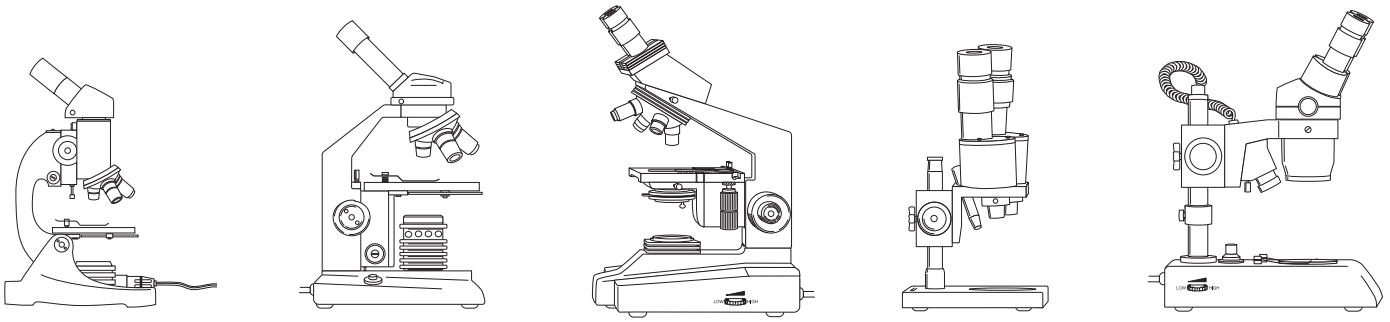
Boreal Microscope Manual

A comprehensive look at the Boreal Microscope line with a focus on:

• Set-Up • Maintenance • Use and Troubleshooting • Features • Accessories and Supplies • Student's Workbook



Boreal Microscope Manual

Teacher's Guide

Thank you for purchasing a Boreal microscope. We work hard to bring you microscopes that offer years of excellent service. All of our microscopes come with a lifetime warranty against any defects in material or workmanship.

Please use this manual to help you and your students get the most out of your new microscope. This manual is presented in two sections. Part one is a Teacher's Manual designed to show you the features and set-up for your new microscope. It also gives you helpful tips on the correct use and maintenance of microscopes. The second part is a Student Workbook. It presents general information about microscopes and microscopy, and detailed instructions on using microscopes. Please feel free to copy this section for use by your students.

Both sections are further divided into discussions on compound microscopes and stereomicroscopes. We hope this makes it easy for you and your students to get just the information you need!

Care and Maintenance of Compound Microscopes

Your microscope was shipped with a dust cover. Whenever the microscope is not in use, it should be protected with the dust cover. It is advisable to keep dust covers on microscopes even if they are stored in a cabinet.

Make sure lenses are clean before your microscope is put away. Always use optical lens paper dampened with optical lens cleaner on the lenses. It is a good idea to first blow off any dust with a rubber syringe (such as the one in our microscope maintenance kit) or a product like Dust-Off®. Sometimes it is effective to lift off small bits of dust with a camel hair paint brush. Then clean the lens with the dampened lens paper. You may find the following technique effective for stubborn dirt: Take a piece of lens paper about 10 cm by 15 cm. Fold it in half and grasp it with forceps in the center of the folded side about halfway onto the paper. Fold the loose part of the paper over the forceps, leaving about a millimeter of paper above the tip of the forceps. Now spin the forceps to roll the paper around them. Dampen the end of the paper-clad forceps with lens cleaning solution. Clean the lens by starting in the center and wiping in a spiral motion to the outside. For high power objectives with very small lenses, wrap the paper very tightly around the end of the forceps, forming a paper point that will fit into the lens opening. Always make sure lenses are cleaned after every use. Allowing dirt to dry onto a lens can make it very difficult to clean later. Be especially careful that the 100x oil immersion lens is free of oil at the end of every use. The bottom lens of these objectives is very tiny and is recessed into the metal housing. Care must be used to remove the oil completely after each session.

When using compound microscopes, make sure that no lens other than a 100x oil immersion lens comes into contact with a liquid. An oil immersion lens is sealed into the lens housing. This prevents oil from getting inside the lens system. The new Boreal2 compound microscopes also have sealed 40x objectives. However, many microscopes do not have sealed 40x objectives and you must be especially careful with these.

High power objectives have a very small working distance, which means that the lens is very close to the cover slip when the specimen is in focus. They also have multiple lenses in the lens housing. If they are not sealed and the bottom lens is allowed to come in contact with a liquid, the liquid may move by capillary action into the space between the first two lenses. In such a case, you may find the lens eventually needs to be disassembled by a technician to be cleaned. If your students are making wet mounts, be sure they always use a cover slip. Then instruct them to use absorbent paper to wick excess water off of their preparation. They should never use a slide with water on the cover slip or drops of water on the slide to either side of the cover slip.

About once a month you should move the focus mechanisms of the microscopes through the full range of their movement, both coarse and fine focus. This prevents the lubricant that covers the surfaces that slide against each other during focusing from building up small hardened ridges over time. Such ridges will eventually keep the focus mechanism from moving past them. Moving the microscope through its full focus range spreads the lubricant over the entire surface and keeps ridges of dried lubricant from forming.

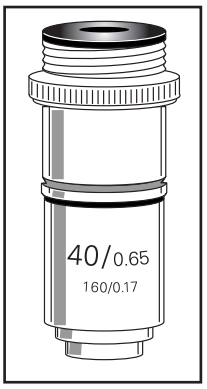
If you wish to clean the finish of your microscope, use a soft cloth dampened with mild detergent. Be sure to wipe the microscope dry.

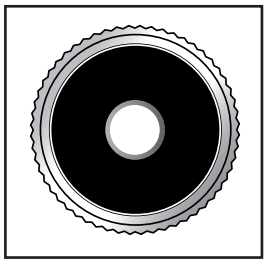
Taking these simple precautions should ensure years of trouble- free use. About once every three years, your microscopes should be disassembled, thoroughly cleaned and re-lubricated. This is generally done by a trained technician.

The most important aspect of caring for your compound microscope is to keep it clean and dry.

Annual Microscope Preparation

Before you begin to use your microscopes each year, there are a few things you should check and adjust.



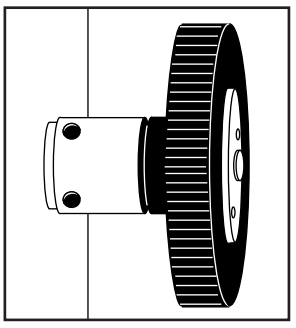


protective backing plate

1. Make sure the lenses are all in place.
Check the objectives carefully to make sure they are screwed firmly into the nosepiece. If you have Boreal2 microscopes, the "C" wrench that came with the microscope must be used to loosen the objectives, so there is little likelihood that they will not be all the way tight. If you see a gap between the top of the objective housing and the nosepiece, unscrew the objective and check to make sure if there is a backing plate (usually black), it is tightly in place. Tighten it and replace the objective.

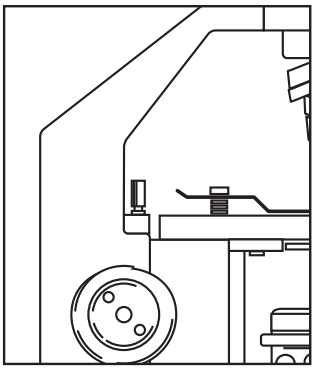
Adjust the eyepiece set screws so the eyepiece will rotate, but will not pull out. Most eyepieces are made with a groove to accommodate the eyepiece set screw. If the eyepiece won't rotate, the set screw is too tight. Boreal microscopes all come with a small hex wrench to adjust this set screw, on other models you may need to use a jeweler's screwdriver to loosen the set screw enough to remove the eyepiece. Make sure that the two halves of the eyepiece are screwed firmly together, then replace it and tighten the set screw just enough so the eyepiece will not pull out, but can still be rotated in the eyetube without unscrewing.

2. Check the coarse focus tension.
The coarse focus knob should turn easily to change the focus, but the body tube or stage should not move on its own. If the tension is either too tight or too loose it must be adjusted. Look at the shaft housing for the coarse focus. On most of the microscopes you will see a collar with four small holes in it. That is the coarse focus tension collar. One of the four small holes has a set screw in it. Boreal2 microscopes come with a hex wrench that will fit that set screw, and will also serve as a handle to move the tension collar. On older Boreal microscopes the set screw can be adjusted with the same small hex wrench that is used on the eyepiece set screw. Otherwise you may need a jeweler's screwdriver to loosen the set screw. Turn the collar towards the arm of the microscope to increase the tension, or towards the coarse focus knob to decrease the tension (while making sure the collar does not rub on the knob). Older Boreal microscopes also come with a "C" wrench that can be used to help turn the collar. Once you have the tension set where you want it, tighten the set screw to keep it at the proper setting. Don't over-tighten the set screw as that will cause additional tension that will then make it hard to turn the focus.



On very old microscopes without coaxial focusing and with no tension collar, the tension is adjusted by tightening or loosening the knobs against the ends of the coarse focus shaft housing. To increase the tension, take both coarse focus knobs at once and turn the one in your right hand so the top is moving away from you, and the one in your left hand so the top is moving towards you. To decrease the tension, turn both knobs the opposite ways. Some coaxial focusing microscopes have a tension ring. Look for a separate ring between the coarse focus knob and the arm of the microscope. Turning that ring towards the arm will increase the tension. If there is no tension ring on a coaxial focusing microscope, the tension may be maintained internally by means of a spring and is self adjusting.

3. Check the stage stop.
First locate the stop. If you have a microscope where the body tube and nosepiece move up and down to focus, look for a thumb screw and nut or a hex screw at the bottom of the focus block, just behind the body tube. On most microscopes where the stage moves up and down to focus, the stop is a hex screw or a thumb screw with a lock nut found just behind the stage. Boreal microscopes have a recessed hex screw in this position. (Some research microscopes do not have a stop; the travel of the stage limits the contact of the objectives with the slide.) Check the stop in the following way: First place any prepared slide into viewing position. If the microscope has fine, as well as coarse focus, move the fine



focus to the center of its range. Put the 10x objective into working position and move the coarse focus until the objective and stage are as close together as they can be. Now, watching from the side, carefully swing the 40x objective into place. That objective should come very close to, but not touch, the slide. Repeat this with the 100x objective, if your microscope has one. The 100x objective must all but touch the slide. To adjust the stop, either adjust the hex screw up or down as needed or first loosen the lock nut, then move the thumbscrew. Adjust the stop as needed and tighten the lock nut, if there is one, to hold it in place. Check the position by focusing on the slide. Make sure you can bring the slide into focus on the highest power objective, and then move just past the focal point so you know you can accommodate thinner slides than the one you're testing. If you're checking many microscopes, you will learn to tell just by looking at the very small space between the slide and the objective when the stop is set correctly.

Troubleshooting Guide

Following are some common problems encountered in microscopy and some ways to correct them.

Coarse focus knob won't change focus.

1. Check and adjust tension (#2 in the Annual Preparation section).
2. Push gently on stage or body tube to loosen grease.

Fine focus knob won't change focus.

Move fine focus both ways through its range. If it will move the stage or objective up but not down, the problem may be that the grease has stiffened. Move the fine focus mechanism until it is all the way down, then gently push the stage or body tube down. Repeat about three times.

Focus moves, but one or more objectives will not come into focus.

1. Check and adjust stop (#3 in the Annual Preparation section).
2. Check to make sure objectives are all the way in (#1 in the Annual Preparation section).
3. Check to make sure the retraction device is working on 40x and 100x objectives. You should be able to push the metal housing at the tip of the objective and have the tip move up about 2 mm. When you let go, the tip should return to its original position. If it does not, the objective must be repaired or replaced, usually this is a simple repair.
4. If either the 40x or the 100x objectives produce a fuzzy image, they may be dirty. Try removing the objective from the nosepiece and inspecting the small lens. Boreal2 microscopes come with a "C" wrench that is used to loosen the objectives for removal. You may want to use a hand lens (or remove the eyepiece and use it as a hand lens, look through it from the bottom and hold it and the objective close to your eye) to check. You can also get a much clearer view if you look through the back of the lens at a light background once the lens is off the microscope. If water or oil has been allowed to contact an unsealed 40x objective, there may be dirt between the lenses. The lens will have to be disassembled to be cleaned by a technician. Boreal2 40x lenses are sealed (all Boreal 100x lenses are sealed) so any dirt between the lenses would be covered under the warranty).

Image "drifts" out of focus during viewing.

