

Appendix B: The Microscope

If you need to familiarize you with the use of the microscope this will acquaint you with some basics. You may find it helpful to work with someone else on the microscope.

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, but do not be afraid to clean them. There are booklets of lens paper on every table. If the view gets foggy (as it probably will sometime during the semester), and lens paper will not clean it, call your laboratory instructor.

(1) Structure of the Compound Microscope

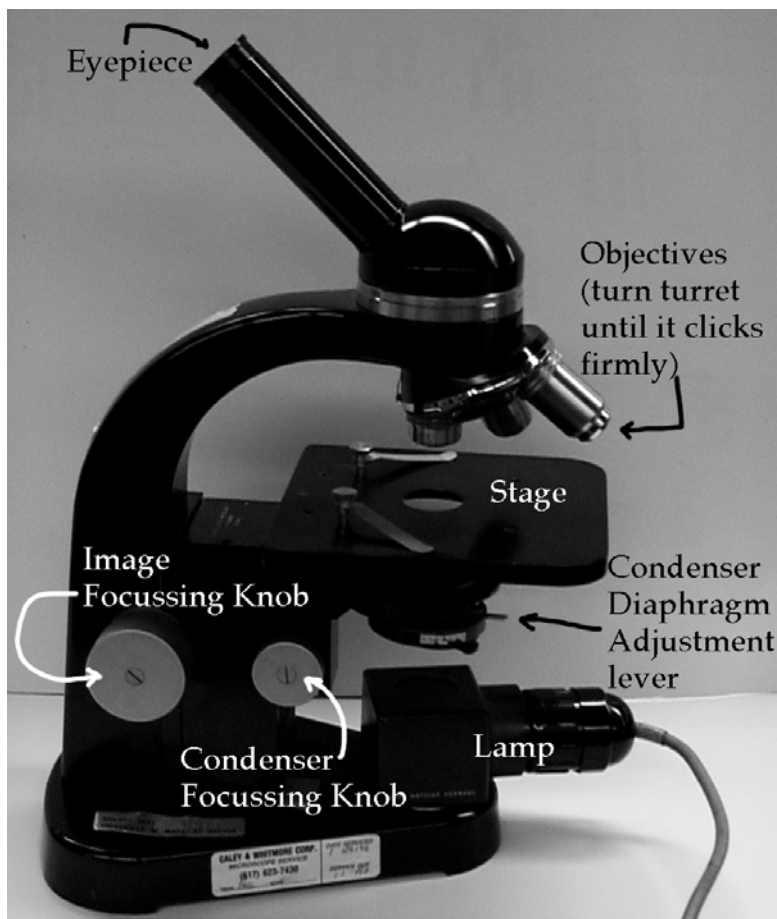
It is very important that you familiarize yourself with the parts of the microscope and their function. Before you use a scope locate all of the parts named in the diagram on the next page, all types of microscopes have these basic parts and you should know their names. Place the microscope so that it is at a right angle to you as you look at it.

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 sub-units:

- A. The system involved in illuminating the object to be viewed, i.e., **light**, diaphragm and condenser.
- B. The lens system - eyepiece, body tube and objective lens which magnify the object.

A. The System of Illumination. Keeping the microscope in the same position: (1) plug it in, (2) turn on the light and (3) move the diaphragm lever as far to the left as possible. 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. 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). 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 eyepiece lens. Basically, the objective lens magnifies the object and forms an image in the tube which is further magnified by the eyepiece 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 so if you are trying to focus on something and much further away or closer, you cannot see it clearly. 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 Total magnification is roughly the product of the magnification of each lens (objective x eyepiece). With 10X objective and 10X eyepiece 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 (3.5 X or 10X objective).

B. Depth of Field The compound microscope has a very limited depth of field. 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.

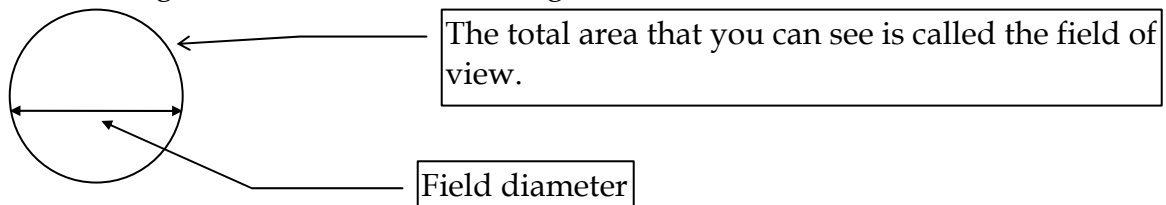
Now turn the microscope around so that you can look through the eyepiece. We are now ready to look at something. You should practice with one of the prepared slides in the lab.

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 move the stage upward when first getting the object in focus (i.e. beware of smashing 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.

How big is it?

You can use the microscope to measure the approximate size of the objects you are looking at. Given the magnification, the table below gives the diameter of the field of view. See diagram:



Once you know that, you can estimate the size of what you're seeing. If the field size is 450µm and the thing you're looking at it half as wide as the field, then it's about 220µm wide. For the microscopes we use:

Magnification shown on objective lens	Actual magnification		Field size (millimeters)	Field size (microns (µm))
3.5x	35x	⇒	5.1	5100
10x	100x	⇒	1.8	1800

40x	400x	⇒	0.45	450
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Tips to Get the Best Use of Your Microscope

1. While obtaining a microscope, carry with one hand around the arm and the other hand under the stage.
2. Plug in the microscope and turn on power using the power switch located on the light source.
3. Make sure the lamp is burning.
4. With **lens paper** clean the eyepiece and objectives. Turn the objective until it clicks to the lowest power. (do not use anything else to clean them with)
5. Obtain a clean slide and coverslip.
6. Pipet 2 to 3 drops of liquid specimen or 1cm² of dry specimen onto the slide. When attempting to obtain fast-moving specimens: On a clean slide make a ring about 1/2 inch in diameter of "Proto-slow". Drop a drop of the specimen in the middle of the ring.
7. Put a coverslip over the specimen:
 - 1) hold it by the narrow edge at an angle
 - 2) let the other edge touch the slide and drag to the border of the sample
 - 3) Lower the coverslip slowly onto the sample, it will result in fewer bubbles under the coverslip.
8. Place the slide onto the stage.
9. Secure the slide with the stage clips, and raise the stage up as high as it can go without touching the lenses.
10. Look through the eyepiece with both eyes open.
11. Move the slide back and forth using low power until your specimen sample is in the center.
12. Use the image focusing knob to bring the specimen into focus, while in low power. Do this by turning it towards you very slowly.
13. Use the Condenser Diaphragm Adjustment lever to control the light flow and improve the contrast.
14. When done viewing lower the stage downward with the Image Focusing knob completely and put objectives into lowest power.
15. Remove the slide and dispose of the specimen properly, and clean the slide.
16. Clean the lens with the lens paper.
17. Turn power off; unplug the microscope and return to proper place.

Other Tips:

- Don't move the microscope around after you start to view.
- Control the amount of light entering the microscope - the contrast at which you view the specimen will control how much detail you view.
- Keep the lens clean. A clean microscope will work better.
- Keep an eye on the stage while using the Image Focusing Knob so that you don't put the objective through the slide.
- Use a clean pipet and slide for each specimen. This will cut down on contamination.
- Become familiar with how the microscope works before trying to view a specimen.
- To help with finding dust: turn the eyepiece, if particles move, then there is dust on the eyepiece that needs to be cleaned. Repeat this process for each Objective lens, Condenser Diaphragm Lever, and Image and Condenser Focusing Knobs.

- Ask for help.