A. Introduction

The typical compound light microscope (Fig. 1) is capable of increasing our ability to see detail by 1000 times so that objects as small as 0.1 micrometer (um) or 100 nanometers (nm) can be seen. Electron microscopes extend this range further allowing us to see objects as small as 0.5 nm in diameter or roughly 1/200,000th the size we can see with a naked eye. Needless to say, development and use of microscopes has vastly improved our understanding of cells and their structure and function.

Figure 1. Binocular compound microscope.

B. Magnification, Resolution, and Working Distance

*Magnification* is simply a function of making an object appear bigger, such as when we use a hand lens to enlarge printed word. Merely magnifying an object without a simultaneous increase in the amount of detail seen will not provide the viewer with a good image. The ability of a microscope (or eye) to see detail is a function of its *resolving power*. Resolving power is defined as the minimum distance between two objects at which the objects can just be distinguished as separate and is a function of the wavelength of light used and the quality of the optics. In general, the shorter the wavelength of the light source, the higher the resolution of the microscope.

*Working distance* is the distance between the objective lens and the specimen. At low magnification the working distance is relatively long. As you increase the magnification the working distance *decreases* dramatically. Oil immersion lenses practically touch the specimen. Be aware of this change in working distance with increasing magnification so as to prevent damage to your specimens.
C. Parts of the Monocular Compound Light Microscope:

Please take time to familiarize yourself with your microscope and its proper use. The controls of the two makes of microscopes we use in our courses are shown below (Fig. 2).

1. Ocular lens or eyepiece: ours are 10x magnification. The scopes we will use are monocular (one eyepiece only.)

2. Body tube: contains mirrors and prisms which direct the image to the ocular lens.

3. Nosepiece: holds the objective lenses, rotates, note the positive stops for each lens.

4. Objective lenses: usually 3–4 on our scopes, 4x, 10x, 43x, 100x oil immersion (red banding). Total magnification = ocular power x objective power.

5. Stage: platform on which slides are mounted for viewing; some scopes have mechanical stages. Learn how to clip the slide in position properly.

6. Diaphragm: the diaphragm controls the amount of light which passes to the specimen and can drastically affect the focus of the image. LEARN TO USE THE DIAPHRAGM AS QUICKLY AS POSSIBLE. MOST PROBLEMS YOU WILL HAVE FOCUSING WILL BE DUE TO INCORRECT ADJUSTMENT OF LIGHT.

We have two types:

- *iris diaphragm*: Look for a lever just under the stage near the front.
- *dial type*: Just below the stage is a rotating dial having different size apertures (holes); this type is useful for creating a pseudo dark field effect.
7. Focusing knobs: Located on side of microscope; outermost is the fine focus and innermost is the coarse focus.

8. Light source: our scopes have built in light sources. The pushbutton switch is located (most often) behind the light lens on the base.

D. Care and Handling of the Compound Microscope

There are only a few ABSOLUTE rules to observe in caring for the microscopes you will use. Taken care of, these instruments will last many decades and continue to work well. Please report any malfunctions immediately to your instructor.

1. ALWAYS use two hands to carry the scope - one on the arm and one under the base - NO EXCEPTIONS! NEVER carry the scope upside down, for the ocular can and will fall out.

2. Use lens paper to clean all lenses before each lab session and after using the oil immersion lens. DO NOT EVER, NOT NOW, NOT EVER, USE ANYTHING BUT LENS PAPER TO CLEAN THE LENSES. Other papers are too impure and will scratch the optical coating on the lenses. Also, do not use any liquids when cleaning the lenses - LENS PAPER ONLY!

3. Always use the proper focusing technique to avoid ramming the objective lens into a slide - this can break the objective lens and/or ruin an expensive slide.

4. Always turn off the light when not using the scope.

5. Always carefully place the wire out of harm's way. Wires looped in the leg spaces invite a major microscope disaster. Try sliding the wire down through the drawer handles beside your bench space.

6. Always replace the cover on the microscope when you put it away

E. Focusing Procedure: Monocular Compound Microscopes

1. Turn on the light source.

2. Switch to the 10x objective lens.

3. Back off on the coarse focus to raise the nose piece.

4. Place the specimen slide on the stage and secure in the proper position. Look at the slide and place it so the specimen is over the light aperture in the stage.

5. Lower objective lens to lower limit (close to slide). Raise the lens using the coarse focus knob until you see the image come into focus and then go out again, then focus back until you find center focus. Adjust fine focus similarly.

6. Center the image and adjust the light using the diaphragm.

7. Re-center and adjust focus, first coarse, then fine focus as in step 5.
8. Readjust diaphragm as needed.

9. Now switch objectives to the 43x if a higher magnification is needed. Readjust fine focus and light (diaphragm) as needed.

Our scopes are parfocal which means that when you switch from low (100x) to high (430x) power, a focused image at low power will remain more or less in focus at the higher power. Most likely you'll have to readjust the fine focus and diaphragm slightly.

F. Oil Immersion Procedure

On some of our monocular, and all of the binocular compound microscopes, we have 100x oil immersion lenses. These can be identified by a red band around the lens housing. At magnifications greater than about 500x light is refracted too much as it passes through air to yield good resolving power. Thus, optics for these higher magnifications are made to use with a high grade mineral oil as the medium for transmitting light. It is imperative that you use only immersion oil and that you clean the lens thoroughly with lens paper after each use.