Adjust tension (see #2 in the Annual Preparation section).

There is dirt in the field of view, but you can't locate it.

1. Move the slide to make sure the dirt isn't on the specimen slide.
2. Spin the eyepiece. If the dirt moves it is on the eyepiece.
3. You may only see the dirt through one objective. To check if the objective is the source, carefully loosen the objective (use the "C" wrench on a Boreal2 model), and just begin to unscrew it to see if the dirt moves. You may see dirt on one objective because the magnification is high enough or the field of view large enough to show it, but the dirt may not be on the objective.
4. If you have a focusable Abbe condenser, move it.

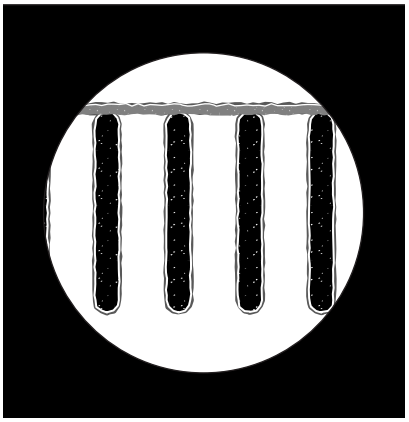
Measuring with a Non-Digital Microscope

Note: *The software that comes with Boreal Digital Microscopes provides direct accurate measurement on the computer image.*

Measuring the field of view under 40x total magnification using a transparent ruler It measures 4.5 mm or 4500 µ in diameter.

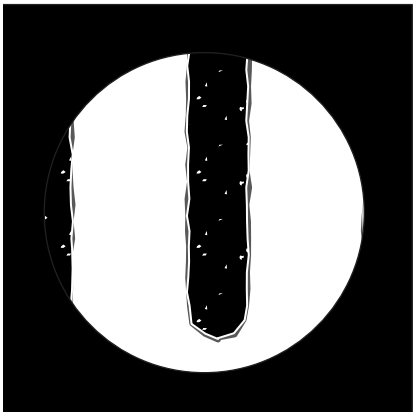
This field of view seen with 100x total magnification is about 1.8 mm or 1800 µ in diameter.

Teaching students to measure with their microscopes helps them to become better microscopists. It also helps them to better relate what they are viewing with what they are studying. It helps them to be better microscopists because it makes them more aware of the image they are viewing; whether it's centered, how much of the field of view it covers, etc. It helps them to relate what they see to what they are learning because understanding the relative sizes of organisms, cells and structures can help them to create better mental pictures of how these different elements relate as they interact and function.



Measuring the field of view under 40x total magnification using a transparent ruler. It measures 4.5 mm or 4500 m in diameter.

No matter what equipment you have, your students can learn to measure with their microscopes. The first step is to calibrate their microscopes. If you have no equipment except microscopes, you can still calibrate the microscopes with transparent plastic millimeter rulers. Have the students focus with their low power objective on the rulers. This works best if they position the edge of the ruler across the diameter of the field of view, and then line up the center of one millimeter mark with the edge of the field of view. They will have to interpolate between the mm markings to get their measurements.



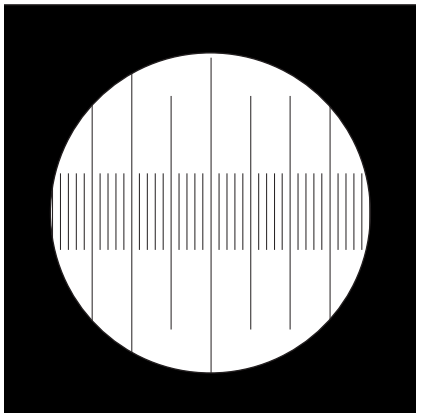
This field of view seen with 100x total magnification is about 1.8 mm or 1800 m in diameter.

If your microscopes have stage clips it is easy to slip the ruler under them. It is not so easy for the students to manipulate the ruler into position, but it is a good practical lesson in the orientation of the image to the object. It also gives them a "feel" for the difference in the field of view, since a very small movement of the ruler brings a large displacement of the image under higher powers. If your microscopes have mechanical stages you may not want to force the rulers under the brackets. You can make a set of microscope stage rulers by cutting 15 cm rulers in half. Each half will then fit in the brackets of the mechanical stage and can be manipulated like a slide. Very thin flexible rulers may fit under the brackets without cutting and without straining them.

This direct measurement will work until the total magnification is over 100x (10x objective with 10x eyepiece). At a magnification of about 150x or more the total field of view will be less than a millimeter. The width of that field of view can be computed by dividing the field of view of a low power objective by the quotient of the magnifying power of that low power objective divided by the magnifying power of the high power objective. For instance, let's say your field of view measured 4.5 mm with the 4x objective and a 10x widefield eyepiece. With a 40x objective and the same eyepiece the field of view should be 4.5 mm divided by 40x/4x, or 4.5 mm divided by 10, or 0.45 mm. This is a good time to introduce the micron (µ), which is one millionth of a meter, one thousandth of a millimeter. Expressed in microns, the field of view would be 4500 microns for the 4x objective and 450 microns for the 40x objective.

If you have 4x and 10x objectives on your microscopes you can do both measured and computed diameters for the field of view under the 10x objective, and discuss the variability of lenses (which are usually manufactured to a tolerance of about 2%) as well as the limits of measurement. While your students may not be impressed with the level of accuracy of these measurements, having them record the diameter of the field of view for each objective on their microscope, and insisting that they draw diagrams in the context of the field of view indicating the total magnification of the image and with a scale on the drawing will go a long way towards increasing the accuracy of their observations. You can then have them consider the relative sizes of the things they view in order to better place them in perspective.

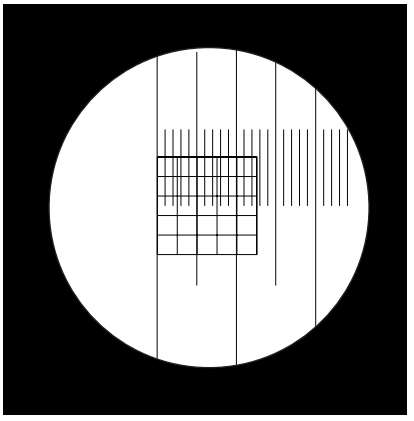
You can extend the direct measurement of the field a view if you have some stage micrometers for your students to use. These are slides with a scale etched on them, typically 1 mm or 2 mm in length and divided in tenths or hundredths of a millimeter. Have your students use the ruler with their lower power objectives until they reach a magnification where the field of view is less than 1 mm, then put the stage micrometer in place of the ruler and measure the field of view in the same way. Some care must be taken to make sure the units are correct.



This field of view with 400x total magnification measures .40 mm or 400 m in diameter when measured with a stage micrometer.

The most accurate measurements can be made using an ocular, or eyepiece, micrometer. This is a glass disc that fits in the eyepiece and has a scale or grid etched on it. It goes by several other names, such as reticle, reticule, graticle or graticule. The eyepiece micrometer remains in place during use, so you will need one for each microscope being used for measurement. It is placed on the diaphragm of the eyepiece. The diaphragm will be a ridge inside the objective where the disc can rest. This ridge or shelf is located so that anything held in that position is in focus with the image in the eyepiece. If your eyepiece has a pointer, the pointer is at the diaphragm (and will have to be removed and replaced by the ocular micrometer). You may have to reach the diaphragm from the bottom of the objective, in which case you will need a spring to hold the ocular micrometer in place. You may have to reach it by unscrewing the eyepiece into two halves. Try this carefully, with the eyepiece held upside down, because in some designs unscrewing the eyepiece allows the two lenses to fall out. A little careful observation and experimentation should allow you to find the eyepiece diaphragm. Pointers may be held in place with a spring or with a threaded ring, and whatever is used to hold the pointer in can be used to hold the ocular micrometer in place, also.

The divisions on an eyepiece ocular must be calibrated for each objective using a stage micrometer. Start with the low power objective, focus the stage micrometer and turn the eyepiece so the scale or one axis of the grid is adjacent to or superimposed on the stage micrometer. Then move the stage micrometer until the beginning of the scale lines up with the edge of the eyepiece reticle. Look for another division on the reticle that coincides with a division on the stage micrometer and compute the measurement for each division of the eyepiece micrometer from the ratio. Lets take an example where the stage micrometer is in hundredths of a mm and you are using a 40x objective with a 10x eyepiece for a total magnification of 400x. You find that the second division on the eyepiece reticle lines up with the fifth division of the stage micrometer. In this case each division of the eyepiece reticle is equal to 2.5 divisions of the stage micrometer, which would be .025 mm or 25 microns.



An eyepiece grid superimposed on a stage micrometer at 400x total magnification showing a segment is 25 m on a side.

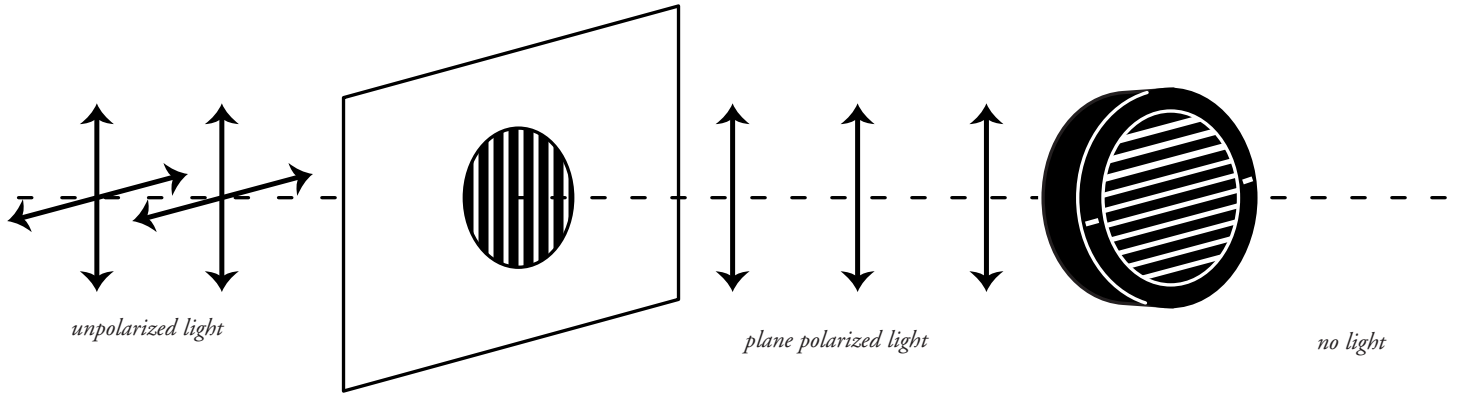
Students should record the microscope number and the calibration of the eyepiece micrometer for each power of the microscope in their laboratory notebooks. They should always use the same microscope and should then be able to include a scale for any drawings they do of microscopic images. It can be an interesting exercise to have them do sketches of different organisms on the same scale.

Ideally each student will do his or her own calibrations, since the calibration will differ with focus, and the focus will differ slightly depending on the eyesight of each student. If you are lucky enough to have binocular microscopes available for some or all of your students (or for

your own use) you should check to see if the interpupillary adjustment (moving the eyetubes apart or closer to accommodate the spacing of your eyes) changes the tube length. Adjust the microscope to fit your eyes and to be in focus. Line up the eyepiece and stage micrometers and find the second point where the markings overlap. Now move the eyepieces closer together or further apart (you may have to look with only one eye for this). If the orientation of the eyepiece and stage micrometers changes, you will know that the tube length is changing with the interpupillary distance. If this is the case, each user must also record the reading of the scale that shows the interpupillary distance and set the microscope back to that setting for each use. This is also a quick way to readjust the microscope for different users.

This may all sound time consuming, but it can be done as part of a laboratory exercise introducing the use of the microscope, and it will improve the results and understanding of all future laboratory exercises using the microscope.

Using the Polarizing Kit for Standard Compound Microscopes

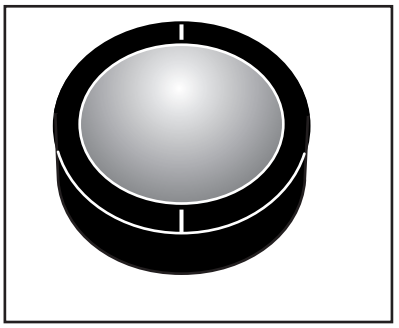


The Polarizing Kit can be used to view unstained and transparent biological specimens or to look at thin sections of rock. This is done by using two polarizing lenses, which when adjusted so the planes of polarization are perpendicular, will block out all the light, as shown in the drawing above. The specimen to be observed is placed between the lenses. The specimen will bend the light after it has been plane polarized, so that some of the light that has been altered by the specimen will come through. Even specimens that are transparent when viewed in normal light will be visible. They will form a light image against a black background. Polarized light is useful for viewing thin sections of rock because the rock crystals modify the light in the direction of their faces, so the crystal structure is emphasized.

