract.
Food vacuole: cavity of the paramecium responsible for digestion.
Micronucleus: one of the less important central organelles of a paramecium.
Oral groove: canal of the paramecium used to ingest nutrients.
Gullet: cavity of the pharynx.
Ectoplasm: vitreous superficial layer of a paramecium.
Endoplasm: central part of a paramecium.
Large nucleus: the most important central organelle of a paramecium.
Canals of contractive vacuole: division of the contractile cavity of a paramecium.
Trochocyst: root of a vibrative cilium of a paramecium.

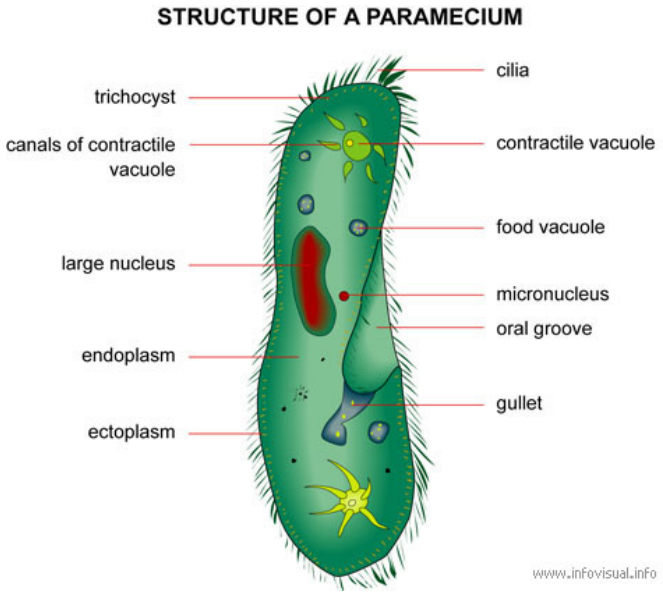

Figure 4. ***Paramecium.***

***Draw examples of what you see for both *Amoeba* and *Paramecium* cultures.**

Aggregate and Colonial Organisms

Materials
microscope slides
coverslips
Protococcus and *Volvox* cultures

Introduction
Unlike unicellular organisms, which live independently of each other, colonial organisms are cells that live in groups and are to some degree dependent on one another. The following organisms show an increasing degree of interaction among cells. *Protococcus* (Figure 5) is a terrestrial green alga that that forms loose aggregates on the bark of trees grows on the north sides of trees and is often referred to as "moss". The size of the cell groupings is random, and there are no permanent connections between cells. Each cell is surrounded by a cell membrane and an outer **cell wall**. *Volvox* (Figure 6) is an aquatic green alga that also is common in aquaria, ponds, and lakes. In this complex colony, the individual cells are interconnected by cytoplasmic strands to form a sphere. Small clusters of cells, called daughter colonies, are specialized for reproduction that the cells of this organism form a large complex colony. Approximately 500 to 50,000 cells (depending on the species) are permanently united, there are cytoplasmic connections between cells, and some cells are specialized for reproduction.

Procedure
1. Prepare wet mounts of both *Protococcus* and *Volvox* samples.
   a. Identify the following structures in *Volvox*:

   Individual cells all possess the following structures: **cell wall, nucleus, vacuole, chloroplasts, flagella (two per cell).**
   Cytoplasmic strands form connections between adjacent cells.
   Daughter colonies are smaller spheres within the larger colony. These are produced asexually, and when they are large enough, they will be discharged from the parent colony into the surrounding environment.

Figure 5. *Protococcus*.

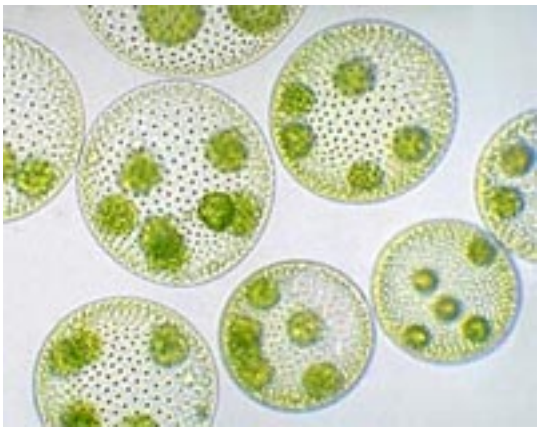

Figure 6. *Volvox*.

