Microscopy Primer

A. Introduction:

The microscope is a vital scientific tool that will be used often to study plants. We shall begin our studies of plants with a brief primer in the proper handling and use of both the compound light microscope and the dissecting scope. For a review of the key parts of a compound light microscope refer to Figure 1.1 below.

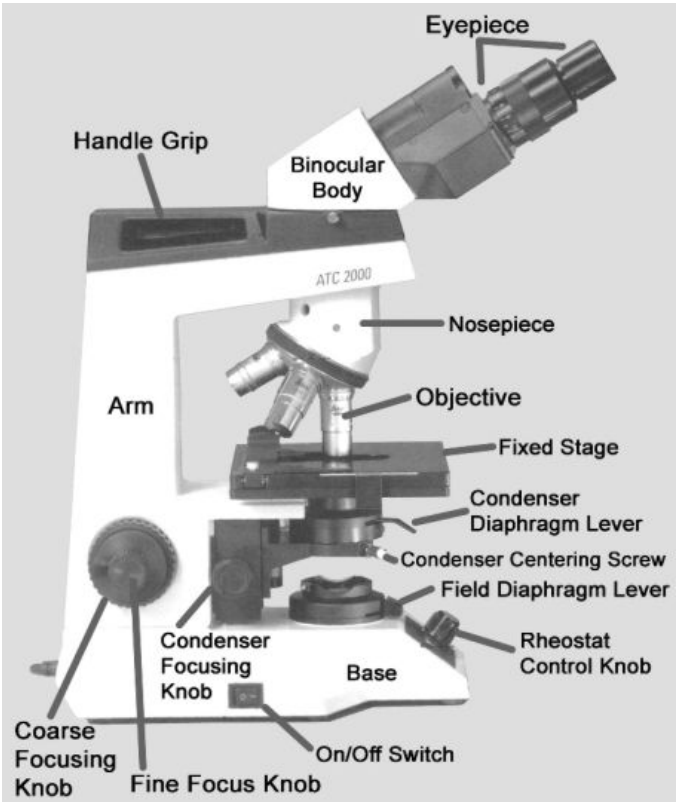


Fig. 1.1. A compound light microscope with important parts labeled.

ARM: Mechanical structure that supports the stage, power pod, etc.; serves as a handle.

BASE: Provides the support and bears the weight of the microscope.

CONDENSER: Focuses the light from the illuminator on the specimen, increases clarity.

FOCUS KNOBS: The fine and coarse focus knobs move the stage up and down. The coarse focus knob should only be used with the 4X and10X objective lenses. Bring the specimen into focus with the 4X or 10X objective and then change to the 40X or 60X for more detail. Only use the fine focus for the 40X or 60X objective lenses or you will ram the stage into the objectives.

IRIS DIAPHRAGM: Regulates the quantity of light illuminating the specimen.

LIGHT SOURCE: An electric bulb light source directed upward toward the stage.

OBJECTIVES OR OBJECTIVE LENSES: Magnification lenses located just above the stage and attached to a revolving nosepiece. These pieces will be labeled with their magnification (e.g., 4x, 10x, 40x, 100x). The maximum for light microscopes is typically 100x.

OCULARS OR OCULAR LENSES: Also called the eyepieces. Provide a location from which to view the specimen. They also magnify the image coming from the objectives by a factor of 10. To determine the total magnification, you multiply the objective mangification times the ocular magnification (e.g., a 40x objective plus a 10x ocular amounts to a total of 400x magnification).

POWER POD: The image passes through a series of prisms to the ocular lenses.

