

The Microscope



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The history of the invention of the microscope will probably never be completely settled. The use of lenses for spectacles is reported as far back as the 1200's in Florence, Italy. It's known that in the 1500's the Dutch became adept at the construction of lenses. Hans Martensz Jansen (d. 1592) or his son, Sacharias Jansen (1588-1631?) are credited by various sources as the inventor of the microscope. Both were Dutch lens makers. In any case, the telescope came on the scene close to this time.

In 1608, Hans Lippershey applied for a patent for a magnifying tube which made objects far away appear very close. It was Galileo who made modifications to the telescope and used it in a systematic way to study the heavens. In 1625, John Faber, an English naturalist is credited with naming the instrument. It's a little muddled as to whether the first microscope was a simple microscope with a single lens or a compound microscope using two or more lenses. Around 1665, Robert Hooke began to use a compound microscope for scientific observations and around 1668 Anton van Leeuwenhoek began grinding lenses to fashion a simple microscope with single lenses. It's also reported he used the magnifying properties of water to produce his first microscope. Van Leeuwenhoek is considered to be the father of microbiology for his work with the microscope.

Getting Your Microscope to Your Position

You will be assigned a microscope for use throughout the semester. You are responsible for making sure the microscope is in good repair and is always returned to its correct position in the microscope cabinet. Always carry the microscope with one hand under the base of the scope and one hand on the arm (there's a convenient hand catch on the back of the arm of the microscope). Always carry the microscope with two hands - one on the base, one on the arm - in an upright position. Never tilt the scope. The eyepieces or oculars may fall out and break.

Once you have the scope at your position, remove the cover, fold it and place it somewhere out of the way. Unwrap only the amount of electrical cord necessary to reach the plug. Any excess cord simply serves as an accident waiting to happen.

Parts of the Microscope

You need to be able to speak the language of the microscope. Most instructions on the use of the microscope will assume you know the terminology.



Eye Piece or Ocular

Eye pieces or oculars are what you use to look through the scope. You are using a compound microscope so there will be other lenses used in conjunction with the oculars. Some microscopes have only one ocular, others may have one for the student and one pointing straight up for the instructor (a teaching microscope) and yours is a binocular microscope - you have two eyepieces through which to look. Look carefully at the oculars. You should see a number 10 written somewhere on the oculars. This means that a single ocular, by itself, will magnify an image 10x its normal size. Also note that one of the eyepieces has a ridged ring around it. This will be discussed later.

Body Tube

Depending on the microscope, the body tube can be quite elongate or very short. In most modern day scopes, the body tube has been reduced to a very short section just beneath the oculars. In your scope, the body tube is a triangular, black structure just beneath the oculars. The body tube contains a triangular shaped piece of glass (a prism) which bends light from the light source at the base of your microscope towards your eyes through the oculars. Note your oculars are angled towards you. If it were not for the prism, the oculars would have to be straight up and down in alignment with the objectives and light source. The prism very simply allows you to sit at your desk and view an image vertical to you. Microscopes are often very well constructed, however the body tube is a weak point. The prism is held in position by a series of clips. If you knock the scope about too much, the prism will be knocked out of alignment and you will not see anything through the oculars.

Revolving Nosepiece

Just below the body tube and oculars is the revolving nosepiece. It's so named because it rotates around the axis of the body tube.

Objectives

Attached to the revolving nosepiece are four teat-like structures called the objectives. These are another set of lenses used in conjunction with the oculars. The four objectives are, in order of lowest to highest magnification: the scanning lens, low power, high power and oil immersion.

- Scanning Objective. Rotate the revolving nosepiece to the shortest objective. Make sure it LOCKS or clicks into position directly over the hole in the stage. It should be a red banded objective and have a mark somewhere on the objective the number 4. This means used by itself, the scanning objective would magnify an image 4x its normal size. However, remember you are using the objective in conjunction with the 10x ocular. Used together, the scanning objective and the eyepiece provide 40x magnification.
- Low Power. Next, rotate to the next shortest objective and lock it into position directly over the hole in the stage. This is the yellow banded objective. You should see the number 10 written somewhere on the objective. This means, in conjunction with the ocular, you should be magnifying an image 100x its normal size.
- High Power. Rotate and lock into position the next shortest objective, the blue banded one.