***Draw examples of what you see for both *Protococcus* and *Volvox* cultures.**

Multicellular Organisms

Materials

microscope slides	coverslips
dropper bottles of water	*Elodea*
toothpicks	methylene blue

Introduction

Multicellular organisms are composed of groups of specialized cells, called **tissues** that together perform particular functions for the organisms. Tissues, in turn, may be grouped to form **organs**, and organs may be grouped into **organ systems**. In this lab study, you will examine some of the cells that compose the basic tissue types of plants and animals**.**

Procedure

Plant Cells

1. The major characteristics of a typical plant cell are readily seen in the leaf cells of *Elodea*, a common aquatic plant (Figure 7). Prepare a wet mount and examine one of the youngest (smallest) leaves from a sprig of *Elodea* under the compound microscope**.**
2. Identify the following structures:

The cell wall is the rigid outer framework surrounding the cell. This structure gives the cell a definite shape and support. It is not found in animal cells.

Protoplasm is the organized contents of the cell, exclusive of the cell wall.

Cytoplasm is the protoplasm of the cell, exclusive of the cell wall.

The **central vacuole** is a membrane-bound sac within the cytoplasm that is filled with water and dissolved substances. This structure serves to store metabolic wastes and gives the cell support by means of turgor pressure. Animal cells also have vacuoles, but they are not as large and conspicuous as those found in plants.
Chloroplasts are the green, spherical organelles often seen moving within the cytoplasm. These organelles carry the pigment chlorophyll that is involved in photosynthesis. As the microscope light heats up the cells, cytoplasm and chloroplasts may begin to move around the central vacuole in a process called *cytoplasmic streaming*, or *cyclosis*.
The **nucleus** is the usually spherical, transparent organelle within the cytoplasm. The structure controls cell metabolism and division.

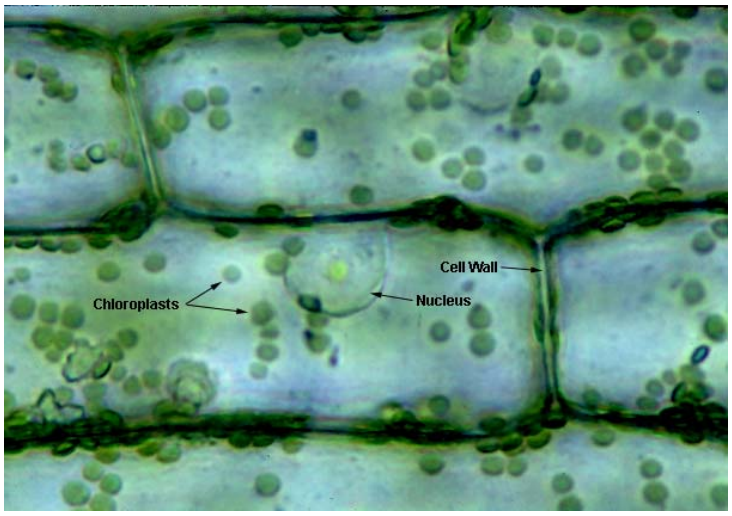

Figure 7. ***Elodea***. *Elodea* is an aquatic plant commonly grown in freshwater aquaria. The cell structures may be difficult to see because of the three-dimensional cell shape and the presence of a large central vacuole.