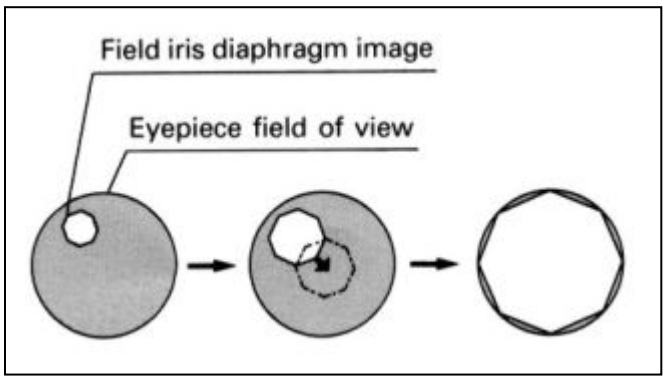
STAGE: Supports the glass slide over an opening that permits illumination of specimen.

B. Proper Use of a Compound (or Light) Microscope:

Before outlining the method for obtaining an optimal image with your microscope, there are a few rules of common sense. The lens, prisms and mirrors inside your scope are very sensitive to bumps and even vibrations. Always carry the scope with two hands, one hand on the microscope arm and the other hand under the base. WHEN YOU MOVE YOUR SCOPE ON THE LAB TABLE, DO NOT SLIDE THE SCOPE INTO POSITION. THE VIBRATIONS FROM THIS SLIDING CAN BREAK LOSE THE MIRRORS & INTERNAL LENSES LOCATED IN THE MICROSCOPE. Another big cause of microscope problems is dirt in vital places, especially on the eyepiece and objective lenses. Please put the plastic cover on your scope when you finish the lab exercise. If you wear eye make-up, clean the eyepiece lens after each use. **DO NOT CLEAN ANY OF THE LENSES WITH ANYTHING EXCEPT LENS PAPER (NOT PAPER TOWELS, TOILET PAPER, KIMWIPES, MICROWIPES, FACIAL TISSUE, ETC.).** If you can not get the lens clean with lens paper, notify your professor.

When using a microscope, an optimal image can be achieved by using the following procedure.

1) Turn the lamp on by pushing down the black **on/off toggle switch** on the left side of the **microscope base**, and use the **rheostat control knob** to adjust the light intensity to a level that is comfortable to your eyes.
2) Make sure the low-power **objective** (4X) is pointing straight down (is in its usable position), and use the **condenser focusing knob** to move the **condenser** to its highest possible position; make sure that the **condenser diaphragm** and **field diaphragm** are wide open (maximum amount of light is getting through).
3) Place a microscope slide in the **slide-clip** and position it (with the **mechanical stage traverse knobs**) such that the cover slip on the slide is directly beneath the low power objective.
4) Use the **coarse** and **fine focusing knobs** to bring the image into sharp focus (use only the right eye for this focus).
5) While using only your right eye to look through the right **ocular**, focus on the slide first using the outer, larger focus ring (**course focus knob**) and then the inner, smaller focus ring (**fine focus knob**).
6) Now close your right eye and as you look through the left eyepiece with your left eye, rotate the base of the eyepiece around until the image is in focus for your left eye.
7) While looking through the scope with both eyes, adjust the distance between the two oculars to match the distance between your eyes by pulling the oculars apart or pushing them together.



8) While looking through the microscope, slowly start closing the **field diaphragm** lever until the field iris diaphragm image is only a small fraction of the total eyepiece field of view (forming a smaller circle of light in the field of view).

9) Turn the **condenser focusing knob** until the inner edges of the field iris diaphragm

image is in sharp focus (the condenser should be very close to its highest possible position just below the fixed stage).

10) Use the two **condenser centering screws** to center the diaphragm image (circle of light) within the field of view.
11) Open the diaphragm back up slightly until the field iris diaphragm image extends just outside of the eyepiece field of view.
12) When you need to increase magnification to 400X, simply rotate the nose-piece such that the 40X objective is pointing straight down. The image should stay close to focus since this microscope should be *parfocal*.
13) You can alter the contrast of the resulting image by adjusting the condenser diaphragm level (below the stage). When observing unstained specimens (e.g. leaf epidermises from epidermal peels), you should decrease the opening of the condenser diaphragm; when observing stained specimens, you might want to open up the condenser diaphragm.

C) Field of View, Depth of Field, Plane of Focus, and Focus Control:

1) Letter "e" slide: Mount this slide and focus on the letter "e", starting at low magnification.

