Topic 3. Instructions for the Use of the Compound Microscope

Introduction: This is intended to impart a familiarity with the one most important tool you will use in general botany. Our goals encompass both learning a set of factual information about the microscope, and in developing skills in its use as outlined below.

I. The parts of the microscope.

When removing the microscope from the cabinet by grasping the handle of the scope with one hand and placing the other hand below the base. Be careful not to hit the top of the cabinet with the oculars!!! Gently place the scope directly in front of you on your lab bench.

Using the illustration on the next page identify the following parts of your microscope. You should know these parts by their name and function.

The ocular lenses: These are the lenses through which you view. They have the number 10x on their side representing their magnification.

The objective lenses: Each microscope has three objective lenses. Together the objective lens and the oculars form the magnified image to be viewed. The magnification of each objective is written on their side (4x, 10x , 40x).

The mechanical stage: The stage is the platform on which the slide rests. The mechanism attached is the mechanical stage, and allows you to easily move the slide in a controlled fashion.

Condenser lens: This lens is situated below the stage. It is an important optical component of the compound microscope. It focuses light from the light source through the subject and into the objective lens. Its position can be adjusted by a knob on the right side of the microscope body below the stage (locate this knob).

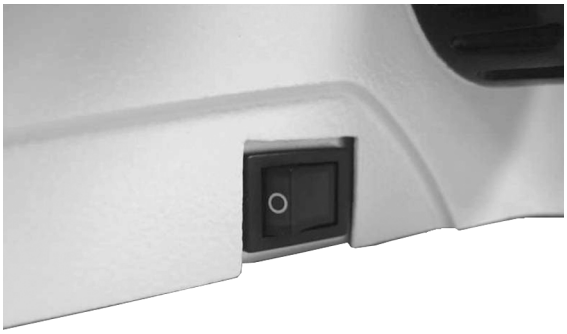
Condenser iris diaphragm lever: The condenser lens has an iris similar to the ones used in cameras. This controls the aperture of the lens. This diaphragm should not be used to control the brightness of the field of view, because this will decrease the detail to be seen in your subject. Use it, instead, to decrease glare to increase contrast (to make the whites whiter and the blacks blacker).

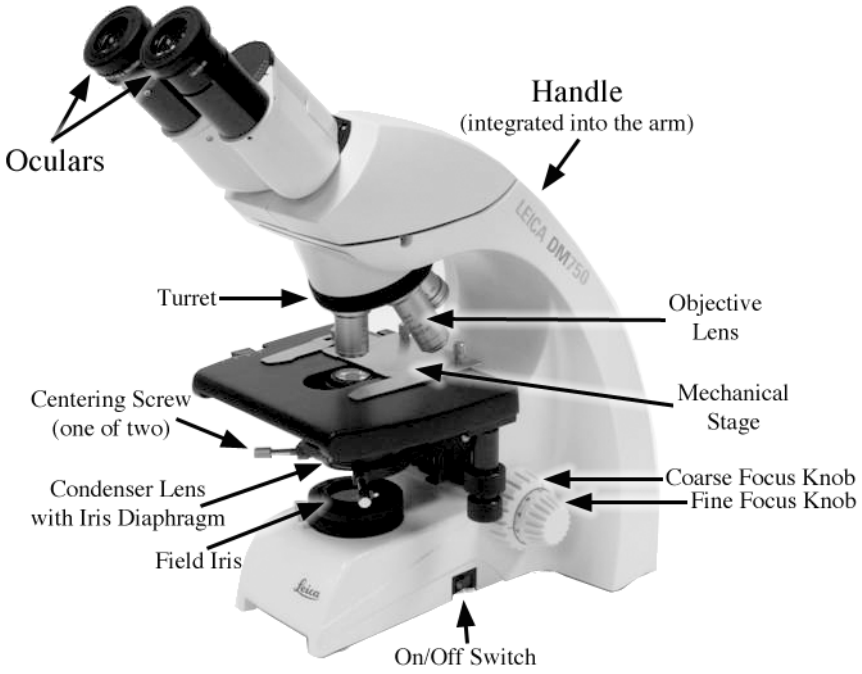
The coarse focus knob: This larger knob is used to get the subject into rough focus. It is to be used only with the low power lens (the 4x lens).

The fine focus knob: This smaller knob is concentric with the coarse focus knob. It allows for fine adjustment of the focus. Because the objective lenses are **parfocal**, this knob is all that is required to bring a subject into focus when switching from one objective to another (All three objectives are designed to bring a subject into focus using the same working distance - lenses with the same working distance - length from slide to lens when the subject is in focus - are **parfocal**) .

