

The Microscope

The microscope is an essential first line tool in the clinical microbiology lab. It is used to obtain some basic information about the clinical sample:

- Is there more than one organism in the sample?
- What is their shape and arrangement?
- How does the organism(s) respond to standard stains such as the gram stain?

This information is important for the microbiologist in deciding what further tests are appropriate in identifying the organism. There is considerable fiscal pressure to do the minimum number of tests necessary.

You will be using bright-field illumination in this lab. There are several other microscopy techniques used in clinical or research labs. Carefully read the section in the text on the principle of how bright-field microscopy works.

- Where does the light come from?
- How is it focused on the specimen?
- How do you determine the actual magnification of the specimen you are looking at?

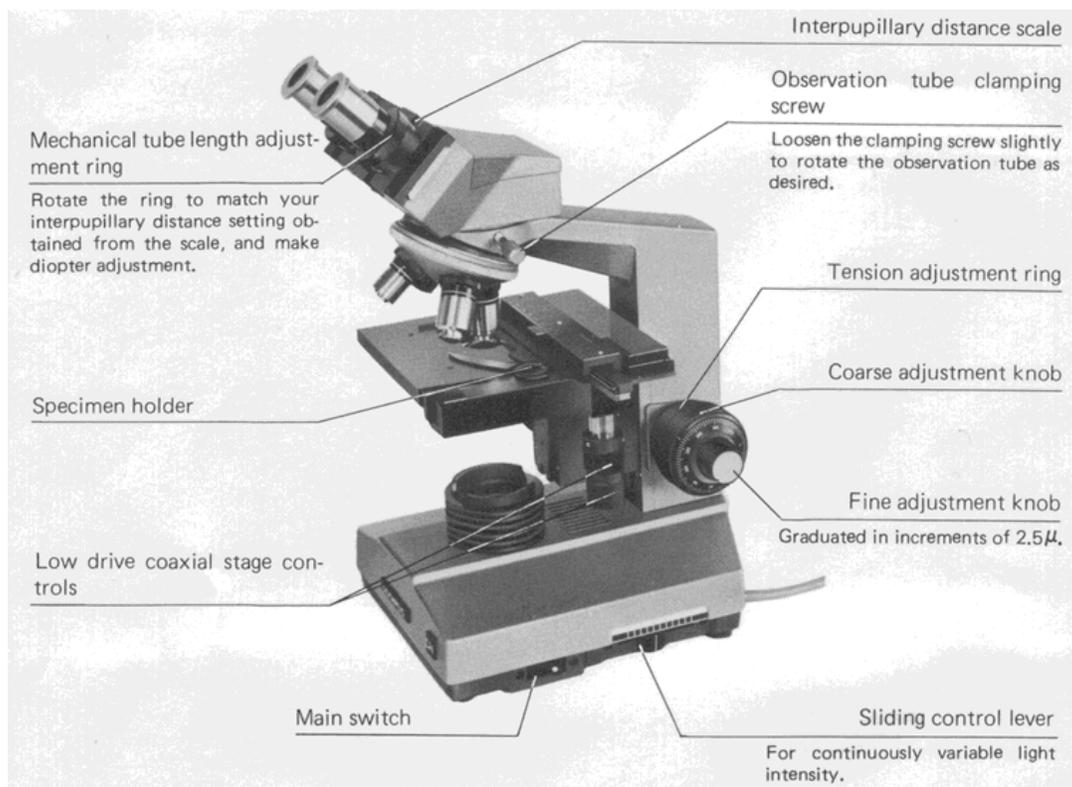
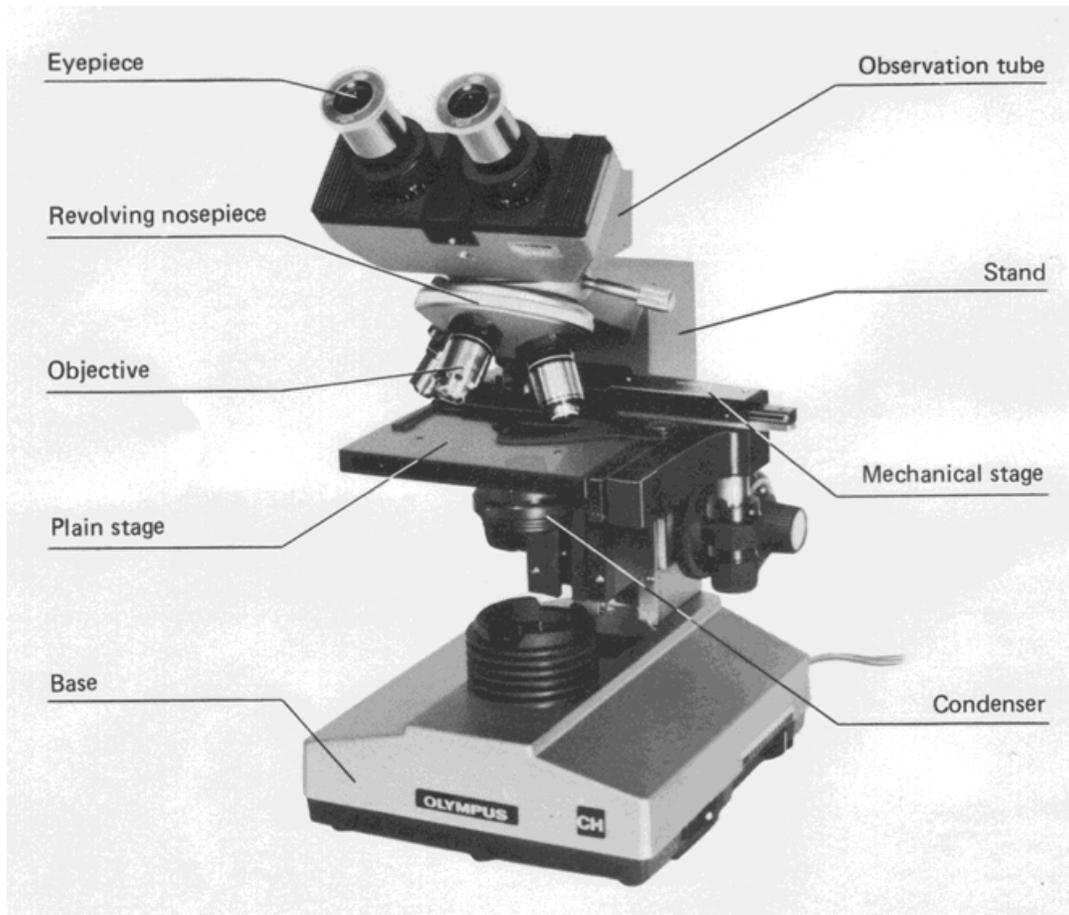
This lab focuses on the practical aspects of using the microscope for microbiology. Hopefully, this is a review of what you learned about the microscope in your previous biology labs.