Notice how the e is only in focus when the stage is raised or lowered to the position of the focal plane for the given objective lens.

Compare the orientation of the "e" when viewed with on the stage with the naked eye, vs. when viewed through the oculars (eyepieces). How are they different?

2) Colored thread slide. Use this slide to become proficient at adjusting the coarse and fine focus. Determine which colored thread is on top and which is on the bottom. HINT: as you move the stage up or down, each colored thread will come into focus as it passes through the focal plane.

The code on your slide is _____.

The _____ thread is on the bottom.

3) Prepared Tissue Mount. Use the provided slide of a "typical monocot leaf" to investigate different cells. In the space below, record and draw two different cells. Use the cell structure terms defined in the next section to properly label the cells…

D. Wet Mounts:

1) TECHNIQUE: Often it is useful to observe living, unstained plant tissue. In this case, we prepare wet mounts. The only criterion for a wet mount is that the tissue or other subject be thin enough for light to pass through it. The basic ingredients are as follows:

1) clean glass slide
2) clean glass cover glass
3) water (or some staining solution) and dropper
4) tissue or other subject (e.g., solution with algae in it).

In a *wet mount*, the specimen is placed at the center of the slide with one (or two) drops of water and the cover glass placed over the specimen. In some preparations (such as looking at pond water for microscopic critters), the object being prepared for viewing is contained within water.
Once the specimen and water are combined on the slide, the cover glass is added. The cover glass should be placed at an angle to the slide, one edge touching the slide, and then lowered as if hinged there. If done properly, the water will force out any air as the cover glass closes over it, and no bubbles will be trapped beneath the glass. Although an occasional bubble might be tolerated, large numbers will make viewing the specimen difficult. Adhesive forces between the liquid and the glass will hold the cover glass firmly in place. Generally, only one drop of water is sufficient. Adding too much water will create a problem, as the affixing of the cover slip to the slide will depend on much weaker cohesive forces (see "Problems and solutions" below). There should be no excess water (water outside the cover slip) and the cover slip should remain in place when the slide is moved to the **stage** of the microscope, where it is held in place by **stage clips** or some other holding mechanism.

2) PROBLEMS and SOLUTIONS:

a) Mostly air under cover slip – too little water was used (increase by 1 drop) or cover glass was improperly dropped onto specimen. Adding a drop of water to the slide at the very edge of the cover glass will result in water being taken in under the cover glass via capillary action. If numerous air spaces are still evident, it is best to start over.

b) Too much liquid under cover slip – cover glass slides around easily on glass slide, and may fall off if slide not held perfectly level. Using a small piece of absorbent paper (paper towel or tissue), touch edge of paper to excess water at edge of cover slip; repeat until cover glass affixes to the slide.

c) Cover slip "rocks" on specimen or is clearly not laying flat – either the specimen or something in the sample (a grain of sand for example) is preventing the cover slip from coming down far enough to adhere to the slide. Focusing on this slide will be difficult. Possibly the specimen is not thin enough or evenly sliced. If a grain is present, remove it and re-mount the specimen.

d) Your view of the subject through the microscope is dark and cell structures are difficult to discern. This is often due to your specimen being too thick; however, it will not always be necessary to make a new section since there will always be some portion of your section that will be thin enough as it tapers towards the edge of the section (Fig. 1.3 below).

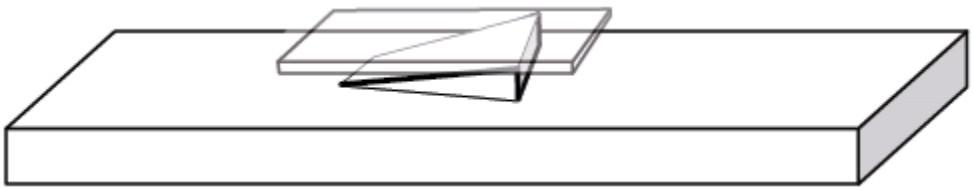


Fig. 1.3. Diagram of a wet mount in which the subject matter (e.g., a wedge of red pepper, carrot, potato or banana) may be too thick in parts. One can either try to obtain another, thinner free-hand section or simply make your observations in a portion of the tissue that is thinnest (i.e., the left side of the wedge of tissue in the diagram).

