

How to Use a Microscope and Introduction to Cells

Part I: The Microscope Learning Goals: After completing this laboratory exercise you will be able to:

1. Name and give the function of the parts of a light microscope.
2. Explain magnification, depth of field, and resolution.
3. Calculate the diameter of field and magnification of each lens setting.
4. Prepare biological material for analysis by microscopy.
5. Correctly use a microscope in the analysis of biological material.

Introduction: If a biologist has a universal tool, it is certainly the light microscope. Most biologists use microscopes in their work as a means of investigating small objects. A certain amount of skill with a microscope acquired at an early stage in one's biological training will be of use in this course and in further biology courses. Always treat the microscope with great care. Make certain that you do not touch any part of the lens system with anything abrasive (such as a slide or dirty water) or greasy (such as even the cleanest fingers). Never clean a lens with anything except clean lens paper! If the view gets foggy (as it probably will sometime during the semester), and lens paper will not clean it, call your laboratory instructor.

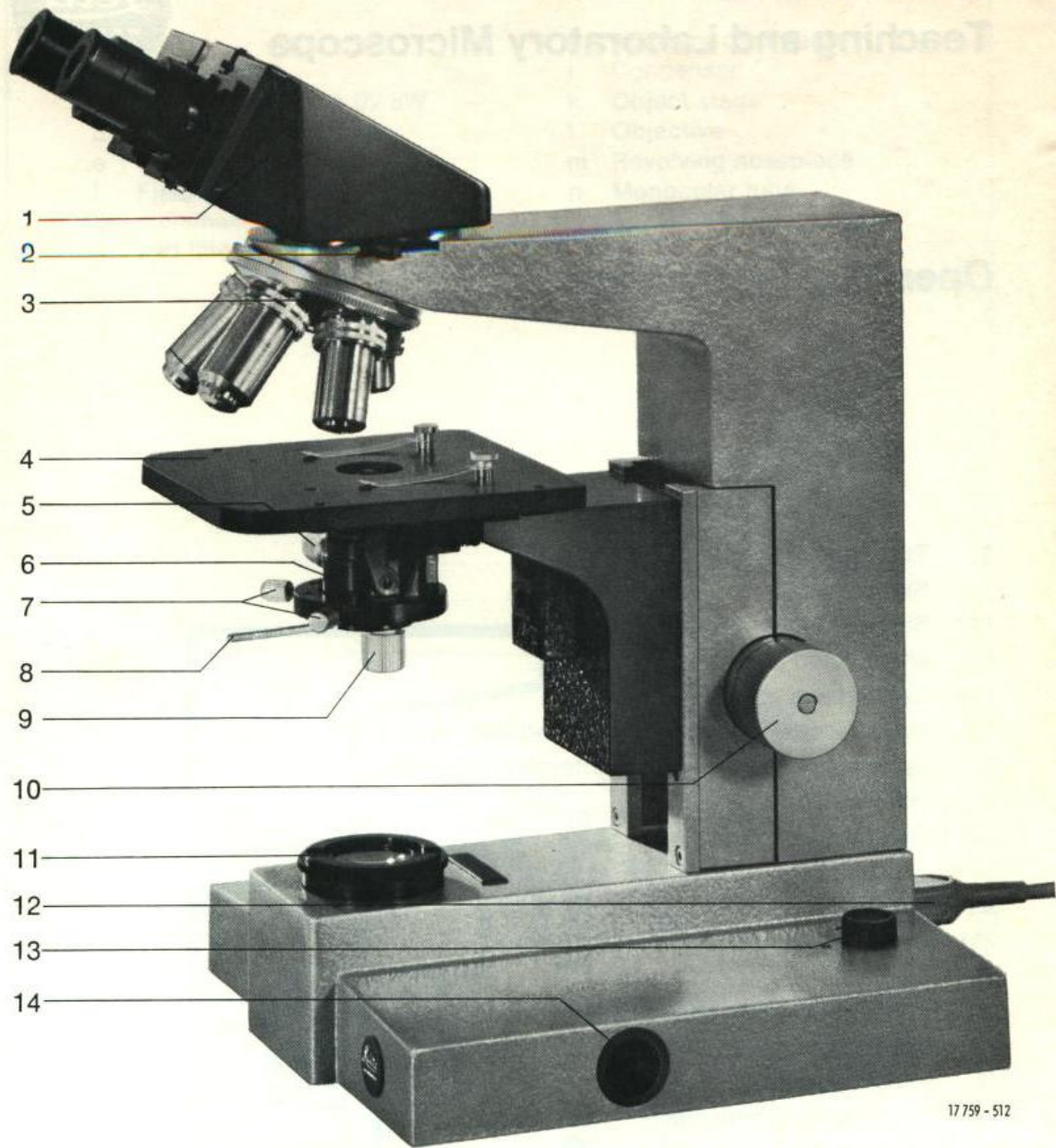
I. Structure of the Compound Microscope

It is very important that you familiarize yourself with the parts of the microscope and their function. Your first task is to locate all of the parts named in the diagram on the next page. Place the microscope so that it is at right angles to you. In addition to the stand (arm & base) and a movable stage by which the object can be positioned and focused for viewing, the microscope consists of the following two sub-units:

1. Object illuminating system: light, diaphragm and condenser.
2. Lens system (magnifies the object): ocular, body tube and objective lens

A. The System of Illumination.

Keep the microscope in the same position: plug it in, turn on the light, move the diaphragm lever as far to the left as possible.



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Fig. 1:
SM-LUX with binocular tube S and object stage No. 24a, with permanently mounted condenser

- 1 Binocular tube S
- 2 Tube changing lever
- 3 Revolving nosepiece with objectives
- 4 Object stage No. 24a with permanently mounted condenser
- 5 Lever for swing-out lens of condenser

- 6 Condenser
- 7 Centring screws
- 8 Aperture-stop lever
- 9 Control knob for vertical adjustment of condenser
- 10 Single control knob for coarse and fine focusing
- 11 Protective glass with field diaphragm
- 12 Mains cable