The Polarizing Kit consists of a polarizing lens that is fitted into a stage attachment and an analyzing polarizing lens in an eyepiece adapter. The axis of polarization of these lenses is indicated by white hatchmarks. The plane of polarization of the stage attachment is set up to be in a line from front to back of the microscope.

Place the stage attachment under the stage clips so it is square to the stage. Notice that the attachment has its own set of specimen clips for your sample. Since the polarizing lens will decrease the intensity of the illuminator light by half, be sure to have the disc or iris diaphragm set to admit the maximum amount of light from the illuminator.

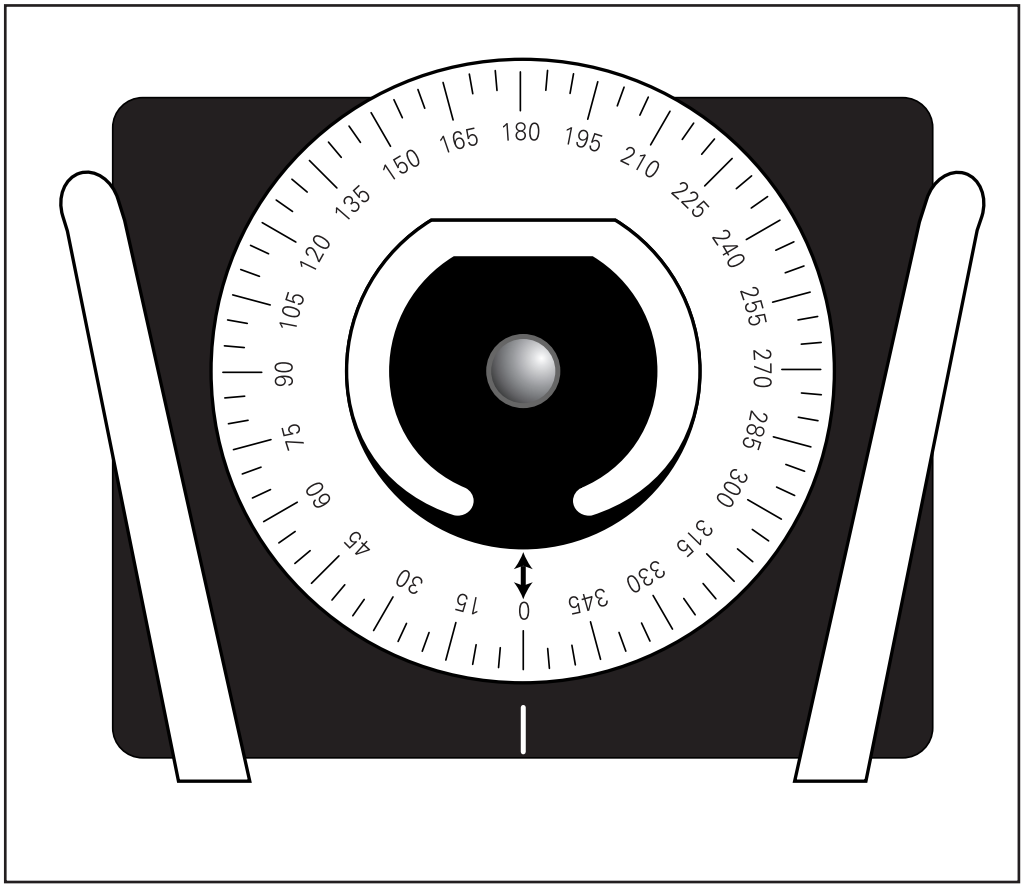
Place the analyzing lens over the eyepiece. Align the hatchmarks on the analyzing lens so they are parallel with the mark on the stage attachment. Look through the eyepiece. There should be light coming through. Now turn the eyepiece analyzer. The light should dim until at one point little or no light is visible. That is the point where the



The eyepiece analyzer for the polarizing kit has hatchmarks that indicate the plane of polarization.

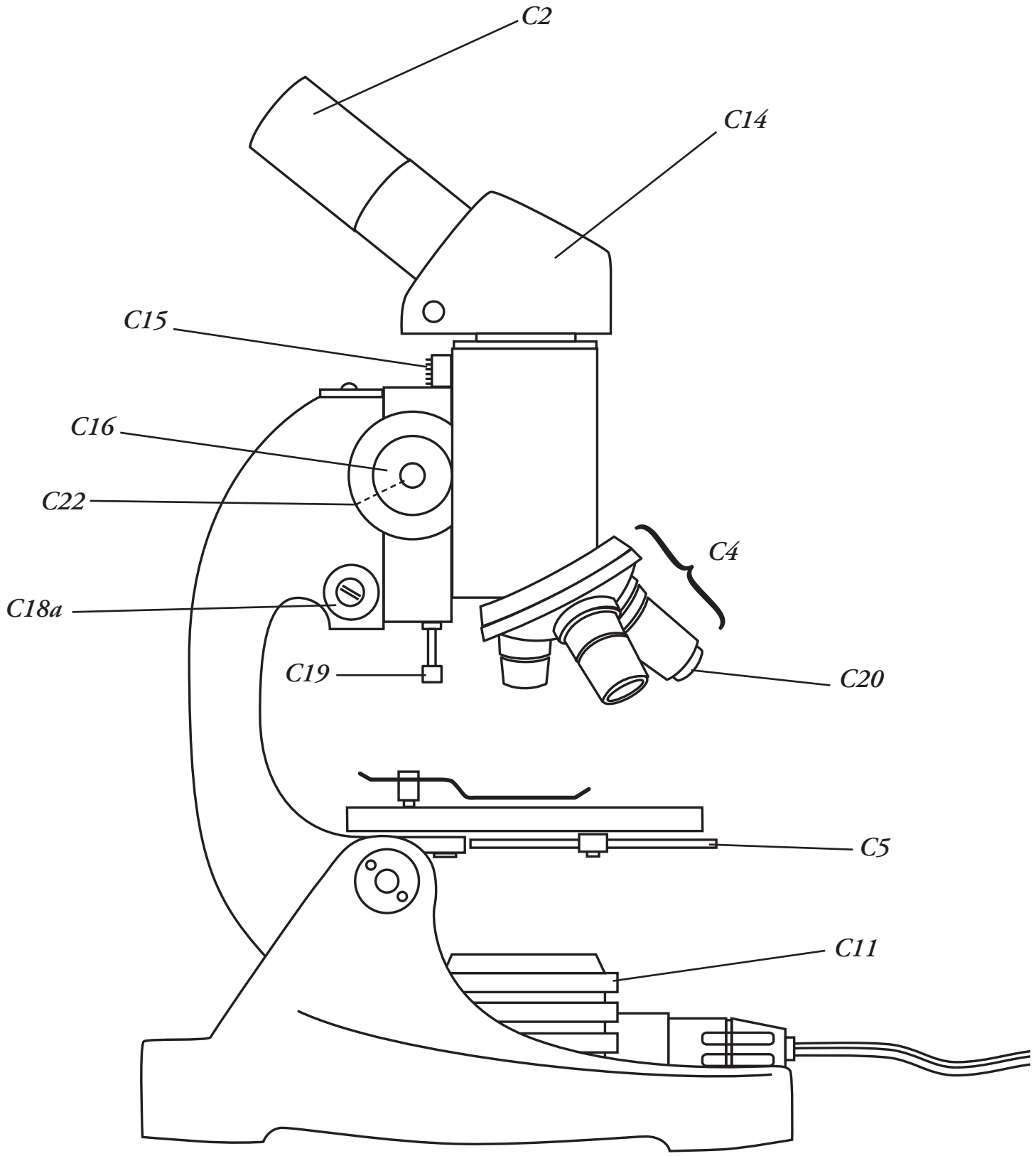
hatchmarks, and the plane of polarization of the lenses, are perpendicular.

Now place a sample on the stage, keeping the eyepiece analyzer perpendicular to the polarizing lens in the stage attachment. Make sure the specimen clips of the stage attachment are set so the protractor reads 0° (see drawing below). If the sample rotates the light, some of the light will now pass through the analyzer forming a bright image on a dark background. This image will be formed even by transparent specimens. By rotating the sample clips on the stage attachment, you can see at what orientation light is allowed through the analyzer. The angle of the sample compared to the polarizer is given by the protractor mounted beneath the sample clips. This can give some insight into the internal structure of crystals in thin rock sections.

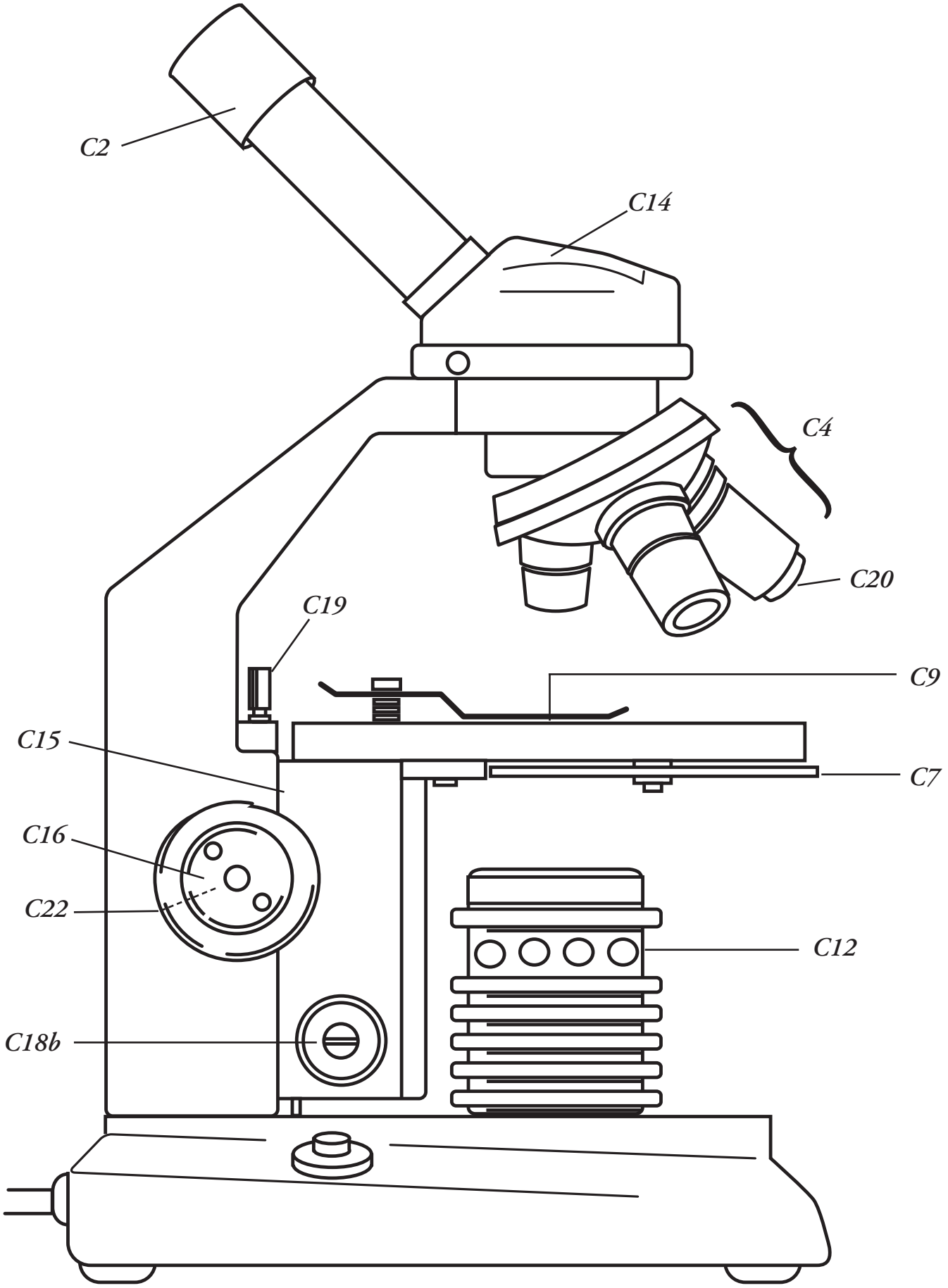


Stage attachment for the polarizing kit showing the specimen clips oriented to the zero degree position.

