

# INTRODUCTION TO THE MICROSCOPE

## LAB A

Our goal is to become familiar with the wide range of cell types using the best tool for directly observing cells, the microscope. First we will learn to use the microscope well and then we will look at a variety of cells, both living and unstained, and fixed and stained.

### I. USING THE LIGHT MICROSCOPE

#### Introduction

One of the most important tools used by biologists is the microscope. It is an exquisitely sensitive arrangement of lenses that, when used properly, permits us to examine living and fixed material that we would not be able to see otherwise. A light microscope, by virtue of its lens system, extends our vision a thousand times, so that objects as small as  $0.1\ \mu\text{m}$  ( $10^{-7}$  meters) in diameter can be seen. Since average cells range in diameter from  $1\ \mu\text{m}$  (bacteria) to about  $30\ \mu\text{m}$  (epithelial cells), the microscope is a perfect tool for directly observing cells. Although three hundred years have passed since its invention, the standard light microscopes of today are based on the same principles of optics as microscopes in the past.

Microscope quality depends upon the capacity to resolve, not magnify, objects. **Resolution** is the ability of the microscope to distinguish detail. Merely magnifying an object, without increasing the amount of detail seen, is of little value to the observer. Modern microscopes increase both magnification and resolution, simultaneously, by matching the properties of the light source and precision lens components.

Even with sufficient magnification and resolution, a specimen can only be seen on a microscope slide if there is sufficient contrast among its parts. Contrast is based on the differential absorption of light by these different parts. Often a specimen will consist of opaque parts or will contain natural pigments, such as chlorophyll. Cells or subcellular structures containing pigments are visible, but what happens with the majority of biological materials that are highly translucent structures or very thin sections of specimens? The microscopist may improve contrast by the use of stains or dyes that bind to cellular structures and chemicals or may elect to use nonspecific stains which stain all structures. The study of staining is a field in itself called cytochemistry or histochemistry.

Other types of microscopy allow us to probe cells and their organelles in different ways. Consult your text to develop an understanding of transmission electron microscopy, scanning electron microscopy and atomic force microscopy (pp. 86-89).

#### Materials:

- binocular compound microscope
- lens paper
- prepared slide with crossed threads
- ruled stage micrometer (2 mm with 0.01 and 0.1 mm divisions)

## Methods:

### **Examining and adjusting the compound microscope**

Before you begin, check to make sure that your microscope is ready to use. It should be plugged in with the light source off and the rheostat set at 1 or the lowest number. The shortest objective (lowest magnification power) should be in place and the stage lowered. Make any necessary corrections before turning on the light source and increasing the light intensity.

1. The *oculars* are the lenses you look through. The oculars can be adjusted to match the distance between the eyes of different observers (interpupillary adjustment). Should you wear your eyeglasses when using a microscope? The answer to this question is qualified. If you are nearsighted or farsighted, you should not wear your glasses for microscopic observations as the adjustments made in focusing the microscope compensate for these eye problems. You *should*, however, wear your glasses if you have astigmatism, since this problem is not corrected by the lenses of the microscope. Note the magnification stamped on the housing of the oculars on your microscope.

2. Attached to a rotating nosepiece, or turret, at the base of the body tube are a group of three or four *objectives*. Rotate the nosepiece and notice that a click is heard as each objective comes into position. The objective gathers light from the specimen and projects it into the body tube and through the oculars. Each objective has numbers stamped on it. One of these numbers identifies the magnification of the objective. Note the magnifications on each of the objectives of your microscope.

3. The total magnification is calculated by multiplying the magnifications of the ocular and objective lenses on the microscope being used. Calculate the total magnification for each ocular-objective combination on your microscope. How large would a 30  $\mu\text{m}$  object appear if it were magnified 40-fold? 100 fold?

4. Light travels from the objectives through a series of magnifying lenses in the body tube to the ocular. In some microscopes, the body tube is straight. In others, the oculars are held at an angle, and the body tube contains a prism that bends the light rays so that they will pass through the oculars.

5. The surface or platform on which you place the microscope slide is the *stage*. Note the opening (*stage aperture*) in the center of the stage. On some microscopes, the stage is movable and is called a mechanical stage. Movement is controlled by two knobs located on the top, side or bottom of the stage.