- 13 Control knob for transformer
- 14 Lamp socket

1. Place a clean slide on the stage over the condenser and put a piece of white paper about 25 mm square on top of the slide.
2. Now slide the condenser knob and move the condenser up and down while observing the light on the piece of paper (do not look through the microscope but continue to look at the paper with your naked eye).
3. Note that you see a fairly intense small circle of light when the condenser is at its uppermost position and that this circle gets larger and more diffuse as one lowers the condenser. For most work with the 10X and 40X objectives it is best to have the condenser near the top of its travel.

Put your eye at table level and look up at the bottom of the condenser. Now move the diaphragm lever and observe what happens. This is an iris diaphragm. Why do you suppose it is called this?

Look at the piece of paper again while opening and closing the diaphragm. The **diaphragm** serves to regulate the amount of light passing through the condenser. It also serves to cut down stray light. Later when you look through the microscope you will see that the diaphragm can be kept partly closed without cutting down on the light passing through the lens (i.e., only light beyond the field of the lens is being blocked). Further closing of the diaphragm will cause less light to enter the lens and decrease the resolving power of the lens while increasing contrast in the viewed object. **Resolving power** is how well specimen detail is preserved. **Contrast** is the ability to see particular detail against its background. Control of the light entering the microscope is very important.

B. The Lens System. Light passes through the condenser, through the object which is placed on the slide and into the lens system. The lens system consists of: (1) an objective lens the revolving nosepiece of your microscope has at least two of these, (2) a body tube -in your microscope the body tube has prisms in it to allow the tube to be inclined and (3) the ocular lens. Basically, the objective lens magnifies the object and forms an image in the tube which is further magnified by the ocular lens. The objective lens is the most important (and most expensive) part of the microscope and the quality of a microscope is largely a question of the quality of its objective lens. The ones in your microscope are very good indeed and deserve care. The 10X objective (low power) has a working distance (the distance from lens to object when the object is in focus) of about 4 mm. The 40X objective (high power) has a working distance of about 1 mm.