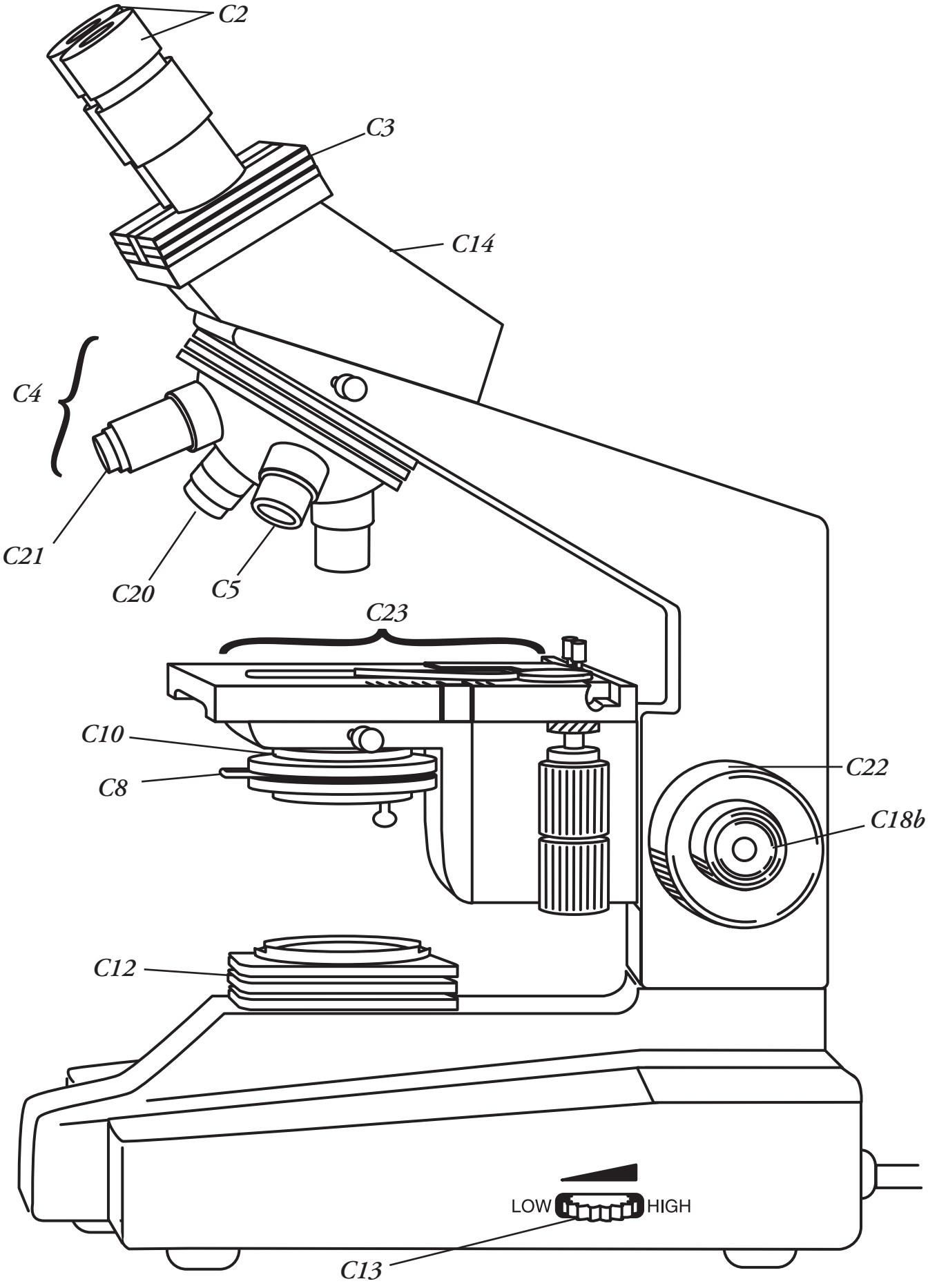
Student Microscope



Standard Microscope



Advanced Microscope



Features of Compound Microscopes

C1. All-glass coated achromatic optics.

Glass gives a clear image and resists scratching. The coating minimizes reflected glare. Achromatic optics are corrected for the way that light bends in going from one medium to another with different optical density (glass and air, in this case). Light of shorter wavelengths (the blue-violet end of the spectrum) bends more than light of longer wavelengths (red light). If the glass lenses don't take this into account, the edges of everything you see through a microscope tend to have a rainbow effect, which makes it harder to discern detail. Achromatic optics are made to bring the focal points of blue and red light together, so there is less of a rainbow and better clarity.

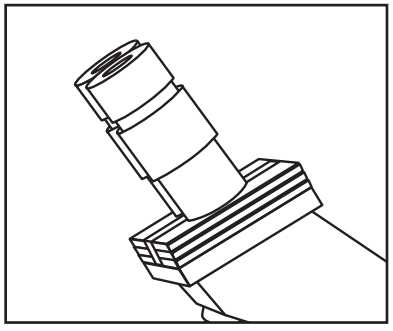
C2. Widefield eyepiece.

A widefield eyepiece creates a larger image than older types of eyepieces. The eyepoint is slightly above the top surface of the lens. The bigger image is easier to see and students can comfortably use the microscope while wearing glasses.

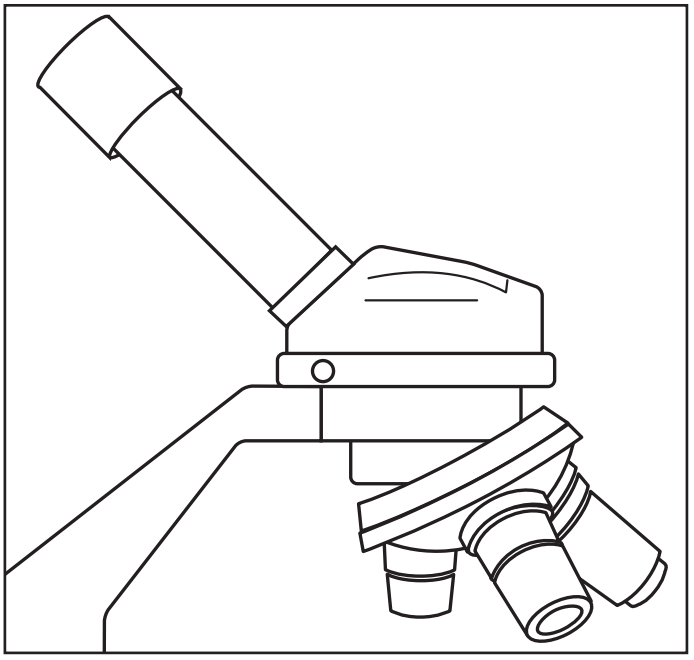
C3. Binocular head.

A binocular head uses prisms to split the image from the objective into two identical images, one for each eyetube. The distance between the eyetubes (interpupillary distance) can be adjusted to fit different faces. A diopter adjustment allows the eyetubes to be focused separately to accommodate differences in vision between the eyes. A binocular head on a compound microscope still shows a single, two-dimensional image, but the ability to use both eyes is more comfortable for long periods of viewing. Seidentopf binocular heads allow you to change the interpupillary distance without affecting the focus.

C4. Parfocal objectives on ball bearing nosepiece.



Parfocal objectives are made so the image stays in focus when the nosepiece is rotated from one objective to another. Use the coarse and then the fine focus to bring the specimen into sharp focus with the lowest power objective. Make sure the area of interest is in the center of your field of view, and then rotate the nosepiece to bring the next higher objective into place. With parfocal objectives, the specimen will be visible and will require only a small adjustment of the fine focus to bring it into sharp focus. The ball bearing nosepiece ensures smooth movement as the objectives are rotated.



C5. Semi-plan 4x objective.

The field of view is largest in the lowest power 4x objective. A semi-plan objective is a more expensive type of optical element that shows a greater portion of the field of view in sharp focus. This gives a flatter field of view.

C6. DIN optics.

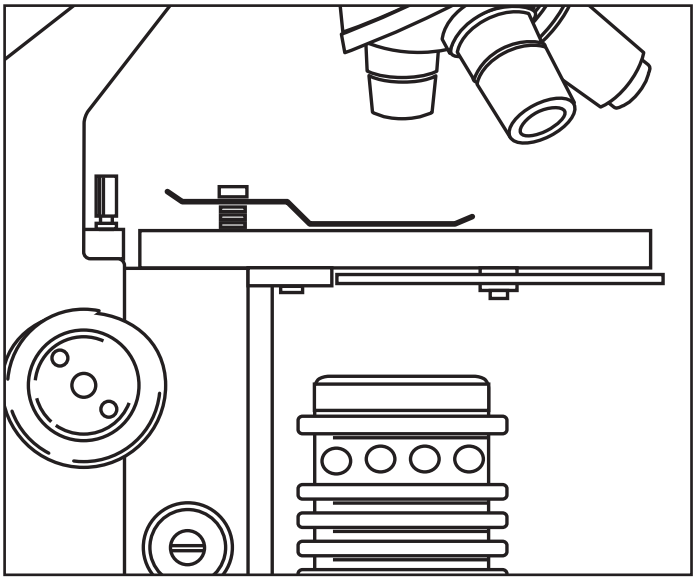
DIN stands for Deutsche Industrie Norman, a worldwide standard for the size, thread and focal distance of microscope optical elements. Eyepieces or objectives made to DIN specifications will fit in and focus on any other microscope made to these standards. DIN optics may be identified by having DIN or D or the number 160 printed on them, meaning that they are built for a body tube length of 160 mm.

C7. Disc diaphragm.

A disc diaphragm lets you control the amount of light coming through your specimen by turning the disc to bring one of five or six different sized holes in line with the stage opening.

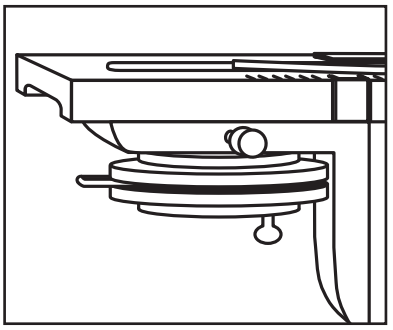
C8. Iris diaphragm.

An iris diaphragm is made up of intermeshed metal leaves that open and close like the shutter of a camera. A lever controls the size of the opening.
An iris diaphragm allows you set the size of the opening for the illuminating light over a wide range.



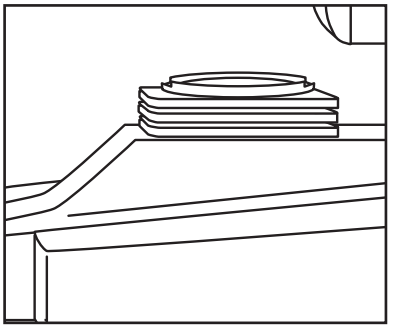
C9. 0.65 N.A. fixed field condenser.

A condenser gathers and focuses light before it passes through the specimen. N.A. is the numerical aperture of the lens. This is the refractive index of the medium between the object and the objective (air or oil) times the sine of the half angle of the cone of light entering the objective. The smallest resolvable detail is determined by the wavelength of the illumination divided by 2 times the N.A. Thus you can increase the detail you can see if the numerical aperture is greater. You can increase the numerical aperture by increasing the size of the cone of light entering the lens or the refractive index of the medium (thus using an oil immersion lens). Also, if the wavelength of the illumination decreases, the size of the smallest detail discernible will decrease (resolution will increase). That is one reason to use blue filters in the illumination system. You should note that objectives, as well as condensers, have numerical apertures, and it does no good to increase the numerical aperture of the objective beyond the numerical aperture of the condenser. In order to get the resolution the objective was designed for, the numerical aperture of the field condenser should be greater than or equal to the numerical aperture of any objective being used with it. The 40x objectives on our standard and advanced microscopes have a numerical aperture of 0.65, the same as this fixed field condenser. A fixed condenser is mounted in the stage in the proper position for use.



C10. 1.25 N.A. focusable Abbe condenser.

An Abbe condenser has two lenses and a greater numerical aperture than our fixed field condenser (see the explanation of numerical aperture under C9). The N.A. of 1.25 gives it the ability to be used with higher resolution 1.25 N.A. oil immersion 100x objectives. These condensers can be moved up and down in the path of the light to optimize the image. Generally, the image will be of the best quality with the top lens of the condenser very close to the top surface of the stage. Movement of the condenser is controlled either by a lever that moves in a sloped groove on the holder or by a rack and pinion gear for the holder of the condenser.