On/Off Switch: Turns the light source on or off.

Voltage control dial: Varies the light intensity of the source. Use this dial to control the brightness of the field viewed through the ocular lenses.





II. How to calculate total magnification.

Total magnification is the product of the number written on the ocular and that written on the objective.

X

=100 x

III. Step by step procedure for the use of the microscope.

1. Always start with the low power objective (4x). It allows for a larger field of view and has a longer working distance. Use the coarse focus knob to bring your subject into rough focus and then into crisp focus using the fine focus knob.

2. Adjust for the distance between your eyes by changing the position of the two oculars on the binocular head by gently pulling them apart or pushing them together. Other students use this scope so you will need to make this adjustment every time you use the scope!

3. Adjust for the difference between the acuity of your eyes. Close your right eye and bring the subject into sharp focus for your left eye using the fine focus knob. Now close your left eye and bring the subject into sharp focus using only the focus adjustment ring associated with the right ocular. Other students use this scope so you will need to make this adjustment every time you use the scope!

4. Our objectives are parfocal. When your subject is in focus at 40x, the other two will be positioned to be roughly in focus. When switching to the 10x or 40x objective you need only use the fine focus knob to bring your subject into crisp focus.

5. Adjust the intensity of the brightness of the field using the voltage control dial not the iris diaphragm of the condenser.

6. Adjust the field iris with viewing with your 40x objective.

7. When you are through, reposition the 4x objective into place, wrap the cord around the base and carefully return to the cabinet.

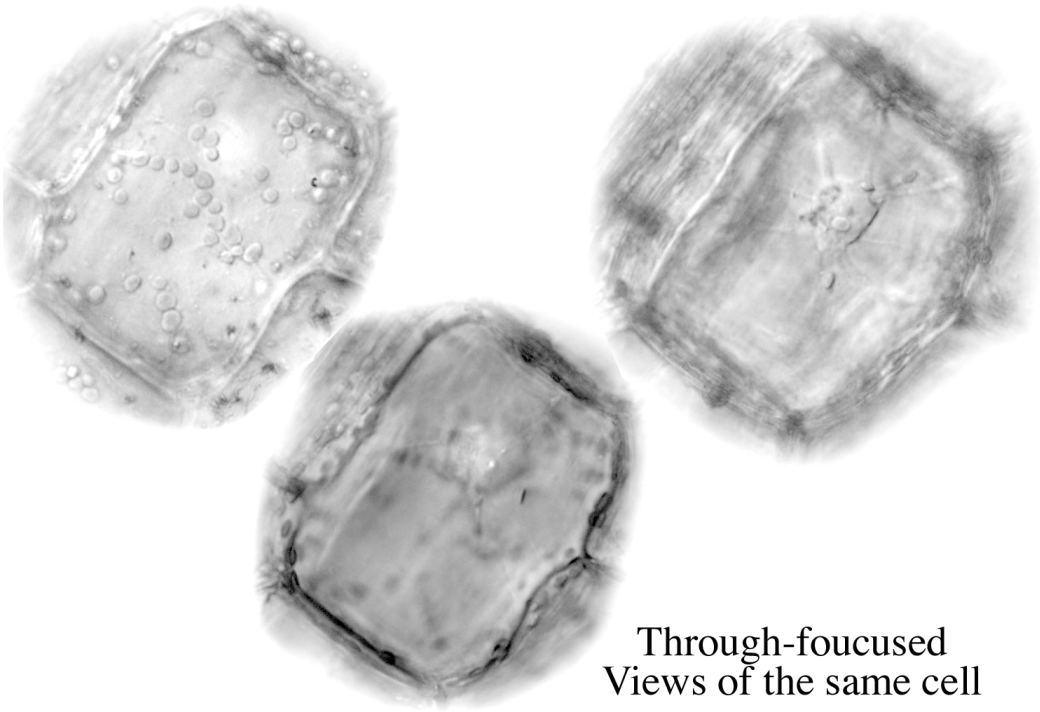
IV. Viewing Newsprint.

Place a piece of dry paper on a slide and view it using the procedure outlined. Note that images are reversed.

V. Through-focusing.

With a microscope, at any one focus setting, you are viewing structures all of which are located in one plane perpendicular to the line of sight. Everything below or above this plane is out of focus and invisible. To "see" the three dimensional structure of your subject, you should be constantly moving your fine focus knob to change the plane of view up and down. This idea can be easily demonstrated using a slide of crossed threads.