While still looking at the microscope from the side, move the stage down well

clear of the objective lenses by turning the coarse adjustment knob. Now rotate the nosepiece and notice that each lens clicks into the proper position. Move the 10X objective into position. Next move the stage up until the lens is about 4 mm from the slide. Notice while doing so that the knob you are turning is both a coarse and fine adjustment (most microscopes have separate knobs for these) and that extreme movement of the knob moves the stage rapidly, but immediately after you reverse the direction of movement, the stage moves almost imperceptibly for a short distance. This fine adjustment allows precise focusing.

II. Principles of Microscopy

A. Magnification is essentially the product of the magnification of each lens (objective x ocular). With 10X objective and 10X ocular the magnification should be 100X. A microscope with a straight body tube is designed to project the image to the level of the bench surface. Your microscope, since it has an inclined tube, projects the image at an angle to the bench. The magnification refers to the increase in size of this apparent image over the object on the slide. Put the microscope on low power (10X objective).

B. Depth of Field is very limited when using a compound microscope. It is necessary to continually focus up and down to get an impression of depth. Make a slide using colored threads which cross over each other, i.e., put a red thread on the slide and a blue cross over it to form an X. Add water and a cover slip and observe. Notice that you can focus clearly on only one of the threads at the point where they cross and must focus up or down to get the other clearly in view. This is especially noticeable under high power. To get an impression of depth with the microscope, one continually focuses up and down and takes optical sections through the object.

C. Resolution refers to the ability to discriminate between two objects which are very close together. Draw two heavy lines on a piece of paper less than 1 mm apart. Put the paper on the wall on the other side of the room. If the paper is far enough away, you will see the two lines as one, but as you walk toward the paper you will be able at some distance to resolve those lines as two distinct lines. At its best, the naked eye can resolve lines which are about 100 microns apart (very close to the eye), but has difficulties with objects closer together than that. What limits the resolving power of your eye? What limits the resolution of a camera?

Resolution in a microscope can be limited by many things. The most important as far as the quality of the microscope is concerned is the quality of the objective lens. It is fairly easy to make a condenser and light system of good quality and an ocular lens of good quality, but good objective lenses, properly corrected, are difficult to make. Magnification is of secondary importance in a microscope. Your microscope will magnify a well resolved object 400X, a Mickey Mouse microscope will magnify a blur 400X.

The ultimate limit on the resolving power of the light microscope is the wavelength of light itself. The shorter the wave length, the better the resolution. A good light microscope will resolve objects about 1/2 micrometer (micron) apart. Now turn the microscope around so that you can look through the ocular. We are now ready to look at something.

III. Preparing a Slide and Making Observations.

Your lab instructor will show you how to make a slide. The great art here is to avoid air bubbles when you lower the cover-slip! Make a water mount of a piece of paper with a small letter "e" on it. Place the slide on the stage. Then check to be sure that the "e" is in the circle of light coming through the condenser and that the 10X objective is in place and about 4 mm from the surface of the slide. Look through the ocular and by moving the focusing knob get the paper in focus (having the objective slightly closer than 4 mm and focusing upward by moving stage downward until the image appears is the best way to do this). When the paper is in focus, move the slide back and forth and up and down until the "e" comes into view. Is the image of the letter oriented in the same way as the actual letter on the slide? When you move the slide to the left does the image move to the left? As you move the slide about it is necessary to constantly keep adjusting the focus. A good microscopist will always keep his or her hand on the focus adjustment and continue to make the fine adjustments in focus as he or she looks at an object.