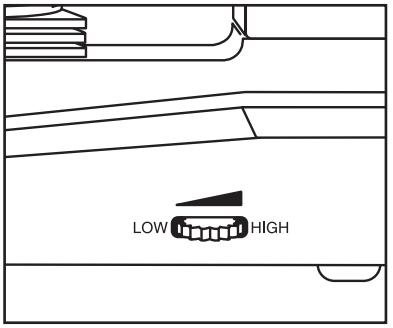


C11. Substage illuminator.

An illuminator is usually much easier for students to use since the light is aimed correctly for the lens system. Tungsten and halogen illuminators use blue filters to send light of shorter wavelengths through the specimen. This shows truer colors in the specimen and also helps to increase the resolution of the image, since the resolution is limited, in part, by the average wavelength of the illumination (this is why so much greater resolution is available with an electron microscope, since the wavelength of electrons is much shorter than light). Today many Boreal microscopes have LED illuminators. The light from these low power sources is bright, white and even. A further advantage is that there is virtually no heat from the bulb.

C12. In-base illuminator.

An in-base illuminator is built into the base of the microscope. It cannot be removed and replaced with a mirror. It is built to line up properly with the optical system of the microscope.



a. Tungsten bulbs. Tungsten was for many years the standard filament for light bulbs. Provide good illumination for most needs and is inexpensive. Generally used with a blue filter.
b. Fluorescent bulbs. Provide cooler, whiter light than tungsten.
c. Halogen bulbs. Provide bright hot white light.
d. LED bulbs. Newest technology, provide bright white, cold light. Have very low power consumption.

C13. Rheostat dimmer control.

The rheostat allows you to dim the light on the illuminator. Using the rheostat to control the intensity, along with the diaphragm (either disc or iris) which controls the size of the column of light coming through your slide, allows you to adjust the illumination to obtain the best contrast for your specimen.

C14. Inclined, 360° rotating head.

The inclined head is easier to use with the stage in a horizontal position. A horizontal stage is especially helpful when viewing wet mounts. A rotating head allows two people to use one microscope without changing places.

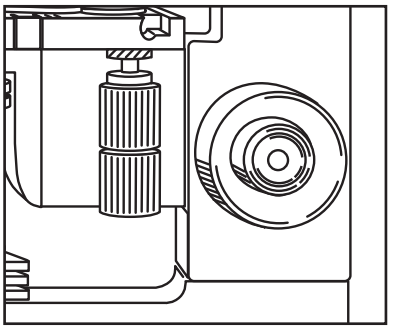
C15. Rack and pinion coarse focus.

The coarse focus knobs turn a pinion gear, which moves the body tube or the stage up and down via a rack. Rack and pinion gearing is simple, sturdy and makes for a positive connection between the knobs and the body tube or stage.

C16. Slip clutch coarse focus knobs.

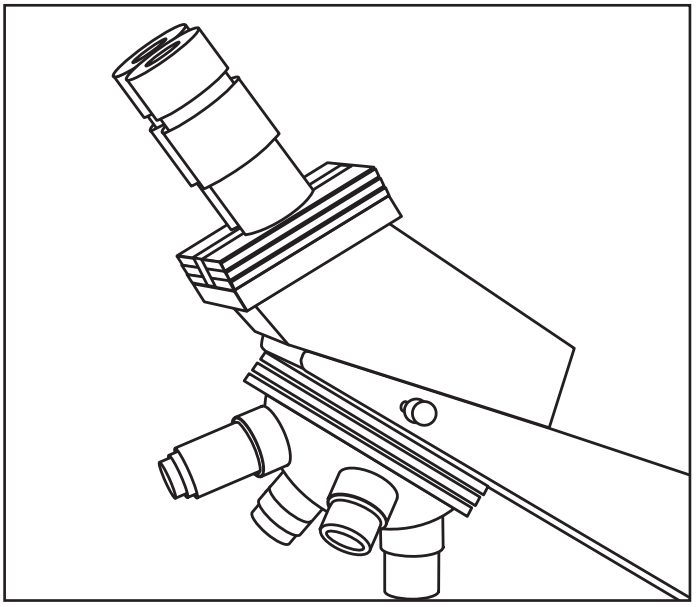
These knobs have a spring washer to transfer the motion from the knob to the pinion shaft. If a student attempts to turn the knobs past their range of travel, the knobs will slip and the pinion gear won't be damaged.

C17. Coaxial coarse and fine focus.

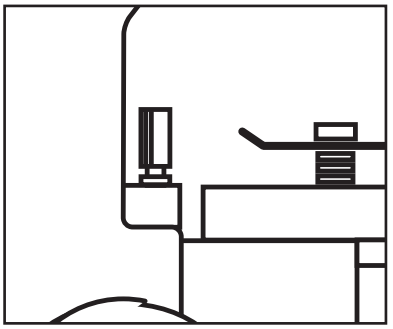


As the name implies, with coaxial focusing both the coarse and fine focus knobs are on the same axis. The coarse focus knobs are larger and are located in towards the arm of the microscope. The fine focus knobs are smaller and are mounted outside the coarse focus knobs. Having both sets of knobs on the same axis makes it easier to switch from coarse to fine focus and easier to locate the focus knobs when your attention is on the specimen slide.

a. Nylon spiral coarse focus with ball bearing fine focus. This is a new design to make coaxial focusing very dependable. The coarse focus operates by having a steel pin ride in a nylon spiral gear. It is virtually unbreakable. The fine focus is driven from a small shaft turned by the action of three large bearings. Turning the fine focus past its travel simply causes the bearings to rotate while the shaft stays stationary in the middle. It cannot damage the mechanism.
b. Gear driven coarse and fine focus. This design offers very precise and smooth action of the coarse and fine focus.



C18. Fine focus.



Having a fine focus adjustment makes it easier for students to focus on precisely that part of the specimen they wish to view. Under high power (400x and above) the depth of field is so small that it helps to focus up and down through a specimen to see all the detail. This is much easier if there are fine focus knobs. There are several different types of fine focus mechanisms:

a. Ball bearing fine focus is found on the Student SKope by Boreal. This is a very sturdy arrangement that is not easily damaged. The fine focus block rides on a ball bearing that moves on a tapered shaft controlled by the fine focus knobs.

b. Lever-action fine focus is common on microscopes with a separate fine focus shaft. In this arrangement a lever rides against a machined shoulder on the fine focus shaft. As the threaded shaft is turned the lever is pushed. Either a pin or shaped point at the top of the lever pushes a block attached to the stage bracket or body tube which raises them. A spring is mounted above the block which pushes it back down when the shaft is moved in the other direction.

C19. Adjustable stop screw.

The stop screw can be adjusted so a slide will easily focus on high power, but the body tube or stage will stop before the objective lens comes into contact with the cover slip of the specimen slide. The stop screw on Boreal microscopes is a recessed hex screw to keep students from changing this adjustment.

C20. Retractable 40x objective.

The higher the power of the objective the closer the objective lens must be to the surface of the cover slip to bring the specimen into focus. This short working distance means that there is little room for error in setting the stop to keep the lens away from the specimen slide. If the stops come out of adjustment, a retractable lens provides another protection against objective lens or slide damage. The tip of a retractable lens is held in viewing position by a spring, which allows the lens to move up into the housing if it is pushed against the slide. The 40x objective on Boreal2 microscopes is sealed to prevent moisture from stains or wet mounts from getting between the lenses.

C21. Retractable, oil immersion 100x objective.

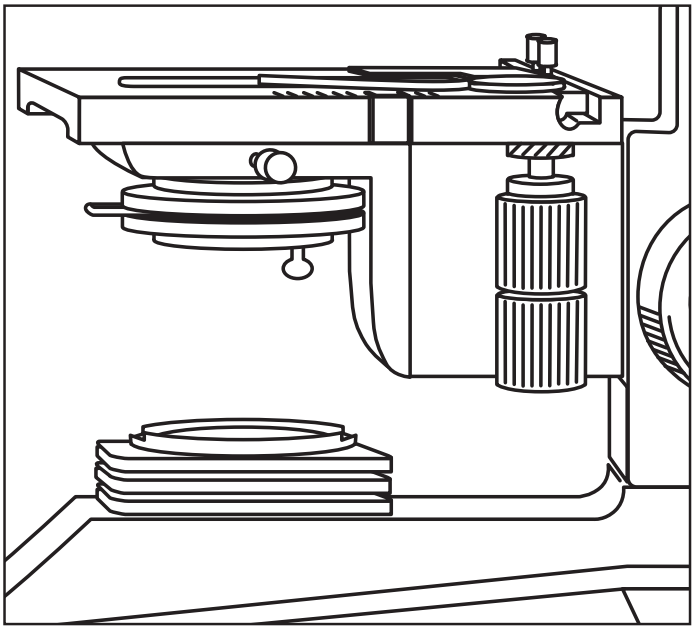
This is a retractable objective just like the 40x objective listed above. In addition, the bottom lens is sealed in the objective case. This allows the end of the objective to be immersed in oil during use, without letting oil inside the lens system. Immersion oil is used to limit the refraction of light on its path through the specimen and slide and into the microscope. Immersion oil has a refractive index of 1.515, which is close to that of the glass of the slide and cover slip. The less the light bends other than in forming and focusing the image, the better the resolution will be.

C22. Adjustable tension collar.

The focusing part of the microscope (either the body tube or the stage) must be easily moved by the focusing knobs, but must hold the stage or body tube in focus for viewing. This is accomplished by setting the tension. The adjustable tension collar is found on the coarse focus shaft housing. Moving the collar towards the arm of the microscope increases the friction between the pinion shaft and the housing to establish the proper tension. A set screw keeps the tension collar in place.

C23. Built-in Mechanical Stage.

A spring clip holds the specimen slide in place. Coaxial controls below the stage move the slide from left to right or forward and back.



Care and Maintenance of Your Stereomicroscopes

The most important aspect of caring for your stereomicroscope is to keep it clean and dry. Your microscope was shipped with a dust cover. Whenever the microscope is not in use, it should be covered with the dust cover. It is advisable to keep dust covers on microscopes even if they are stored in a cabinet.

Make sure lenses are clean before the microscope is put away. Always use optical lens paper dampened with optical lens cleaner on the lenses. It is a good idea to blow off any dust with a rubber syringe (such as the one in our microscope maintenance kit) or a product like Dust-Off. Sometimes it is effective to lift off small bits of dust with a camel hair paint brush such as the one included in our microscope maintenance kit. Then clean the lens with the dampened lens paper. You may find the following technique effective for stubborn dirt: Take a piece of lens paper about 10 by 15 cm. Fold it in half and grasp it with forceps in the center of the folded side about halfway onto the paper. Fold the loose part of the paper over the forceps, leaving about a millimeter of paper above the tip of the forceps. Now spin the forceps to roll the paper around them. Dampen the end of the paper clad forceps with lens cleaning solution. Clean the lens by starting in the center and wiping in a spiral motion to the outside.

About once a month you should move the focus mechanism of the microscopes through the full range of its movement. This prevents the lubricant that covers the surfaces that slide against each other during focusing from building up small hardened ridges over time. Such ridges will eventually keep the focus mechanism from moving past them. Moving the microscope through its full focus range spreads the lubricant over the entire surface and keeps ridges of dried lubricant from forming.

If you wish to clean the finish of your microscope, use a soft cloth dampened with mild detergent. Be sure to wipe the microscope dry.