6. The area under the stage, called the substage, may contain a diaphragm, a condenser, or both.
  - a. *Diaphragm*--The diaphragm regulates the *amount* of light passing from the light source through the specimen and through the lens system of the microscope. By properly adjusting the diaphragm, you can provide better contrast between the surrounding medium and your specimen, thus greatly improving your image of the specimen.
  - b. *Condenser*--The condenser consists of a series of lenses that focus light on the specimen. It is moved up and down by a knob at its side. By properly adjusting the condenser, you can greatly improve the *clarity* of the specimen.
7. Your microscope has a built-in illuminator. It is built into the base of the microscope and controlled by an on/off switch. You can control light intensity by adjusting the voltage of a transformer attached to the illuminator. Use low or medium settings for most microscopic observations.
8. Return the microscope to its "ready" position, i.e., put the 4X objective in place, lower the stage, turn down the illuminator to its lowest setting, and turn off the illuminator power. You may also wish to unplug the cord and wrap it around the base; then cover the microscope if a cover is available.

### **Focusing your microscope**

1. If necessary, clean the oculars and objectives using lens paper. **Do NOT use Kim-wipes, facial tissue, paper towels, or your sleeve!** (These materials all tend to scratch these high-precision lenses.)
2. Obtain a slide with a set of three crossed threads. Examine it with your eyes before placing it on the microscope stage. Now move the slide so that the area with the threads is located over the stage aperture. The 4X objective should be in place and the microscope stage down.
3. Turn on the illuminator, raise the light intensity to a comfortable brightness while looking through the eyepieces. Open the diaphragm halfway. Position the condenser as high as it will go. Readjust the light as needed.
4. Center the specimen into the field of view by using the mechanical stage.
5. Raise the stage (or lower the objective) using the coarse focus knob until the first thread comes into reasonable focus. Look and note how close the objective is to the slide. To adjust the focus for your right eye, close your left eye and use the fine focus knob to bring the middle thread into optimal focus. Now, close your right eye and adjust the left ocular lens until the same thread is in focus. Open both eyes. You should now have a well-focused binocular view of the threads.
6. Switch from the 4X to the 10X objective by turning the nosepiece and watching from the side as you move it into place. **Do NOT hold the objectives while turning;** or you are likely to unscrew them and have them fall out or become misaligned. The objective should 'click' into the

proper position.

7. Re-center the specimen while looking through the oculars using the stage adjustment knobs. (Locate them with your eyes and hands before looking through the microscope.)
8. Adjust the focus using the fine adjustment knob if necessary. Adjust the light as needed.
9. Adjust the condenser to increase the contrast of the image by moving the condenser as close to the stage and slide as possible and then move it back down approximately half a centimeter. You will want to 'play' with this adjustment to optimize the lighting for each specimen and magnification.
10. While looking from the side, switch to the 40X objective by turning the nosepiece. Avoid hitting the slide with the objective.
11. Adjust the focus (fine adjustment knob only!), the light and condenser as needed to optimize your view.

### **Determining the depth of field**

Like the human eye, the lenses of your microscope provide a limited depth of field. This means that only part of the object will be in sharp focus while areas above and below that part will be slightly out of focus or not in focus at all.

1. Go back to the 4X objective and examine the three threads together. Adjust the fine focus knob and note as the three threads in turn come into focus. Which thread is on top, which is in the middle, and which is on the bottom?
  
2. Repeat this examination using the 10X and 40X objectives. Which objective has the greatest depth of field?
  
3. Given that each thread is about 0.3 mm thick, what do you estimate the depth of field to be for each magnification?

### **Measuring the field of view**

1. The ruled stage micrometer that you have is a tiny ruler. It is 2 mm long with 0.1 and 0.01 mm divisions. Put your microscope in the 'ready' position with the 4X objective in place and the stage micrometer slide on the stage with the ruling centered over the aperture and under the objective lens.

2. Focus on the rulings using the coarse and fine focus knobs. Use the rulings to determine the width of the field of view. Record this value in both mm and  $\mu\text{m}$ . Draw a picture indicating how large something 100  $\mu\text{m}$  in diameter would appear in this field of view.

3. Repeat these measurements and drawings using the 10X and 40X objectives.