3) Exercise: Onion, yellow or white

Prepare a wet mount of a living (fleshy) onion bulb epidermis using a procedure called an epidermal peel (Fig. 1.4). Take a length of onion tissue and snap it in half and without tearing the outer (abaxial) epidermis, pull one half toward you and push one half of the onion away from you. You will see a thin, one-celled layer of epidermal tissue pulling away from the onion tissue. Place it in a drop of water on a slide, cut of any remaining portion of the onion tissue and cover with a cover slip. VIEW THE TISSUE STARTING WITH THE 4X OBJECTIVE, then the 10x and 40x.

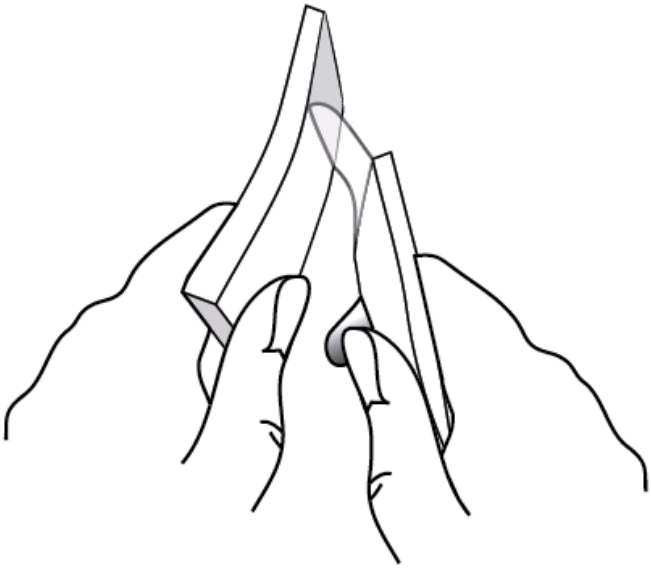


Fig. 1.4. Illustration of peeling off a piece of epidermis from the outer (abaxial) side of an onion bulb leaf.

Identify the cell wall boundaries and the presence of a nucleus. Can you see nucleoli? The partitions separating the cells are the cell walls of adjacent cells. Although they look separate, they are connected via small plasmodesmata.

Stain the tissue with I_2KI to darken the nuclei by adding a drop to one side of the coverslip and placing a paper towel on the other side of the coverslip. The I_2KI will then wick in under the coverslip and into the tissue.

Draw what you see below:

How do the stained cells differ from the non-stained cells?