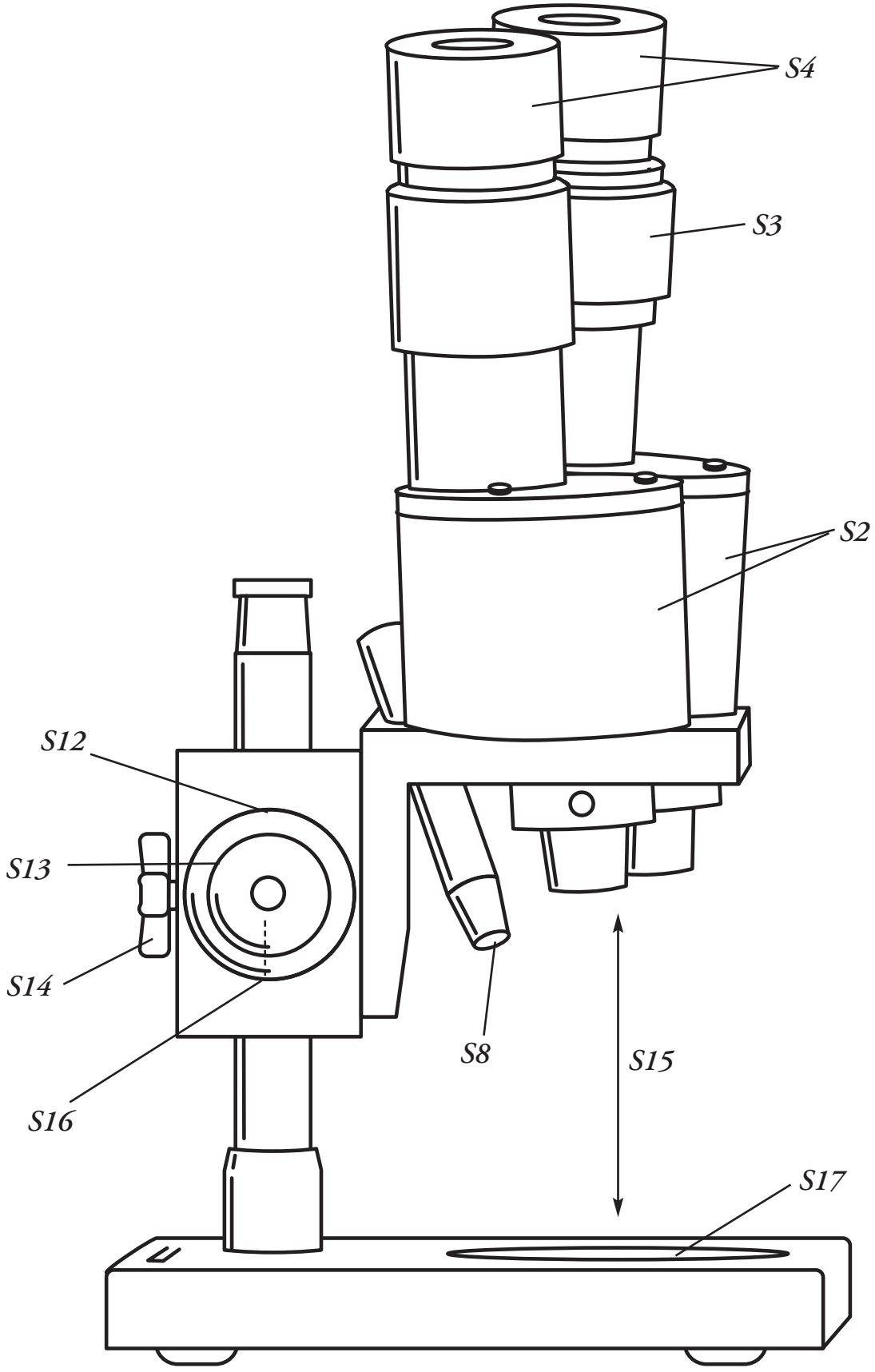
Eventually, you may find that your stereomicroscope won't stay in focus. You focus on the specimen, but if you let go of the focus knobs the image "drifts" out of focus. This means that the tension must be adjusted. Look at the shaft housing for the focus. On most of the microscopes you will see a collar with four small holes in it. This is the focus tension collar. One of the four small holes has a set screw in it. Boreal2 microscopes come with a hex wrench that will fit that set screw, and will also serve as a handle to move the tension collar. On older Boreal microscopes the set screw can be adjusted with the same small hex wrench that is used on the eyepiece set screw. Otherwise you may need a jeweler's screwdriver to loosen the set screw. Turn the collar towards the arm of the microscope to increase the tension, or towards the coarse focus knob to decrease the tension (while making sure the collar does not rub on the knob). Older Boreal microscopes also come with a "C" wrench that can be used to help turn the collar. Once you have the tension set where you want it, tighten the set screw to keep it at the proper setting. Don't over-tighten the set screw as that will cause additional tension that will then make it hard to turn the focus.

If there is no tension collar the tension may be adjusted by tightening or loosening the knobs against the ends of the focus shaft housing. To increase the tension, take both focus knobs at once and turn the one in your right hand so the top is moving away from you, and the one in your left hand so the top is moving towards you. To decrease the tension, turn both knobs the opposite ways.

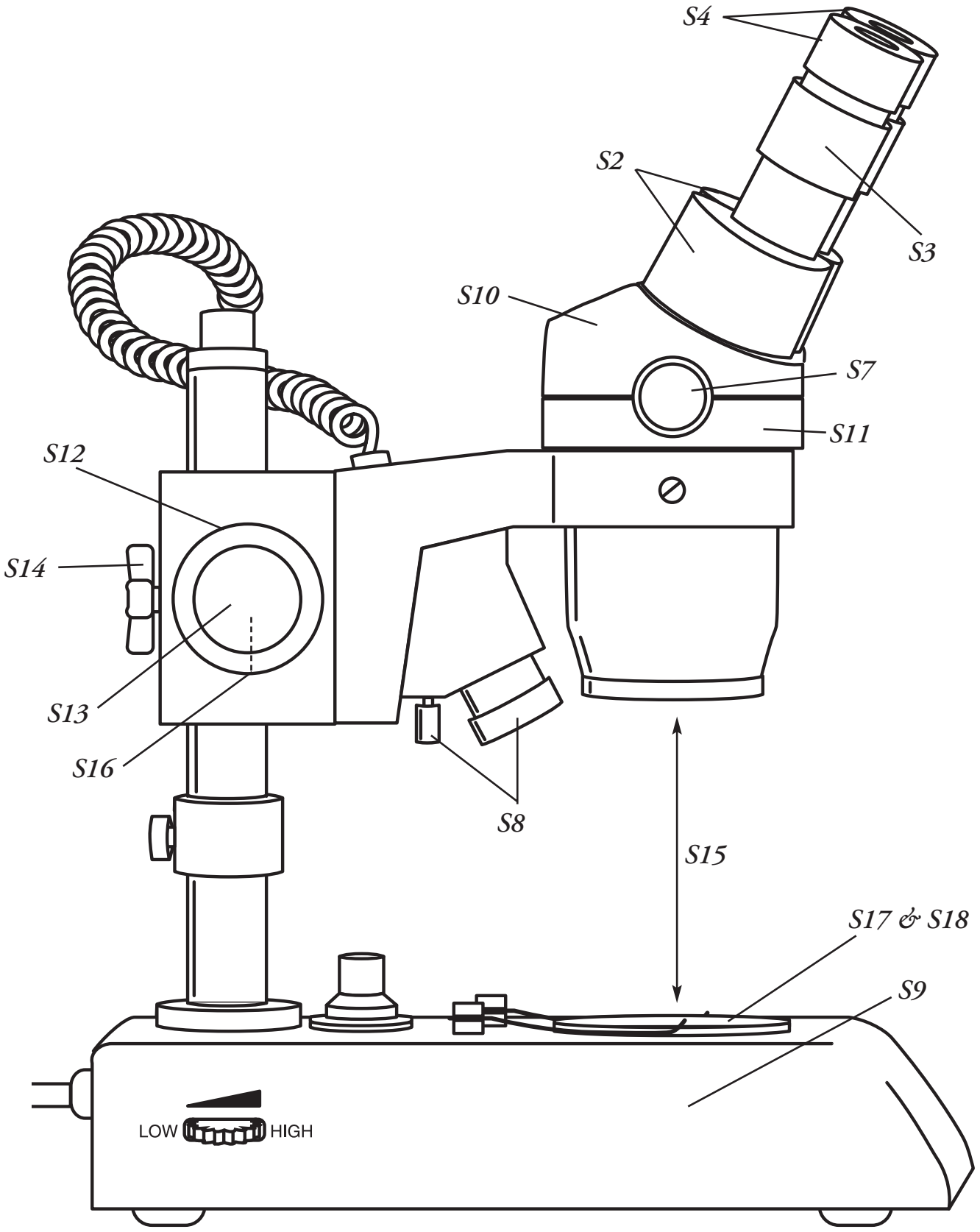
Taking these simple precautions should ensure years of trouble free use. About once every three years, your microscopes should be disassembled, thoroughly cleaned and re-lubricated. This is generally done by a trained technician. If you or someone on your staff is interested in doing this, we recommend that you purchase our Boreal Microscope Maintenance Kit and follow the instructions in the accompanying booklet.

The most important aspect of caring for your stereomicroscope is to keep it clean and dry.

Portable Student Stereomicroscope



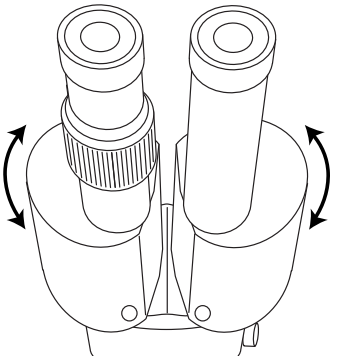
Boreal Zoom Stereomicroscope



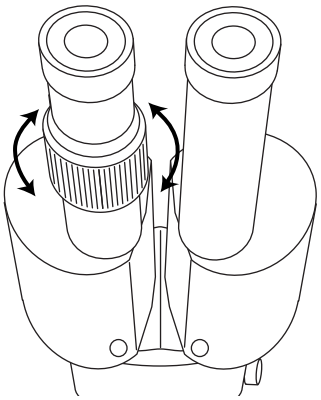
Features of Stereomicroscopes

S1. All-glass coated achromatic optics.
Glass gives a clear image and resists scratching. The coating minimizes reflected glare. Achromatic optics are corrected for the way that light bends in going from one medium to another with different optical density (glass and air, in this case). Light of shorter wavelengths (the blue-violet end of the spectrum) bends more than light of longer wavelengths (red light). If the glass lenses don't take this into account, the edges of everything you see through a microscope tend to have a rainbow effect, which makes it harder to discern detail. Achromatic optics are made to bring the focal points of blue and red light together, so there is less of a rainbow and better clarity.

S2. Adjustable interpupillary distance.
The two eyetubes with eyepieces can be moved closer together or further apart to accommodate the distance between the eyes of the viewer. It is important to have students adjust this spacing so that they see a three dimensional image.



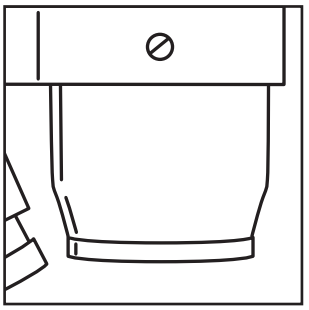
S3. Diopter adjustment.
Many people have different vision in one eye than the other. A diopter adjustment allows one eyetube to be focused separately from the other in order to change the focus to match the vision of the viewer.



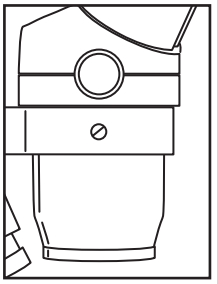
S4. Widefield eyepieces.
Widefield eyepieces create an image that is larger than the eyetube. The focal point is slightly above the top surface of the lens. This makes a bigger image that is easier to see. It also means that students can comfortably use the microscope while wearing glasses.

S5. Auxiliary or optional objective.
Some microscopes offer another lens or set of lenses that can be added to or substituted for the standard objectives to increase the magnification.

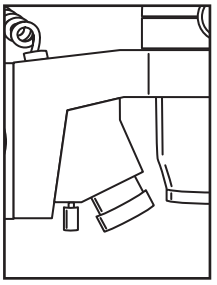
S6. Rotating nosepiece with two sets of objectives.
This feature allows the user to simply turn the nosepiece to change magnification. The nosepiece has two pairs of objectives, with each pair aligned to produce a clear three dimensional image for the viewer.



S7. Zoom magnification.
Zoom stereomicroscopes allow you to change the magnification through a continuous range while you are viewing. It is best to focus first at the highest magnification and then adjust the zoom down or back up as needed.



S8. Incident illuminator.
Light from an incident illuminator shines down onto the sample being examined. Some of our incident illuminators have a thumbscrew that enables the viewer to aim the beam of light. Some models have a rheostat dimmer switch to control the intensity. Some Boreal2 models have LED incident illumination built in to the objective turret, so the light shines directly down on the specimen. These features help to optimize illumination for varied specimens.



S9. In-base illuminator.