Procedure: Place prepared slide of the alga, *Spirogyra* on your stage. Bring the filaments into focus with your 40x objective. Find a pair of filaments that cross. Then, using your fine focus knob, raise your objective so that both filaments are out of focus. Now lower your objective till one of the two filaments comes into focus. This is the one on top. Make a drawing. Now, lower your objective till the second comes into focus. Make a drawing of it.



Through-foucused
Views of the same cell

Draw two views of crossed algal filaments at different focal planes.

VI. Measuring the diameter of the field of view.

It is important to be able to correlate what you observe through the microscope with items observed by the unaided eye. To do this it is essential to gain a sense of scale through the eyepiece.

Procedure: Take the clear ruler from your supply drawer and lay it flat on your stage. View the ruler with the 4x and 10x objective (at 40x and 100x magnifications). Determine the diameter of the field of view for each magnification by directly viewing this scale (in millimeters).

Do not make this observation with the 40x objective (400x magnification). Calculate this value using the following information:

There exists an inverse relation between magnification and the diameter of the field of view, hence

$$\mathbf{M_1/M_2 = D_2/D_1}$$

The equation can be rewritten $\boldsymbol{D_1M_1 = D_2M_2}$

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where D_1= diameter of the field of view at magnification, **M_1**;
and **D_2** = diameter of the field of view at a second magnification **M_2.**

Use your observed diameter at 100x to calculate the one at 400x. Record this value, and the diameter of the other field of views below and on the inside page of the back cover of your lab manual.

40X _____ **100X _____** **400X _____**

VII. Rules for the use of your microscope.

1. Your microscope is a delicate instrument, treat it with care using some common sense. Never slide your scope across the bench as the rubber feet will cause the microscope to vibrate violently!

2. Work with the scope directly on front of you and your notes to the side - not the other way around. By having the scope directly in front of your seat, you can more comfortably and effectively view your subject. Further, there is less chance that the scope may accidentally be knocked off the bench.

3. When sharing a view with your TA, get out of your chair to allow her a view of your subject.

4 Clean the lenses as needed. When the oculars are dirty it is obvious, however, when an objective is dirty, you will simply experience a reduction in the quality of your view. If your view isn't as crisp as you think it should be, check the objective lens. When cleaning lenses use the lens paper provided in your supply drawer and the distilled water in the dropper bottles on each bench. Never use regular tissue, and never rub a lens when it is dry.

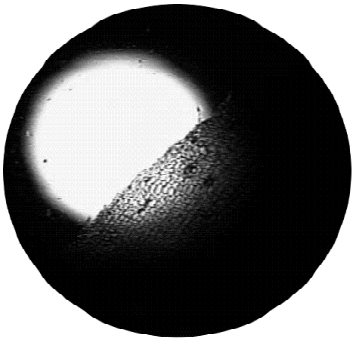
5. If you are experiencing a problem that isn't obvious to you, ask your TA for help.

6. Scopes are assigned to specific seats. Use only the scope at your work station and always return it to the same place. If you discover it missing, inform your TA immediately.

Binocular Head

How to Adjust Your Field Iris

1. Get your subject into focus.

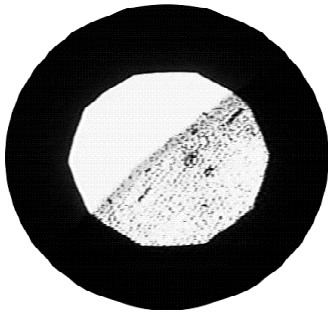


2.

Close down your field iris until the field of view is only partially illuminated

3.

Position your condenser using your condenser knob to bring the outline of the iris opening into focus.

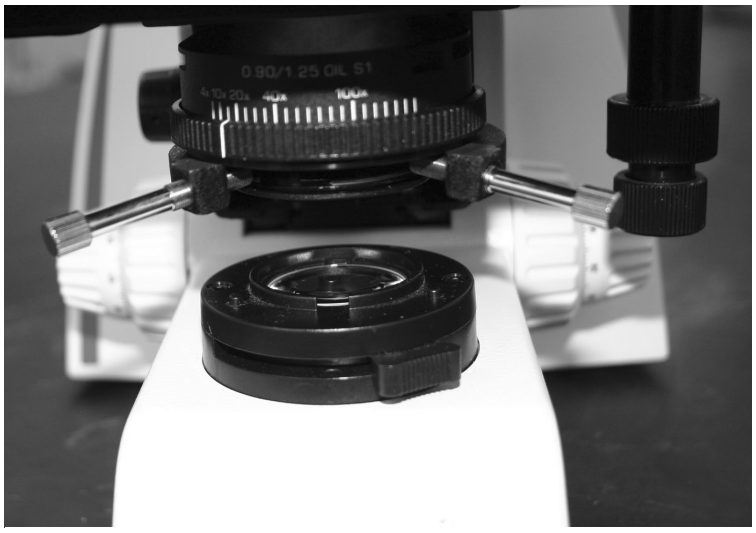


4.