1. Locate the region of interest on your slide and center it at 430x.

2. Raise the objective lens to its limit (i.e., maximize the distance between stage and objectives) and swing the lens out of the way about half way to the next position.

3. Carefully place a small drop of immersion oil directly on the slide over the center of the region of interest.

4. Rotate the oil immersion objective into position and, carefully, while looking from the side, lower it using the coarse focus knob until the lens just makes contact with the oil drop. You will see the drop leap up into a column as the contact is made.

5. Lower the lens a smidgen more and then, using the fine focus and looking through the ocular lens, focus on the specimen.

6. When done, clean lens with lens paper until no more oil comes off and clean slide if it is to be saved.

G. Determining Field-of-View Diameter

You may wish to estimate the size of the specimen (e.g., cells) you will see in lab. The best way to do this is with an ocular micrometer, a precision ocular lens insert that has a ruler etched into glass. The monocular scopes we use in the introductory courses are not so equipped, so we will use an alternative method based upon knowing the field-of-view diameter for your particular microscope. To do this, you must determine:

- the approximate diameter of your low magnification field-of-view for your particular microscope.
- the total magnification for each of your other objective lenses.

Knowing this for each objective lens, you can compare the size of the specimen against the known field diameter and make a reasonable estimate of size. This technique works for any microscope.
1. Obtain a slide scale and position it on your scope. A transparent metric ruler will work as well.

2. Bring it into focus using the 10x objective (100x total). The scale bars are increments of 1mm as shown in the figure below. Thus, a black bar = 0.5mm as does a space.

3. Move the slide such that the edge of an outside black bar is just tangent to the lighted field (see point "A" above).

4. Starting at that edge, estimate how many bars and spaces it takes to cross the field-of-view. You will probably have to estimate the last fraction of a space or bar. For most of our microscopes it is approximately 1.8 - 2.0 mm wide. You must check this on any microscope you use that does not have an ocular micrometer.

5. Record your scope’s ID number and field diameter at 100x in your lab notebook for future reference.

6. Next, calculate the field width at 430x total magnification using the following formula (we refer to the 100x mag as "low power" and 430x as "high power"): 

\[(\text{low power mag}/ \text{high power mag}) \times \text{low power field diameter (in mm)}\]

For example, suppose you determine that the 100x field diameter is 1.8 mm, at 430x, the field diameter would be:

\[(100/430) \times 1.8 \text{ mm} = 0.418 \text{ mm} = 418 \text{ um (micrometers)}\]

Note that the field diameter at high power is proportional to the ratio of the low to high power objectives. That is, as you increase magnification, the actual field of view becomes proportionally smaller.

H. Binocular Compound Light Microscopes

Parts of the light Microscope

1. Ocular lens or eyepiece: ours are 10x magnification. The scopes we will use are binocular (two eyepieces).

2. Body tube: contains mirrors and prisms which direct the image to the ocular lenses.

3. Nosepiece: holds the objective lenses, rotates

4. Objective lenses: usually 3-4 on our scopes, 4x, 10x, 43x, 100x oil immersion (red banding). Total magnification = ocular power x objective power. Most of our binocs have fixed position lenses--the stage moves up and down rather then the lens.

5. Stage: Movable platform on which slides are mounted for viewing; all of our scopes have mechanical stages with X,Y Vernier scales. Focus knobs move the stage up and down.

6. Condensor: A substage lens which focus the light on the specimen. Our binocs have condensers that move up and down to focus the light beam.
7. Iris Diaphragm: the diaphragm is located just below the stage and controls the amount of light which passes to the specimen and can drastically affect the focus of the image.

8. Focusing knobs: outermost is the fine focus and innermost is the coarse focus. On the binocs these knobs control up/down movement of the stage.

9. Light source: our scopes have built in light sources. The rheostat ON/OFF switch is located either on the scope or on the external power supply and is used to regulate light intensity.

I. Focusing Procedure: Binocular Compound Microscopes

1. Turn on the light source. Binocular scopes have either a built in unit or an external power supply.

2. Switch to the 10x objective lens.

3. Adjust the coarse focus to raise the nose piece (or lower the stage).

4. Clip the specimen slide on the stage in the proper position.

5. Look at the ocular lenses of your scope. One lens is fixed and the other has a focusing ring (like a pair of binoculars). Bring the lens as close to the slide as possible, then, looking only through the fixed ocular lens, back off until the specimen just comes into focus. Adjust fine focus similarly for the fixed lens.

6. Now, looking only through the adjustable ocular, adjust its focus using the focus ring around the lens. Look with both eyes (adjust for inter-pupillary distance to see a single round lighted field) and make any minor adjustments to focus.

7. Center the image and adjust the light using the condenser lens, iris diaphragm and light source rheostat.

8. Re-center and adjust focus, first coarse, then fine focus as in 5.

9. Readjust diaphragm as needed.

10. Now switch objectives to a higher power. Readjust fine focus and light (diaphragm) as needed.

Our scopes are parfocal which means that when you switch from low to high power, a focused image at low power will remain more or less in focus at the higher power. Most likely you'll have to readjust the fine focus and diaphragm slightly (increase light at higher powers).