An in-base illuminator is built into the base of the microscope. To use it, remove the black and white reversible stage plate and replace it with a frosted glass stage plate. In-base illumination is helpful for translucent specimens or, combined with incident illumination, specimens with very irregular surfaces.

a. Tungsten bulbs. The standard filament for light bulbs. Provides good illumination for most needs and is inexpensive. May be used with a blue filter.
b. Fluorescent bulbs. Cooler, whiter light than tungsten. Useful for examining live specimens.
c. Halogen bulbs. Provide bright hot white light.
d. LED bulbs. Newest technology. Bright white cold light. Low power consumption.

S10. Inclined head.

A stereomicroscope with an inclined head uses prisms to direct the light from each objective into its angled eyetube. This makes it much more comfortable for viewing.

S11. 360° rotating head.

This allows the head to be turned in any direction for comfort and convenience.

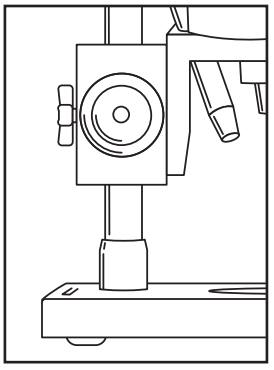
S12. Rack and pinion focus.

The focus knobs turn a pinion gear, which moves the head up and down via a rack. Rack and pinion gearing is simple, sturdy and makes for a positive connection between the knobs and the head.

S13. Slip clutch focus knobs.

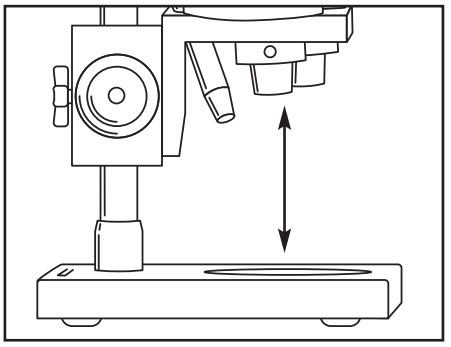
These knobs have a spring washer to transfer the motion from the knob to the pinion shaft. If a student attempts to turn the knobs past their range of travel, the knobs will slip so the pinion gear won't be damaged.

S14. Post and clamp body construction.



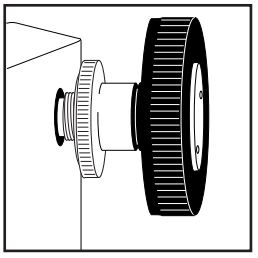
The focus mechanism and head are mounted on a vertical post and held in place by a clamp that is tightened by a knob on the back of the unit. This allows the entire body of the microscope to be moved in relation to the stage, giving you more flexibility in dealing with differently shaped specimens. For very thin specimens, the body can be closer to the stage than for very large specimens. For microscopes with heavy heads there may be a ring on the post that can be moved just below the body clamp and is held in place with one or more set screws. This is to protect the head from falling down the post.

S15. Working distance.



The working distance is the distance between the objective in use and the surface of the specimen in focus. For the same optical system, the working distance is always less for greater magnification (except in a zoom microscope, where it is set for the highest magnification). A longer working distance gives you more room between the specimen and microscope for dissection or other manipulation of the specimen.

S16. Adjustable tension collar.



The head of the microscope must be easily moved by the focusing knobs, but must stay in place to remain in focus for viewing. This is accomplished by setting the tension. The adjustable tension collar is found on the coarse focus shaft housing. Moving the collar towards the arm of the microscope increases the friction between the pinion shaft and the housing to establish the proper tension. On some models a set screw keeps the tension collar in place.

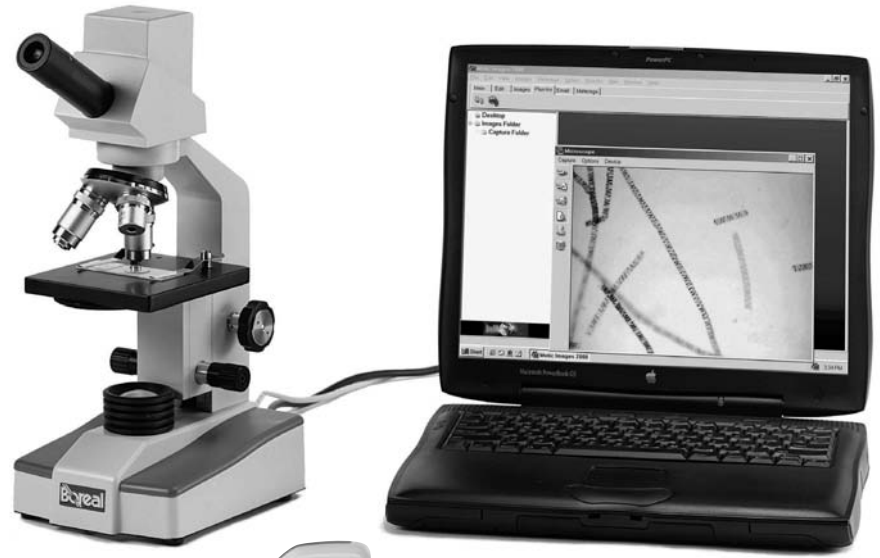
S17. Reversible black and white stage plate.

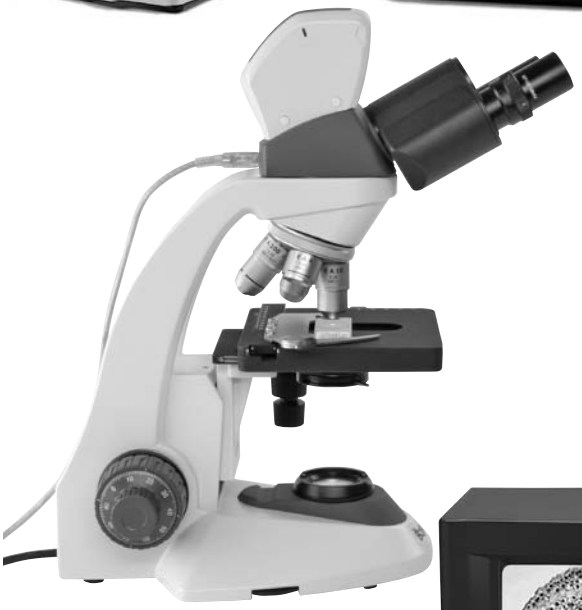
One side of this stage plate is white and one is black. Try a specimen against both backgrounds to find the one that shows detail best. The black side is especially useful for live, translucent organisms (such as daphnia) with incident illumination.

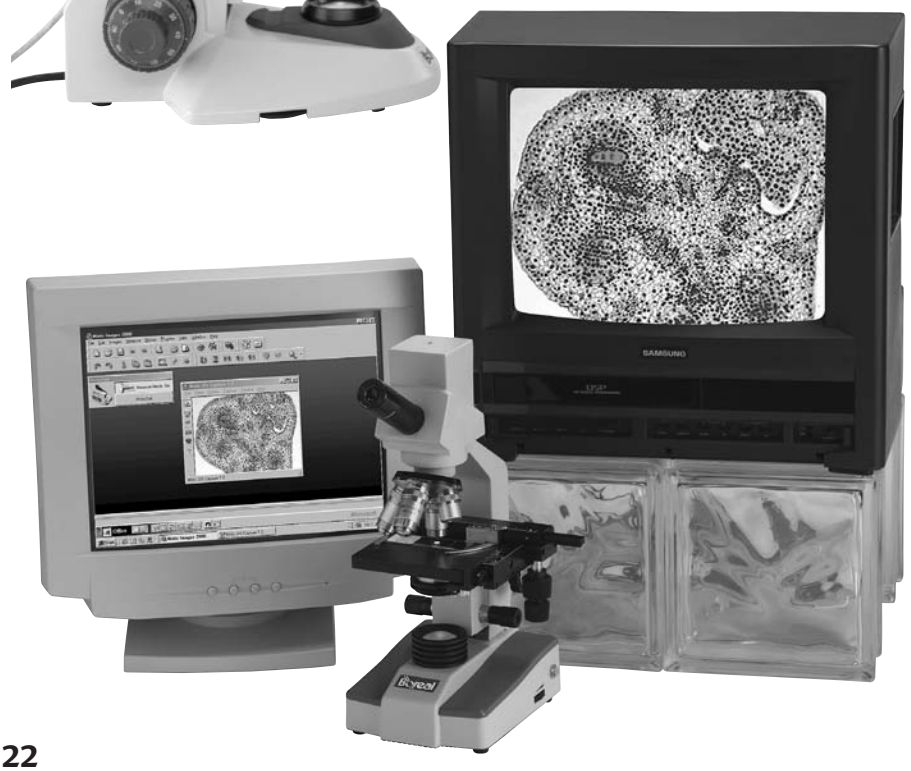
S18. Frosted glass stage plate.

This can be substituted for an opaque stage plate for use with in-base transmitted light. Thin and translucent specimens may be best illuminated using this arrangement.

Digital and Digital/Analog Microscopes





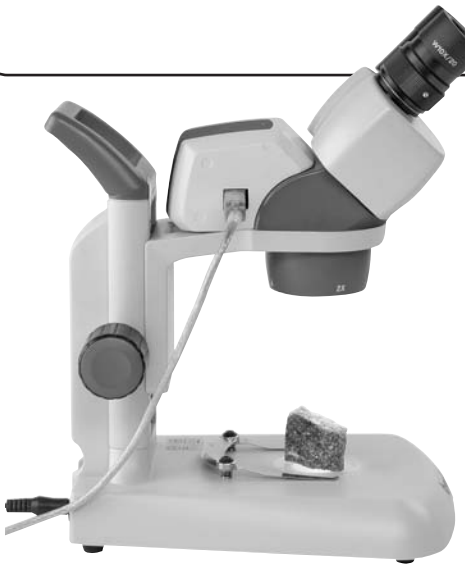


All Boreal digital and digital/analog microscopes have a lifetime warranty for the microscope itself and a warranty of one year for the camera on the microscope.

The bodies of digital and digital/analog microscopes function in the same way as our regular compound microscopes and stereomicroscopes, and the following sections of this booklet are applicable:

- **Features of Compound Microscopes**
- **Care and Maintenance of Compound Microscopes**
- **Annual Microscope Preparation**
- **Features of Stereomicroscopes**
- **Care and Maintenance of Your Stereomicroscopes**
- **Replacing Illuminator Bulbs**
- **Troubleshooting Guide**

For instructions about installing and using the software, please consult the set-up and operation manual that you received with your microscope.



Student Workbook

Light Microscopes

Most of us are familiar with light microscopes, in which the image is formed by light. There are two major groups of light microscopes. These are generally referred to as compound microscopes and dissecting microscopes, or stereomicroscopes (although technically, dissecting microscopes are also compound microscopes).

In a compound microscope, light is transmitted through a specimen that is thin enough to be translucent. The image is focused by two sets of lenses-the objective lenses, located near the specimen, and the ocular or eyepiece lenses, into which you look. The image you see is upside down and reversed from left to right. The image is magnified by both sets of lenses. The total magnification is found by multiplying the number of times the objective magnifies the image by the number of times the eyepiece magnifies the image. So, a 4x objective used with a 10x eyepiece will magnify the image 40 times and a 100x objective with a 10x eyepiece will magnify the image 1000 times. This is the range of magnification common with achromatic (blue-red color corrected) optics on compound microscopes.

In a dissecting microscope, light is usually shone down onto a specimen (although some dissecting microscopes or stereomicroscopes have in-base illumination for transmitted, as well as incident lighting) and reflected by the opaque specimen back into the objectives and eyepieces. The image is oriented in the same way as the object. Stereomicroscopes have paired objectives and paired eyepieces, so each eye of the viewer sees a separate image. These images are aligned so that the combined image appears three-dimensional. (There are binocular compound microscopes with paired eyepieces designed for comfort for extended viewing. Each eyepiece shows the same image from a single objective, and the image is two-dimensional, as it is for all compound microscopes.) The magnification on a stereomicroscope is also the product of the magnification of the objectives and the magnification of the eyepieces. Stereomicroscopes usually have 1x to 4x objectives and 10x or 15x eyepieces, so the magnification on stereomicroscopes is usually from 10x to 60x.

Generally, a stereomicroscope is used to see small details on larger specimens that can be either opaque or translucent. Stereomicroscopes are used for dissection of small, but visible, organisms or just to observe the tiny details used to identify insects, flowers, etc. A dissecting microscope or stereomicroscope has several advantages. First, objects can be examined with no preparation other than holding them in the field of view. Second, the image has the same orientation as the object, making it easy to manipulate the object. Finally, although many details can be seen that were not visible with the unaided eye, the overall object is still recognizable, making it easy to relate the details to the specimen.