Animal Cells

1. Animals are multicellular heterotrophic organisms that ingest organic matter. They are composed of cells that can be categorized into four major tissue groups, epithelial, connective, muscle, and nervous tissue. In this lab study, you will examine epithelial cells. Similar to the epidermal cells of plants, **epithelial cells** occur on the outside of animals and serve to protect the animals from water loss, mechanical injury, and foreign invaders. In addition, epithelial cells line interior cavities and ducts in animals. Examine the epithelial cells (Figure 8) that form the lining of your inner cheek. To obtain a specimen, follow this procedure:

a. With a clean toothpick, gently scrape the inside of your cheek several times.
b. Roll the scraping into a drop of water on a clean microscope slide, add a small drop of methylene blue, and cover with a coverslip.
c. Using the compound microscope, view the cells under high power.

Observe that these cells are extremely flat and so may be folded over on themselves. Attempt to locate several cells that are not badly folded, and study their detail.
2. Identify the following structures:
The **cell membrane** is the boundary that separates the cell from its surroundings.
The **nucleus** is the large, circular organelle near the middle of the cell.
Cytoplasm is the granular contents of the cell, exclusive of the nucleus.

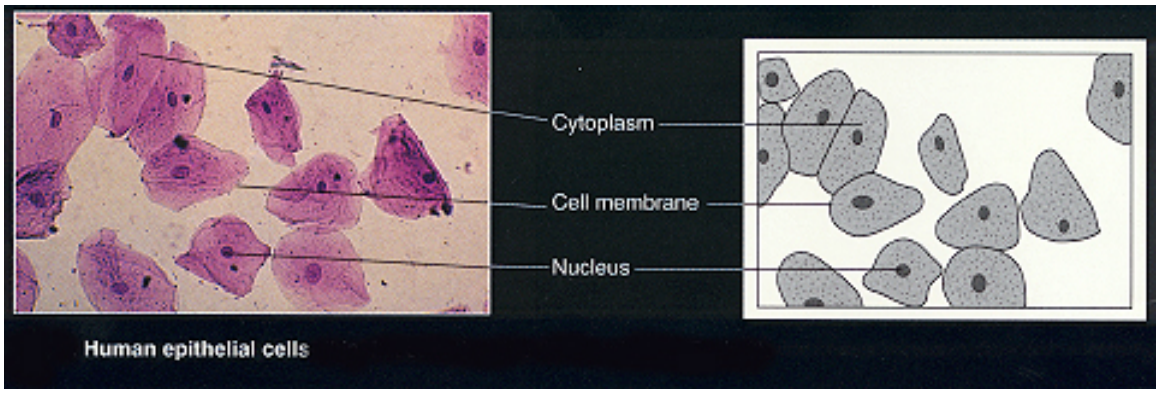

Figure 8. Human epithelial cells.

***Draw examples of what you see for both plant and animal cells.**

BIO201 Laboratory Assignment: Microscopy

Exercise for today:

Follow procedures as outlined in manual and answer the following questions:

Exercise 1. Basic Microscope Techniques

1. If you move a slide slowly to the right, in what direction does the image in the ocular move?

2. Is the image in the ocular inverted relative to the specimen on the stage?

3. Working distance. Is the working distance greater with the 40 X or the 10 X objective?

4. Compute the total magnification of the specimen being viewed (the letter e and crossed threads).

    a) What is the total magnification of the letter e when viewed in focus with the 40 X objective?

    b) What would be the total magnification if the ocular were 20 X and the objectives were 100 X?

    c) Focus on the region where the threads cross are. Are all threads in focus at the same time?

    d) Rotate the 10 X objective into position and focus on the cross. Are all threads in focus at the same time?

e) Does the 4 X or the 10 X objective have a shorter depth of field?

5. Measuring the diameter of the field of view. (Using a clear ruler).

a) What is the diameter of the field of view? (Explain)

b) Measure the diameters of the field of view for the 4X, 10 X and 40 X objectives:

4X = _____ 10 X = _____ 40 X = _____

Exercise 2. The Organization of Cells

Draw examples of what you see for *Amoeba, Paramecium, Protococcus, Volvox, Elodea* and human epithelial cells at 400X magnification.