This is high power and you should see the number 40 written somewhere on the objective. With the eyepiece, this provides 400 magnification.

- **Oil Immersion.** The last objective is the oil immersion objective or simply the oil objective. It is black and white banded and has the number 100 somewhere on the objective. With the ocular, you will obtain 1000 magnification. Actually, this is too much magnification under the conditions of the laboratory. The magnification is so great that air currents in the room distort the image. To prevent this, the objective is used with immersion oil - a very clear oil. You place a drop of immersion oil on the slide and run the objective down into it. This prevents any air currents from distorting the image. You cannot use the oil immersion objective without immersion oil. **CAUTION!** The oil immersion objective is a sealed objective - both water proof and oil proof. The other three objectives, scanning lens, low and high power, are not. NEVER use immersion oil on those objectives. Always remove the oil on the slide before using scanning, low or high power. When finished using the oil immersion, always remove the oil in the manner prescribed under the topic "Cleaning the Microscope."



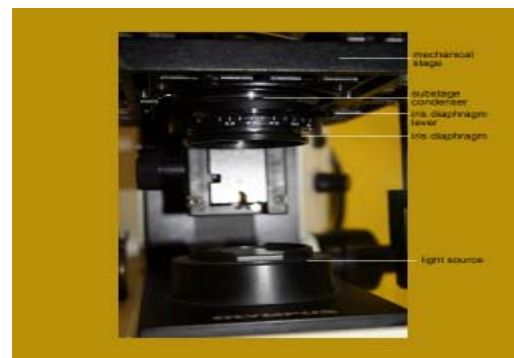
Mechanical Stage

You have the luxury of a mechanical stage. The stage is the platform upon which you place your slide. The slide is attached to the stage by stage clips. Older, simpler scopes used a pair of metal clips located to either side of the hole in the stage. These were very difficult to adjust and use. Your microscope has a simple clamping device. Simply pinch the lever arm of the clamp and place your slide against the clip. Release the lever arm and the slide should be held tight against the back of the clip. Now comes the magic. With the older model scopes the stage clips had to be moved to maneuver the slide from one position to the next. Unfortunately, this was awkward and difficult to do. Your stage is called a mechanical stage because under the stage on the left side is a set of knobs. The larger, top knob moves the stage out from you and back towards you. The smaller, bottom knob moves the stage right and left to your position. Using both knobs you can easily position the slide on the stage in the exact position you desire.



Substage Condenser

Look through the hole in the stage. You should see another lens system. This is the substage condenser and it is designed to take the broad beam of light coming from your light source at the base of the microscope



and condense it into a narrow beam of light coming through the stage.

Iris Diaphragm and Lever

Look up under the stage of the microscope. You should see a lever arm sticking out. Look up under the substage condenser and move the lever arm back and forth. You should see an opening get larger in diameter and smaller in diameter as you move the lever. This is the iris diaphragm.

Just as the irises of your eyes adjust to the light intensity in a room, the iris diaphragm on your scope regulates the

amount of light which enters the substage condenser. Unfortunately, the iris on the scope is not automatic like your eyes. You must adjust the light manually with the iris diaphragm lever.

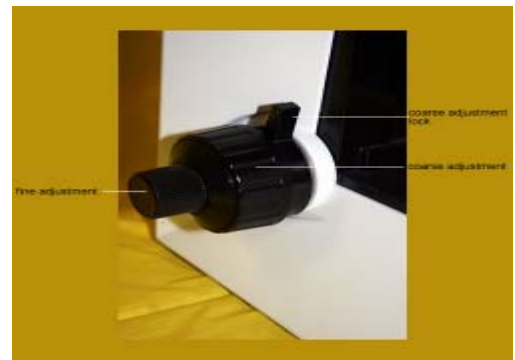


Light Switch and Rheostat

Look on the left side of your microscope. You will see a toggle switch and above it a cylindrical knob. The toggle switch is the on/off switch for your light source and the cylindrical knob is the rheostat for the light. In essence, the rheostat serves as a dimmer switch. Turn on the light and adjust the rheostat to get light coming up through the substage condenser.