Care of the microscope

The microscope is a tool. Your skill in using this tool and the care you (and the rest of the students) give it will directly influence your success in lab. The lab microscopes cost close to \$2,000. The microscope specialist that services our scopes charges over \$100/hour (and you wondered where your lab fee goes!). We have them professionally cleaned and serviced twice a year. Please take care of them.

You will be assigned a scope based on your lab bench seat. The seats are all numbered. If you leave your scope dirty, the next person using it will have problems. They will most likely notify their instructor or IA and the dirty scope will be traced back to you. Leaving your workplace tidy for the next section (or shift) will be greatly appreciated. The following information outlines the proper care of the scope. It would be useful for you to review the labeled picture of the microscope parts.

Parts of the Microscope



Transport and storage of the scope

Always carry the microscope with one hand on the base and your other hand holding the arm. Check to see that the power cord is wrapped around the base of the scope. If it falls loose, stop walking and pick it up before you trip over the cord.

Set the scope on the table with the arm and all the control knobs facing away from you. The ocular head should be facing the inside of the table. Loosen the head screw if necessary and swing the ocular head to face you. Retighten the screw. You will be looking through the scope for a long time. Make your equipment work for you. Pay attention to the ergonomics of the scope and your chair. The only reason the eyepieces are over the arm is to protect the ocular lens and head from smashing against the cabinet during storage. (A new head costs almost \$500.)