Assuming that the microscope is in focus with the 10X objective, switch to the 40X objective by rotating the nosepiece. It is a good idea to look at the nosepiece as you rotate it to be sure that the high power objective does not strike the coverslip. The lens should come into position about 1 mm above the coverslip and be nearly in focus when you look through the ocular. Lenses which come into focus in this manner are referred to as being parfocal. Continued refocusing with the fine adjustment is more important here than with the low power lens. This is because the depth of field is less. When you switched from low to high power, did the object get brighter or dimmer? Why? You may have to readjust the light (open or close the diaphragm). Note that though the object is larger, the field of vision is smaller. Under high power (40X) the diameter of the field of vision is 0.4 mm, under low power (10X) the diameter is 1.35 mm.

Points to Remember: The following are worth remembering as you use the microscope.

1. Be sure that you are using the condenser and diaphragm correctly.
2. Do all preliminary focusing under low power.
3. Do not focus downward when first getting the object in focus (i.e. beware of

smashing the slide and lens together).

4. Try to use the microscope with both eyes open -it will seem hard at first, but is easier in the long run.
5. Use the fine adjustment constantly to keep things in focus.
6. Use lens paper to clean the lenses occasionally, you will find that the microscope works best when clean.

IV. Preparation and Analysis of Biological Samples.

Peel a piece of onion (*Allium cepa*) epidermis from an inner leaf (your instructor will show you how) and make a wet mount of it as you did with the letter "e". After careful inspection of several cells, make a drawing of a "typical" cell indicating the cell wall, cytoplasm, nucleus and vacuole. Your instructor will show you how the drawing should be made and labeled. Assume that a typical onion cell is 10 micrometers in height. Measure the height of the cell in your drawing and calculate the "magnification" -- how much did you magnify the cell when you drew it? (There are 1000 micrometers in one millimeter.)

Ideally the light should be fairly bright, but you will find it difficult to distinguish cell parts. Cutting down on the light will make more parts visible. Cutting down on the light increases the contrast, but reduces the resolution. You will find that you will often have to sacrifice resolution to gain contrast in order to see the various parts of something which looks to be various shades of gray. There are two solutions to this problem:

- 1) to use a special microscope called a phase contrast microscope or
- 2) to stain the object and gain contrast by differential uptake of stain by different parts of the object.

Staining

Stain the onion cells with neutral red stain. This can be done without removing the onion peel from the slide. Add a drop of stain to one side of the coverslip and sop up water with a piece of paper towel from the other side. Different parts of cells take up different dyes in differing amounts. What part of the cell takes up the dye here? Can you now see more detail than before the cell was stained? Can you now use more light to see this detail? Prepared slides can be made with 2 or 3 stains to get a maximum effect. Look at the prepared slide provided and see if you can determine which colors were used and what is stained by each. Do you suppose that staining offers any clue to the chemical composition of the cell?

1. Human Epithelial Cells

Gently scrape the inside of your cheek with the broad end of a toothpick sterilized with 70% alcohol. Stir the scrapings into a drop of water on a slide and cover with a cover glass. To stain the cells, add a small drop of methylene blue at the edge of the cover glass. Locate the cells under low power. They will appear as small masses of granular material. Switch to high power and identify the various parts of the cell. The cells are large, flat (squamous epithelial) cells that form tissue surfaces. Individual cells are best seen at the margins of a group of cells. Note the cell membrane, its junctures with neighboring cells, the granular cytoplasm and the small, rounded nucleus. Note that these animal cells characteristically lack a cell wall, chloroplasts and the large vacuole which is so prominent in most plant cells.

2. The Starch Storage Cells of the Potato Tuber

Within plant (and animal) cells are many structures which may be seen more clearly by using various staining techniques. Examples of such structures are starch grains, formed and stored in the cells of many plants, including the potato tuber. Take a chunk of a peeled potato tuber, rub it on your slide and add a drop or two of water to it to make a wet mount. Observe under low power the many elliptical starch grains. Now add a drop of iodine solution to the slide at the edge of the coverslip so that the iodine diffuses under the coverslip. Wait 1-2 minutes then add a few drops of water to the edge and pull the stain out by holding a bit of paper towel on the other side of the cover slip. (this will give a clear back ground, by removing the stain.) Iodine reacts with starch and turns it blackish or dark brown in color. Structures such as starch grains can be identified as containing starch when the iodine "test" is performed.