Coarse Adjustment

To either side of the microscope towards the base are two sets of knobs, one within the other. The larger knob is called the coarse adjustment. Move the coarse adjustment back and forth and you should see the mechanical stage mover rapidly up and down. The coarse adjustment is used to get object your are trying to view in rough focus.



Fine Adjustment

The smaller inside knob to the coarse adjustment is called the fine adjustment. You will use the fine adjustment to get the object you are trying to see into perfect focus.

Coarse Adjustment Lock

On the left side of the microscope of the coarse adjustment knob is a lever. This is the coarse adjustment lock. You only use coarse adjustment once with each slide. The coarse adjustment lock prevents the coarse adjustment from accidentally being moved once you have an object in focus. More about its use later.

Cleaning the Microscope

You will need to clean your microscope oculars, objectives and substage condenser before beginning. To clean your microscope you will need lens paper. This is a specially designed paper which will not scratch any of the lenses. Remove a single sheet of lens paper from the booklet and fold lengthwise. Place your forefinger on one side and your thumb and middle finger on the other side. Place the lens

paper surface down on one of the oculars. **DO NOT USE A CIRCULAR MOTION!** Instead, press hard against the ocular and make one swipe across the ocular. Now change sides with the lens paper and use the other surface. You never use the same surface of a piece of lens paper more than once. Press hard against the ocular and swipe again. You've now used two of the surfaces of the lens paper. Simply fold the paper back against itself and you now have two new unused surfaces. Clean the other eyepiece as before.

You cannot clean the scanning objective. The lens is recessed and you cannot reach it. You can, however, clean the low, high and oil immersion objectives. Repeat the process for the ocular but in this case you are pushing up against the objectives, not down. It's often easier if you rotate the objective to be cleaned to the front of the scope and lower the mechanical stage so you can get to it easily.

You can also clean the substage condenser. Just don't push as hard. It sometimes pops out if the tightening screw is not adjusted correctly. You will also need to clean your slides - just don't press on the coverslip too hard.

For really stubborn dirt, add a drop of lens cleaner to the lens paper and swipe. Now dry the lens with another fresh piece of lens paper.

How to Begin All Microscope Work in Lab

There are three rules to begin all microscope work in lab. You need to follow all three rules at the beginning of the use of each new slide, otherwise, you will have a very frustrating experience with your microscope work in lab.

1. Begin with the scanning objective locked into position directly over the hole in the stage.
2. Close the iris diaphragm to the lowest possible light coming through the substage condenser.
3. Raise the mechanical stage using the coarse adjustment until it will go no higher.

If you are not at these three positions, you are not going to have much success with the microscope. When you have completed all microscope work in the lab, be sure to return the scope to these three positions prior to putting the microscope back into the microscope cabinet. You want the microscope available to you next lab in the same position.

Some Other Things to Consider with Your Microscope

One feature of your microscope is resolution. Resolution is the ability to discriminate details. Another way of putting it is "How close can you place two dots on a piece of paper and still see two dots?" A microscope with good resolution allows you to separate details at extremely small distances. Your microscope has adequate resolution for the material you will be viewing in lab. The better the resolution of the lenses, the higher the cost of the microscope.

Color aberration is also a problem. All lenses bend light and separate light into its various frequencies. You often run into a problem with living material. Just because an object in a living specimen looks a particular color, it does not mean it is actually that color. Your lenses are not color corrected, therefore there is a slight color aberration. If for example you look at the eyespot of a *Euglena* it

may appear orange in the microscope. It may or may not be orange. It may be more toward the red end of the spectrum or the yellow end of the spectrum. In preserved material, the tissues or cells are often stained a particular color for emphasis. Therefore, in a prepared slide if something looks red, it really is red because it was stained that color.

