PBIO 115: Spring 2006
Lab 1: Initiating Growth of Plants; Microscopy

INTRODUCTION AND OBJECTIVES

In this laboratory you will initiate the growth of radish plants. You will nurture them throughout the quarter and observe their life cycle. You will also familiarize yourselves with the use and care of microscopes so that you can effectively observe small things and understand what you are seeing.

EXERCISE A: ESTABLISHING THE GROWTH OF PLANTS

This exercise initiates the growth of a radish plant, which you will study throughout the course. The data sheets that you will need to record future observations will be passed out in lecture before each lab. They also are available from the PBIO-115 web site.

Planting Radish Seeds.

Flowering plants, including radish, grow from seeds. You will be working **individually** to study the growth and development of radish plants. However, pairs of you will share plants.

1. Following your TA's instructions, and working in pairs, sow three radish seeds between the paper towel and the soil on opposite sides of a plastic cup, mark the cup to identify it for future observation, and water the soil to begin seed germination. Be sure the towel doesn't extend above the soil line because it will cause the soil to dry out too quickly.

2. The life cycle of the soybean plant on page 448 & 449 of your text book is the same as in the radish, although the leaves and other parts of the plant body differ in appearance in the two species. Consult this life cycle periodically as your radish plant grows. You will be observing the growth and development of your plants and recording your observations throughout the quarter.

EXERCISE B: USE AND CARE OF MICROSCOPES

Part 1. Demonstration of Compound and Dissecting Microscopes.

Your TA will now demonstrate the care and use of both compound and dissecting microscopes. Position yourself so that you can follow along and try the various types of adjustments on the microscopes. You will find a dissecting microscope in the center of the bench, and a compound microscope at your side. The TA's will indicate which scopes to use. Use the same compound microscope each lab period throughout the quarter.

Part 2. Using Dissecting Microscopes.

Finding, focusing and estimating the size of things on the stage of a dissecting

microscope are relatively easy tasks. You may place a ruler next to the specimen to measure size through the dissecting microscope. During this demonstration, work in pairs and use the ruler as a specimen.

1. Begin by rotating the zoom knob to the lowest magnification.

2. Place the specimen in the center of the stage.

3. Direct light onto the specimen - reflected light (from above) for opaque specimens, and transmitted light (from below) for transparent specimens.

4. While looking through the objective, center the specimen in your view, focus the image, and then increase the magnification with the zoom knob until the desired part of the specimen fills the field of view.

Part 3. Using Compound Microscopes.

A. Finding, Focusing and Estimating Sizes of Specimens.

Use of a compound microscope takes a bit more care but quickly becomes easy if you follow these simple steps. During this demonstration, work individually and use the "e" slide as a specimen.

1. Always begin by rotating the lowest power objective into the path of light.

2. Place a slide on the microscope stage so that the specimen appears to be centered in the path of the light.

3. Turn up the light until you can see rather bright light through the ocular. **If the light hurts your eyes, it is too bright.**

4. Carefully turn the coarse focus knob up and down until the specimen comes into focus. **Caution! This is where you can crush slides with the objective if you are not careful.**

5. Center your specimen in the field of view.

6. Adjust the iris diaphragm (located on the condenser), if needed. Close down the iris only as much as is necessary. To do this, move the iris lever to the side until the contrast of the specimen begins to increase. (Remember, when you cut down light on the condenser you are increasing contrast at the expense of resolution.)

7. Estimate the specimen's size by deciding what portion of the field of view it occupies (e.g., 50%, 10%, etc.). Now multiply the size of the field of view by that percentage to determine the size of your specimen. The size of the field of view for each objective on your microscope is indicated on the table below.

4X Objective	10X Objective	40X Objective	60X Objective
4.3 mm	1.6 mm	0.45 mm	0.29 mm

8. If you wish to use higher magnification objectives, increase the magnification by one objective at a time. At each increase in magnification repeat steps 4 - 6. **Use only the fine focus at these higher magnifications!**

B. Optical Changes in Orientation (rotating or flipping?).

With a dissecting microscope the specimens appear in the same orientation in the ocular as they are found on the stage. With the compound microscope this is not true. Determine how the image of a specimen is either rotated or flipped (i.e., a mirror image).

To determine how a specimen is reoriented when viewed in a compound microscope:

1. Position a slide with the letter "e" in its normal orientation on the stage.

2. Draw the letter on a sheet of paper in its normal orientation.

3. Now position and focus the letter as described above, and draw what you see beside your previous drawing. How do the two differ? Does the microscope rotate the image? If so, how much (e.g., 90^{o}, 180^{o}, etc.). Does the microscope flip the image either vertically (i.e., from top to bottom) or horizontally (i.e., from side to side)?

C. Optical Sectioning and Vertical Orientation.

Now that you know how to focus the microscope on a specimen, you can use focus to increase your understanding of specimens. Microscopes focus images for only a narrow vertical distance. The higher the magnification, the narrower is this distance (this is called depth of focus). As you focus up and down on a thick specimen, only a small slice of it is in focus at any time.

By focusing up and down through a specimen, you can figure out what is above and what is below. In this way, you can understand the three dimensional shapes of things. To illustrate this and give you practice in using this three dimensional capability of the compound microscope, we have made some slides that consist of colored threads that are stacked on each other.

1. Place one of these slides on the microscope and bring the image into focus as described above.

2. Now focus up and down through the threads until you understand which is on top of which.

3. Make a drawing that illustrates this, and compare it to the drawings made by other students. Are they all the same? If not, look again at the slide with the person whose drawing differs. Together, decide whose drawing is most accurate.

D. Using Microscopy to Observe and Understand.

Now that you understand how to use dissecting and compound microscopes, look at some things.

1. Look at the aquatic flowering plant *Elodea* under the dissecting microscope. Can you figure out what is stem and what is leaf?

2. Look at the surface of a leaf of a geranium (either *Geranium* or *Pelargonium*) with the dissecting microscope. These plants have aromatic compounds that give them a pleasing odor. The compounds are produced in hairlike glands on the surface of the leaf. See if you can see these glandular trichomes (not hairs; animals have hairs, plants have trichomes). Your TA will help prepare a slide that can be viewed with the compound microscope to see these trichomes at higher magnification. Are they constructed of cells?

3. Place a stage micrometer on the stage of the compound microscope. This is a glass slide that has been marked into millimeters, tenths of a millimeter (i.e., 100 micrometers), and hundredths of a millimeter (i.e.,10 micrometers). Can you figure how to determine the size of the field of view for each objective? Are the sizes of the fields of view for the various objectives in the table (above) accurate?

4. Your TA will demonstrate how to make a wet mount on a microscope slide. Using pond water or other material provided, make your own wet mount and examine it with the compound microscope. How many kinds of organisms can you find? Try examining them at different magnifications, and make simple sketches of some of them. Do you have any idea what they are? If not, you may in a few weeks!

You are now ready to use dissecting and compound microscopes as tools to reveal tiny features of life!