You may also want to look at diatomaceous earth, pond water, and freshwater aquarium plants for examples of other biota.

V. Fluorescence Imaging

The use of chemical stains in microscopy, such as methylene blue and iodine (there are *many* more), began in the 19th century. They have been, and still are, extremely valuable in cell biology. Newer methods for highlighting specific structures within cells use fluorescent staining or marking techniques. We don't have the equipment to do fluorescence microscopy in Bio 111, but your TA may show you some pictures of fluorescence imaging. By the end of this semester you will understand some of the principles involved, and when you take Cell Biology in a later semester you'll get to do some fluorescence microscopy yourself.

VI. More Cells and Tissues

Learning Goals:

1. Increase familiarity with the use of the light microscope.
2. Observe and sketch cells and their structures.
3. Use chemical stains to enhance the visibility of cells under the microscope.

Introduction:

The initial distinction between cell types was dependent on either the presence or absence of a nucleus. Those containing a nucleus are eucaryotic and those lacking a nucleus are procaryotic – though remember both cell types contain DNA.

Cells can also be categorized based on their function. For example, a single organism can contain many different types of eucaryotic cells. Tissues are groups of cells that have the same function within a single organism. As you will learn in your study of Biology, the types of genes that are expressed in a tissue or cell type help to establish its function.

You will examine cells from at least 2 of the 3 domains of life (Bacteria, Archaea, and Eucaryotes). Also, you will examine tissues from at least 3 kingdoms within the Eucaryote domain (Protists, Plants, Fungi, Animal).

Record observations:

- I. Comparison of Eucaryotic and Procaryotic cells – Find slides containing cells from at least 2 different domains.
 - A. Make observations of each in your lab notebook, include sketches, estimates of size and labels. Are cell structures recognizable?
 - B. Concluding questions. During lab, record answers to these questions in your lab notebook: Are there differences in size? Are there differences other than just the presence or absence of a nucleus? Can you identify specialized structures within the cells?

II. Comparison of Eucaryotic Tissue Types – Find slides from at least 3 different kingdoms.

- A. Make observations of each in your lab notebook. Include sketches, estimates of size and label which Kingdom it represents.
- B. Concluding questions. During lab, record answers to these questions in your lab notebook: Are there differences in size? Can you identify specialized structures within the cells? Can you determine the function of the cell?

V. The Dissecting Microscope

If time permits, and you did not learn this in an earlier lab, learn to use the dissecting microscope. Your instructor will give you pointers here. A coin is a good object to practice on. Note:



1. That the magnification is much less than with the compound microscope.
2. That the working distance is much greater.
3. That the depth of field is much greater and one has a stereoscopic view of the object.
4. That the image is not inverted.
5. That one can use either transmitted or reflected light for viewing.

Adjustment of the binocular microscope ocular

If you have normal binocular vision, you will want to adjust your binocular dissecting microscope so that you see in three dimensions:

1. Move the two oculars outward or inwards until they are in the correct position for your eyes. People vary in the width of the face, and your eyes may be farther apart, or closer together, than the previous user of your microscope.
2. Note that the left ocular has a knurled ring which can be turned to change focus, while the right ocular is fixed in focus. Put something on the stage of the microscope which you can focus on, close your left eye, and focus on the object, using only your right eye. Use the main focusing knob on the arm of the microscope to achieve a sharp

focus.

3. Now close your right eye, and use the knurled ring on the left ocular to achieve the same sharp focus for your left eye.

4. Now both eyes should be in focus for any object, and you need only to use the main focusing knob to focus on new objects. Since people vary in the adjustments they make, you may have to repeat this entire procedure every time you use a dissecting microscope which has been used by others.