Focusing Your Microscope

Assume you have a slide on your mechanical stage. Remember, you should (1) have the scanning lens locked directly into position over the hole in the stage (2) the iris diaphragm closed down and (3) the mechanical stage run all the way up. Make sure what you are trying to view on the slide is directly over the hole in the stage. While looking into your eyepieces you should lower the stage using the coarse adjustment until something brushes into your field of view. STOP! Remove your hand from the coarse adjustment and use the fine adjustment to get it into perfect focus. If this does not work, move the mechanical stage back to the upper most position, and slowly lower the stage with the coarse adjustment until something brushes past your field of view. Again, use the fine adjustment until it comes into focus. You may or may not have to move the slide somewhat if it doesn't come into focus. Use the coarse adjustment lock lever and push it down to lock the coarse adjustment into position. You will not need to use coarse adjustment again until you change slides.

Adjusting the Oculars

Remember, you are using a binocular scope. The problem is each person's eye width is different. To accommodate the differences in the width of different peoples' eyes, the oculars can be spread further apart or brought closer together. You now need to adjust the eye width of the oculars so they are suited to your eyes. If you get the width correctly adjusted, you should see a single circle of light coming from the eyepieces or oculars. If you see two overlapping circles, something's wrong. You will also need to be concerned about the distance your eyes are from the oculars. Practice moving the oculars' width and the distance your eyes are from the oculars until you get a single circle of light from the oculars. This is essential. Unless you do this, you will end up with a pretty bad headache by the end of the period.



Now that you have a single circle of light coming to your eyes, you need to adjust for the differences in vision of each of your eyes. Some people have different vision in the left and right eyes. Remember the adjustment ring on the left ocular? First, focus with the fine adjustment until the right eye sees the object in perfect focus. Now using the adjustment ring, focus the left eye. At this point both eyes should be in perfect focus even if you see differently in real life through the left and right eyes.

Going From Scanning to Low Power

Once you have your object in perfect focus on scanning, make your observations. If you wish to go to the next highest magnification you will need to maneuver the stage until the object you wish to view is directly in the center of field of view. Then and only then rotate to the low power objective. There is a possibility you will have to increase the amount of light through the iris diaphragm. The higher the magnification, the more light is required in the scope.

Parfocal Microscopes

Your microscope is said to be parfocal. That means you can go from one magnification to another without readjusting the coarse adjustment. Simply focus on one magnification, use the mechanical stage to center the object viewed in the center of field of view and rotate to the next magnification. You should only need the fine adjustment to see it perfectly at the higher magnification.

CAUTION! Don't go from scanning to high. You must go in order: scanning, low, and high.

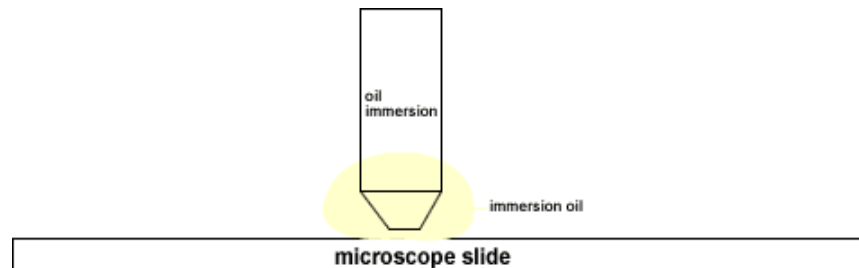
Going from Low to High Power

The same situation applies to going from low to high. On low, get it in perfect focus with the fine adjustment. Use the mechanical stage to move the object to be viewed directly in the center of field of view. Then rotate to high power and refocus using the fine adjustment. Again, you may have to increase the amount of light entering the scope *via* the iris diaphragm. Remember, you only use coarse adjustment once per slide - at the beginning on scanning.