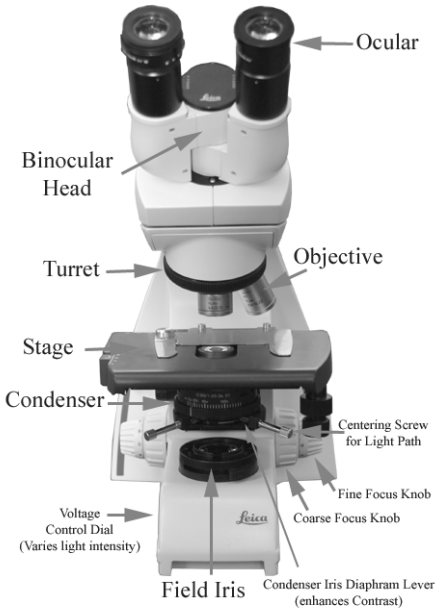
Center the view of this iris using the two screws on the condenser

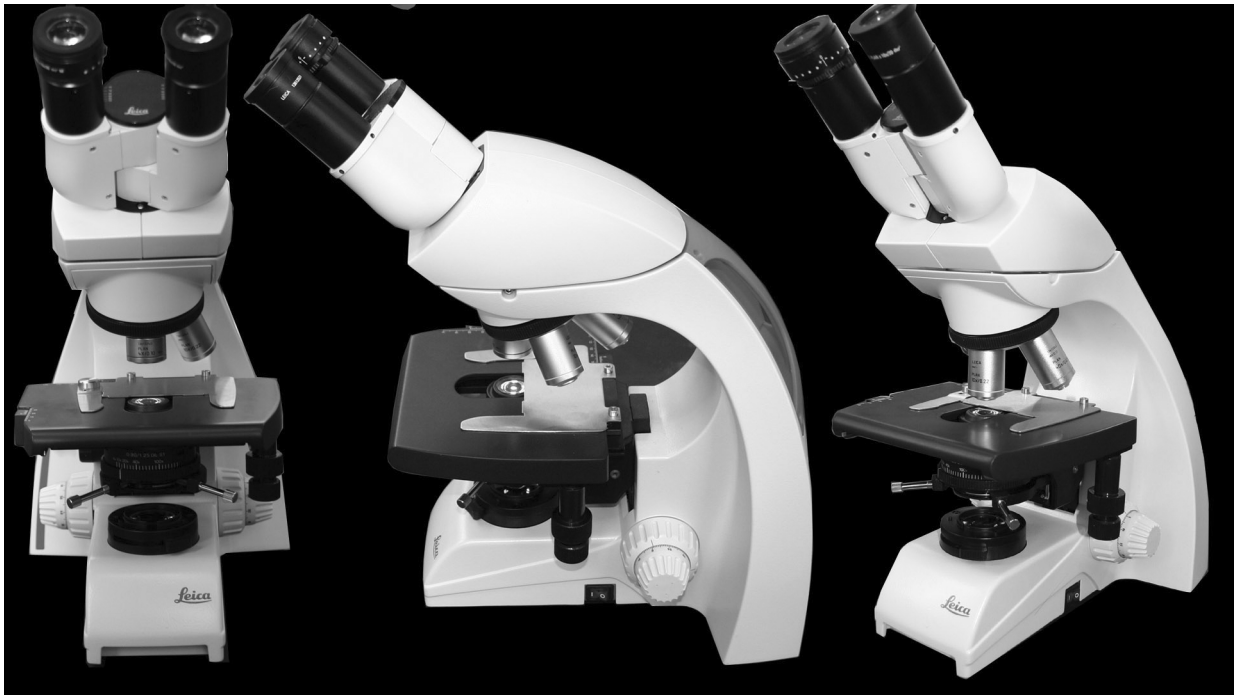
5.

Open up the field iris until the iris image just circumscribes the circle of the field of view.



Screws for centering outline of the field iris





Three Views of Our Microscopes