The outlets at the benches are inconveniently located. Be sure to wrap any extra cord around the table leg so it is not dragging on the floor. We have had more than one student and instructor go sprawling because they caught their foot on the cord as they walked by the bench.

When you are done with your scope take a few minutes to thoroughly clean the lenses, turn the ocular head back onto itself, wrap the cord around the base of the scope, turn the objective lenses so that the 4x lens is facing out, and adjust the slide holder so it is not sticking way out. The 4x is the shortest lens and should not bash against the back of the cabinet. Using both hands, replace the scope in its appropriate spot. The next student to use that scope will appreciate your care.

Viewing your specimen

You will be observing either prepared slides with coverslips or fresh smears that you have made without coverslips. You will mostly view smeared and stained bacteria. They must be viewed with the 100x lens using oil immersion because of their small size. Even then they are still pretty small. It is absolutely essential you learn to view your slides with oil immersion. Do not rely on your lab partner to get your specimens in focus for you.

Place your properly prepared smeared and stained slide in the stage holder. Be sure that you have the smear facing up. If you have forgotten which side of the slide you put the smear on, scratch the surface of it with your loop or needle. If a little of the smear comes off, you have the correct side up. You will not be able to focus your sample if the smear is on the bottom of the slide. This happens more often than you think!

Position the middle of the stained smear directly over the hole above the condenser. Be sure that your scope is turned on. Start with your 4x lens over the specimen and focus in on the smeared blob. You will only be able to get the whole stained area in focus. Look for sharp edge contrast. You will probably do most of your focusing with the coarse focus knob.

Move your 10x lens over the slide. Turn the fine focus knob until the image is once again sharp. If you are viewing bacteria, the magnification is still too low to clearly see the cells. You will still be focusing on a blobby stained area. You should only need to slightly refocus with the fine knob each time you change the objective. You may want to re-center your slide so there is a distinct object directly in the center of view. Remember as your magnification goes up, your field of view gets smaller. If what you want to see is over at the edge of your slide, it will disappear from view as you increase the objective magnification.

Now move the 40x lens over the slide. Again, carefully refocus with the fine focus knob and reposition your image if necessary. If you cannot easily focus your specimen with the 40x lens, the lens is probably smeared with oil. Please ask the IA or instructor to check and clean the lens.

Now this is where it gets a little tricky and real messy if you goof. You have to pay attention to which direction you swing the objective lens. Partially turn the 40x objective to the side. Do not click it into the next spot yet. Place **one** drop of immersion oil on the slide in the very center of the field of view. **Do not let the dropper actually touch the slide or you may contaminate the immersion oil bottle.**

Swing the 100x lens into position. The bottom of the lens will make contact with the oil drop and form a little column. Look at the slide through the microscope. It will most likely be slightly out of focus. Using the fine adjustment knob only, slowly turn the knob to adjust the focus until your specimen is clear. Just move it a little bit, no big movements. If you move it up too far you will lose the connection with the oil. If you move it down too far, you will at best break your slide and at worst ruin the lens. If you have tried everything and still can't get it in focus, your 100x lens is probably dirty. Oil left on the lens will form a hard residue that can only be removed with ether. Your instructor or IA will need to clean the objective.

When you are done viewing your slide, lower the stage, turn the 100x objective lens off the slide so the 4X lens will be over the slide. If you turn the objective head in the 40x lens direction you will probably drag the lens through the oil. Proceed to cleaning up your scope.

Cleaning your microscope

Do not underestimate the importance of cleaning your microscope. Remember that kleenex is cheap in comparison to the cost of a new lens and your frustration with lab if you can't see your specimens! Only use kleenex on the lens. **NEVER** use paper towels.

Use a fresh piece of kleenex and wipe off the excess oil from the lens with one swipe. Continue to fold the lens paper over to a clean section and keep wiping the lens, one swipe per clean spot of kleenex, until you don't see any oil residue.

Moisten a **NEW** piece of kleenex with the lens cleaning solution and wipe the lens off in a circular motion.

Use a **NEW** piece of kleenex and dry the lens off.

Follow the same procedure for your 40x lens if you think it has been accidentally coated with oil.

ALWAYS, ALWAYS use a new piece of kleenex to wipe off your lens. With proper attention when using oil immersion you should never need to clean the 4x or 10x lenses.