You can go from high to scanning but it's recommended you follow the magnifications down in size as well, *e.g.* high to low to scanning. That keeps you from getting too lost in a very small world.

Oil Immersion

Oil immersion does not follow the above outline. You will use oil immersion in lab only when the instructor specifically tells you to do so. To use oil immersion, lower the stage so you can easily reach the slide. Add a single drop of oil to the cover-



slip of the slide. Make sure you have enough light coming through the iris diaphragm. With your eye at slide level, begin to raise the mechanical stage with the coarse adjustment until the end of the oil immersion objective touches the oil. Make sure there is complete contact between the oil and the objective. Now looking into the oculars use the fine adjustment to try to find the object. Repeat the process until you can get the object in focus. You may need further help from your instructor. When finished with oil immersion, remember to clean both the slide and the objective with lens cleaner and lens paper and make sure there is no oil residue anywhere on the objective, slide or stage.

Types of Slides

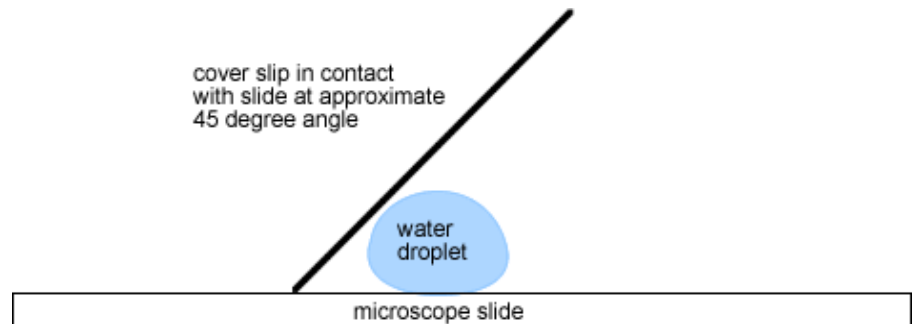
There are two basic types of slides used in lab. First there is the permanent or prepared slide. This one is already made for you with a coverslip and a label. The object on the slide may or may not be stained. What makes the slide "permanent" is the object to be viewed has been mounted in a air proof medium. The mounting medium of choice is Canada balsam - tree sap from the balsam fir. It's almost perfectly clear. When you place any object in Canada balsam it keeps oxygen from the object. Oxygen is what causes degeneration of tissues and cells.

The other type of slide is called a wet mount or temporary slide. You make this for use for that day in lab and at the end of the day you clean the slide off. To make a good wet mount, you need a very clean slide. Even new packages of slides which are labeled "pre-cleaned" are dirty. So first, remove a

blank slide from the pack and wash it thoroughly in soap and water at the sink. Rinse thoroughly and then blot most of the water from the slide using paper towels. Do not dry it completely. Take the slide to your position and use Kimwipes® to completely dry the slide. Kimwipes® have less lint and leave less on the slide.

If the object you are to view in the slide is “dry” add a single drop of distilled water to the slide. It should form a perfect “bead” of water. Now place the object in that bead. To place a cover slip on the slide use the plastic cover slips (you’ll need to clean these also) provided. Take a single clean coverslip and hold it at a 45 degree angle to the slide. Touch the surface of the coverslip to the slide and drag it towards the

water. As soon as the water and coverslip come in contact, drop the coverslip. It should push any air out as it drops and prevent air bubbles from forming in your wet mount. This takes practice but you should have it in no time.



Remember, it’s temporary. At the end of the period, remove the coverslip and discard in the trash. You will need to wash and dry the slide and replace it in the box. Never replace the slide while wet. The slides stick together after that and cannot be easily separated.

After You’re Finished

After completing your microscope work, remember to place the microscope back in its proper place in the microscope cabinet with the scanning lens directly over the hole in the stage, the iris diaphragm closed and the mechanical stage run all the way up.