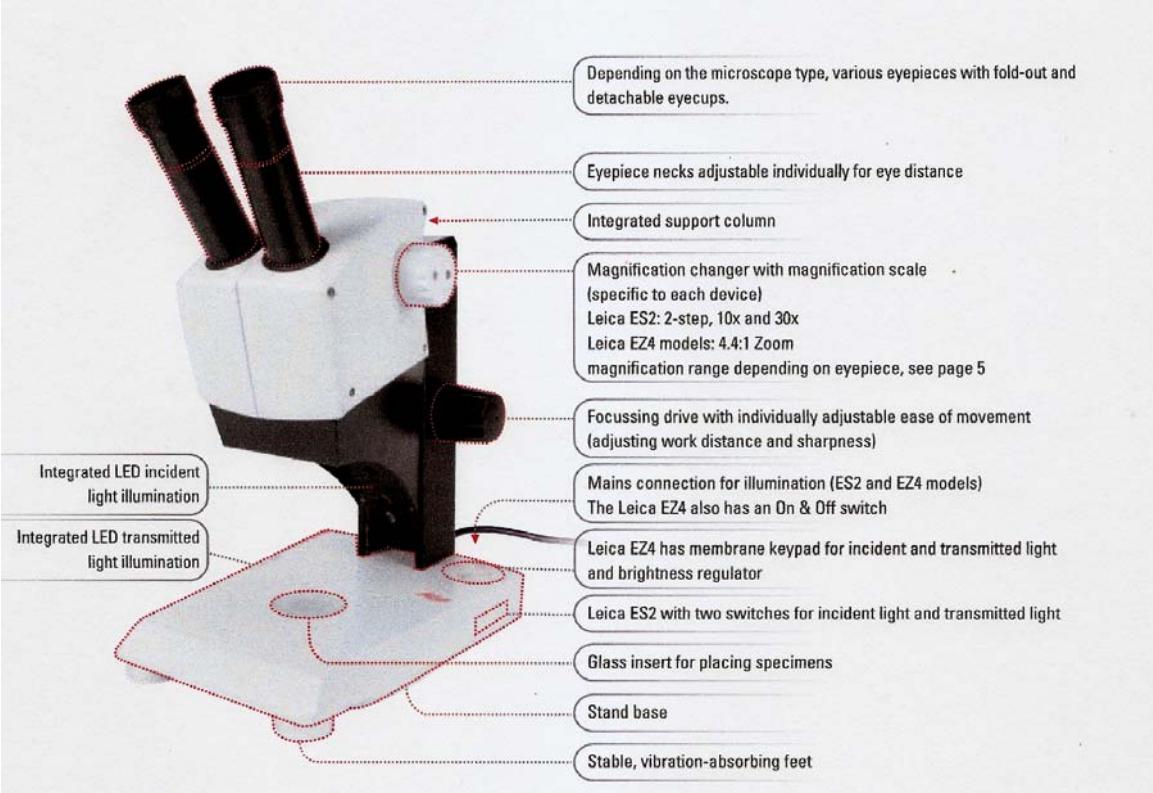
E Proper Use of a Stereo (or Dissecting) Microscope:

The dissecting Leica EZ4 micrscopes have a minimum magnification of 12.8X and a maximum magnification of 56X. This type of microscope (stereoscope) is used to observe the structures present on larger specimens. These scopes can light the specimen from above and below allowing the user to have increased flexibility in making their observations.

The numbers on the top knob of the dissecting scope represent magnification with the lowest magnification at 12.8X and the highest magnification at 56X.

To operate, plug in the power cord, turn on the toggle switch in back, and then press the buttons on the base to illuminate from the top, bottom, or both. The +/- buttons change the intensity of the light.

Place a specimen on the base or in a glass petri dish for analysis. Start your observations at the lowest magnification, focus the image and then increase your magnification. You will need to constantly adjust the focus and light in order to gain the best possible image.



EXPERIMENTAL:

Observe and draw the unique surface structures associated with the provided specimens in lab. Describe what you see:

1) **Stinging Nettle (if available)**: **BE VERY CAREFUL** when handling this plant. The modified trichomes are made of silica and act like hypodermic needles. The tips are coated with acetylcholine and formic acid and cause a strong burning sensation associated with a rash. **ONLY HANDLE WITH GLOVES AND FORCEPS**!!!

Observe and draw the surfaces of the leaves and stem.

2) **Tomato leaves (if available)**: (focus on the variation of trichomes)

3) **Switchgrass or Maiden Hair grass** (focus on the leaf margins and figure out why you can get a paper cut from the leaves)

4) **Crysanthemum** (figure out why they appear soft and have a white coloration)

5) **Leatherleaf Viburnum** (How do the upper and lower leaf surfaces differ? Draw and try to figure out the form of any trichomes you see.)

6) **Avens**. (Note the aggregate of little fruits in this somewhat distant relative of the raspberry. Draw the individual fruitlets and attempt to understand how they collectively work like Velcro to attach themselves to the fur of mammals for dispersal).

7) **Wineberry**. (Observe the trichomes on the stem. How many different types are there? Draw each type. Touch the stem: a sticky texture indicates the presence of glandular trichomes. Do you see any evidence of these, if so draw one).