Exercises

You will view three slides in lab today. Two are prepared or permanent and the other is a wet mount.

Prepared Slide of the Letter “e”

A permanent slide has been made of a newspaper letter “e”. It has been mounted in Canada balsam and a cover slip placed on top. You need to get the slide in focus on scanning, low and high power - in that order. First clean the slide with lens cleaner and a Kimwipe®. Place in on the mechanical stage and lock it into position. Rotate to the scanning lens and lock it into place. Close the iris diaphragm down and raise the mechanical stage upward until it will go no further. Maneuver the stage until you can see the letter “e” through the hole in the stage. Slowly back the stage down with the coarse adjustment while looking into the oculars until it comes into view. Remember to adjust the oculars for your eye width and left/right eye focus. Use the fine adjustment to get it into perfect focus. Adjust the light with the iris diaphragm as needed. You don’t won’t too much light or it will give you a headache. Lock the coarse adjustment into position using the coarse adjustment lever. Call your instructor over and have them check you image on scanning.

Once approved by the instructor, try your hand at low power. Maneuver the mechanical stage clip until the “e” appears dead center in your field of view. Simply rotate to low power and use the fine

adjustment. You may need to increase the light with the iris diaphragm lever.

To go to high power, again maneuver the mechanical stage clip until the “e” is dead center of your field of view. Rotate to high power and use the fine adjustment to get it into perfect focus. You will again need to adjust the light intensity with the iris diaphragm lever. Note how little of the letter you can see on high. Do not go to oil immersion.

What happens to the image in the oculars when you move the stage away from you?

What happens to the image in the oculars when you move the stage to the left?

What you are experiencing is the reversal of movement in the microscope. If you move an image to the left, it shifts to the right in your field of view. If you move it up, the image goes down. This takes a little getting used to.

Prepared Slide of Silk Threads

You will now view a slide of three colored threads, each laying atop the other. Your job is to focus on the threads on scanning, low and high power. However, on low power, you need to be able to tell which thread is on the top, which is in the middle and which is on bottom. You do this by focusing up and down very carefully with the fine adjustment. Remember the image inversion problem. On low power, determine the order of threads and have your instructor confirm your choices.

Wet Mount of Onion Skin

Obtain a blank slide and clean as outlined previously in the lab. Place a single drop of distilled water in the center of the slide. Using a scalpel or razor blade, cut off a small piece of onion - approximately 1 inch in length. Twist the onion between your fingers until it snaps and you get a small piece of onion “skin.” Cut that off and place the onion “skin” in the drop of water on your slide. Cover with a cover slip.

Observe under scanning, low and high power. Once in focus on high power, call your instructor over for confirmation.

Staining the Onion Slide

Live tissue often does not show detail well. In order to better see features in the cell, we often stain the material. Unfortunately, this also generally kills the cell. However, it does allow you to see specific items within the tissue. You will be using a stain today specific for the nucleus of cells. It is called aceto-orcein. CAUTION! Aceto-orcein has concentrated acetic acid in it. Concentrated acetic acid can be very dangerous. Don’t get it on your fingers or hands, eyes, and especially don’t get it on the microscope stage or objectives.

To stain the onion skin, you could remove the coverslip, place a drop of stain on the slide and replace the coverslip and then go through all the refocusing process. However, there is an easier way. While the slide is still on your stage and you have the image in focus on high power, place a single drop of aceto-orcein on the left hand side of your coverslip edge. Place the edge of a Kimwipe® on the opposite side of the coverslip (the right side) and insert an edge of the Kimwipe® under the coverslip

to soak up any water. As the water is pulled into the Kimwipe®, it should pull the stain on the other side of the coverslip up under the coverslip. Keep doing this until a good portion of the stain is pulled up under the coverslip. You will never get all the stain to go but enough will. You may need to move the mechanical stage a little to reposition the image to see stained cells. Look for a stained nucleus in the oculars. Again, let your instructor confirm your image.