Viewing most protozoa or cells from multicellular organisms requires the higher magnification of a compound microscope. More sample preparation is needed. The specimen must be thin enough to transmit light. If you are looking at microorganisms, this usually involves spreading them out in a thin sheet of water. For multicellular organisms, either a smear of loose or liquid cells (for example, cheek or blood cells) or a thin section cut with a microtome or razor is needed. In addition, cells or tissues small and thin enough to transmit light are usually so colorless that staining is needed to show the details. Alternatively, prepared slides of many common organisms, cells and tissues can be purchased. Despite the extra work and expense of using a compound microscope, the detail revealed at the higher magnifications is well worth the effort. It should be noted that 1000x magnification is needed to see any detail of shape for most bacteria. If you are interested in studying bacteria make sure your microscope will give good resolution at 1000x.

Compound Microscopes

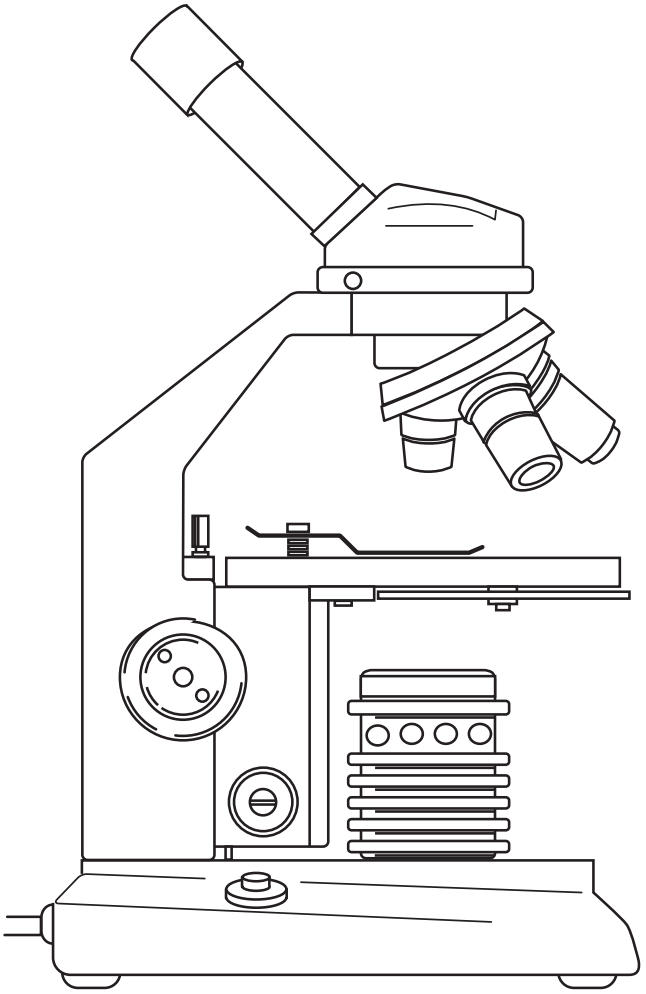
Parts of the Microscope

In a compound microscope light shines up through a very thin specimen. The light passes through one set of lenses (the objective) close to the object in view and then through a second set of lenses (the eyepiece or ocular) close to the eye of the viewer. There is some way to move the lenses to focus the image. We will review common types of compound microscopes and look at how they do these things to make the magnified image.

Following the path of the light, we start with either a mirror, to direct and focus light from some other source, or an illuminator, which is a lamp added to or built into a microscope that shines light up through the specimen. The mirror may have one curved side and one flat side. The curved side is a concave surface, curved inward, to focus more light into a path that will strike the specimen. The curved side is used with indoor lighting, to make the light bright enough for good viewing. The flat side does not concentrate light, and is good for use with outdoor light shining through a window, which may be too bright if concentrated. Direct sunlight is never reflected into the lenses of a microscope because it is so bright it could damage the eye of the viewer. Most illuminators have a lens to help aim the light and a blue filter. Blue light gives images of truer color and has a shorter wavelength than the yellow light given off by many bulbs. The shorter wavelength makes it easier to get an image with better resolution. Better resolution enables you to see the details of the image more clearly.

Most microscopes will have some means to control the amount of light you allow through the specimen. This can be done with a disc that fits under the stage and turns so that different sized holes line up with the hole in the stage. That type of light control is called a disc diaphragm. You can get more control with an iris diaphragm which is made up of overlapping leaves of metal which open and close like the shutter of a camera. Some microscopes also have a dimmer switch on the illuminator to give even more control of the light.

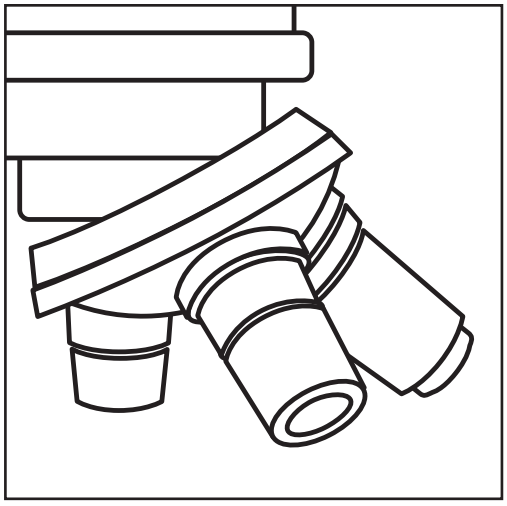
On the simplest microscopes, the light shines directly through the slide with the specimen on it once it leaves the mirror or illuminator. However, some microscopes have another lens or set of lenses below the slide. This lens system is the condenser. The condenser helps to gather light and send it up through the specimen in parallel rays.



This helps to form a clearer image for the viewer. Boreal microscopes offer two types of condensers. The simpler is a single lens fixed beneath the stage in the hole where light will pass to reach the specimen. It is called a fixed field condenser. The other is a two lens system in its own holder that can move up and down. This is called an Abbe condenser, in honor of Erst Abbe, its inventor.

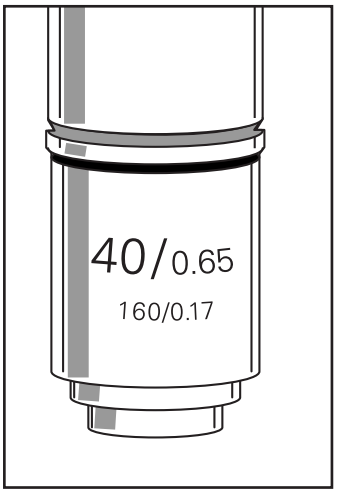
Both types of condensers are located beneath the flat working area of the microscope, the stage. Stages come equipped with some device for holding a slide in place. Stage clips are two flexible metal strips that hold the slide on the stage. The slide has to be moved by hand. A microscope may come equipped with a mechanical stage, either built in or placed on the stage in place of the stage clips. A mechanical stage has a spring arm that holds the slide in place against a metal bracket. Knobs off to the side or below the stage allow you to move the slide bracket and slide forward and backward or from side to side. A mechanical stage lets you move the slide evenly and in small movements. This is especially helpful if you are using high power objectives with a small field of view.

After the light passes through the specimen it goes into the objective lenses. The objective lenses magnify the image. How much the objective magnifies the image is determined by the power of the objective. The power of an objective is indicated on it as a number followed by an "x". The "x" means "times", so a 4x objective magnifies an image four times. Common powers for objectives are 4 or 5 x, 10x and 40x. Some microscopes have 100x objectives. If you look at a microscope with several objectives you will see that as the power of the objective gets greater, the diameter of the bottom lens gets smaller, the length of the objective gets longer, and the distance between the objective and slide (the working distance) when the specimen is in focus gets shorter. It is important to remember that the lens of a higher power objective is smaller. It takes in less light and the image it produces shows less of the specimen. When you are using a microscope, you often must increase the opening size on your disc or iris diaphragm to let more light through the specimen when you use the high power objectives.



Most microscopes have several objectives mounted on a revolving nosepiece, so you can simply turn the nosepiece to bring another objective into place. Modern microscopes have parfocal objectives. The objectives are made and mounted so that the image stays very close to the same focus as you switch from one objective to another.

You might notice several other markings, besides the power, on your objectives. If there is a D, DIN, or 160 marked on the objective it indicates that the objective is made to a standard size and focal length. DIN objectives will fit and focus on any microscope built to DIN standards. The 160 is for 160 mm, the tube length, or distance from the shoulder of the objective to the eyepiece that the objective is designed for. There might also be a decimal number printed next to the power, sometimes followed by the initials N.A. This is the numerical aperture of the lens. That is a measurement of the resolving power and light gathering ability of a lens. A larger numerical aperture denotes a lens that will give an image with greater detail. Condensers also have numerical apertures. The numerical aperture of the condenser must be greater than or equal to the numerical aperture of any objective used with it in order for the objective to work properly. Our fixed field condenser has a numerical aperture of 0.65, and so will work with any objective up to and including the 40x objective, which also has a numerical aperture of 0.65. The 100x objectives have a numerical aperture of 1.25 and should be used with a microscope with an Abbe condenser, which also has a numerical aperture of 1.25. You should know that common objectives and eyepieces will not give good results if the total magnification is more than 1000 times the numerical aperture. So a microscope with a 0.65 N.A. fixed field condenser is designed to give good resolution up to 650x and a microscope with a 1.25 N.A. condenser up to 1250x.



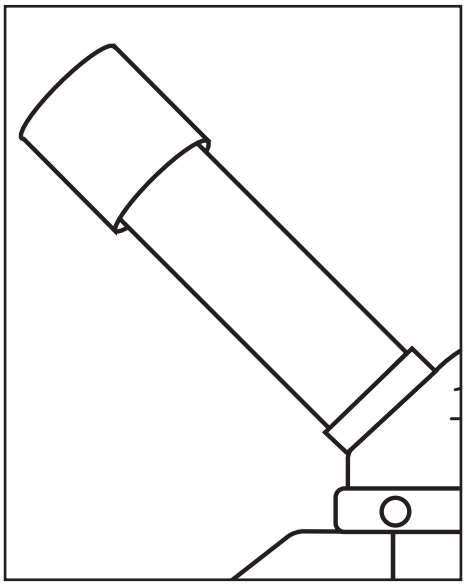
There may be one other number on the high power objective. This is a decimal such as 0.17 and is the thickest cover glass the lens is designed to work with (measured in mm). Remember, as the power of an objective increases, the working distance decreases. The cover glass number tells you how thick a cover glass your lens can focus through. A thicker cover glass may prevent that lens from focusing on the specimen. Cover glasses commonly come in three thicknesses:

Number 0 = 0.09 to 0.12 mm
Number 1 = 0.13 to 0.17 mm
Number 2 = 0.18 to 0.25 mm

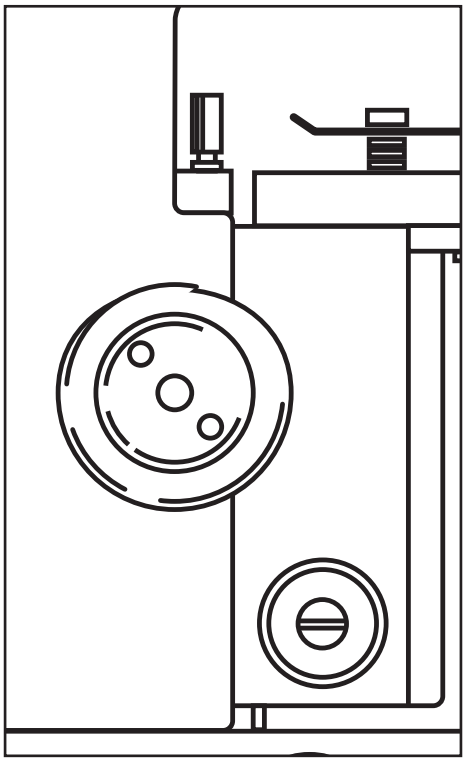
An objective lens rated 0.17 would work with a number 0 or number 1 cover glass, but not with a number 2 cover glass.

Before we finish discussing objectives we should talk about oil immersion lenses. Most 100x objectives are oil immersion lenses. Light bends when it travels from one thing to another with a different index of refraction. (The index of refraction is the ratio of the speed of light in a vacuum to the speed of light in another transparent material.) The index of refraction of air is 1.0003, that of glass is around 1.5. Immersion oil has an index of refraction of 1.515, close to that of glass. You use immersion oil to form a column between the cover slip and oil immersion

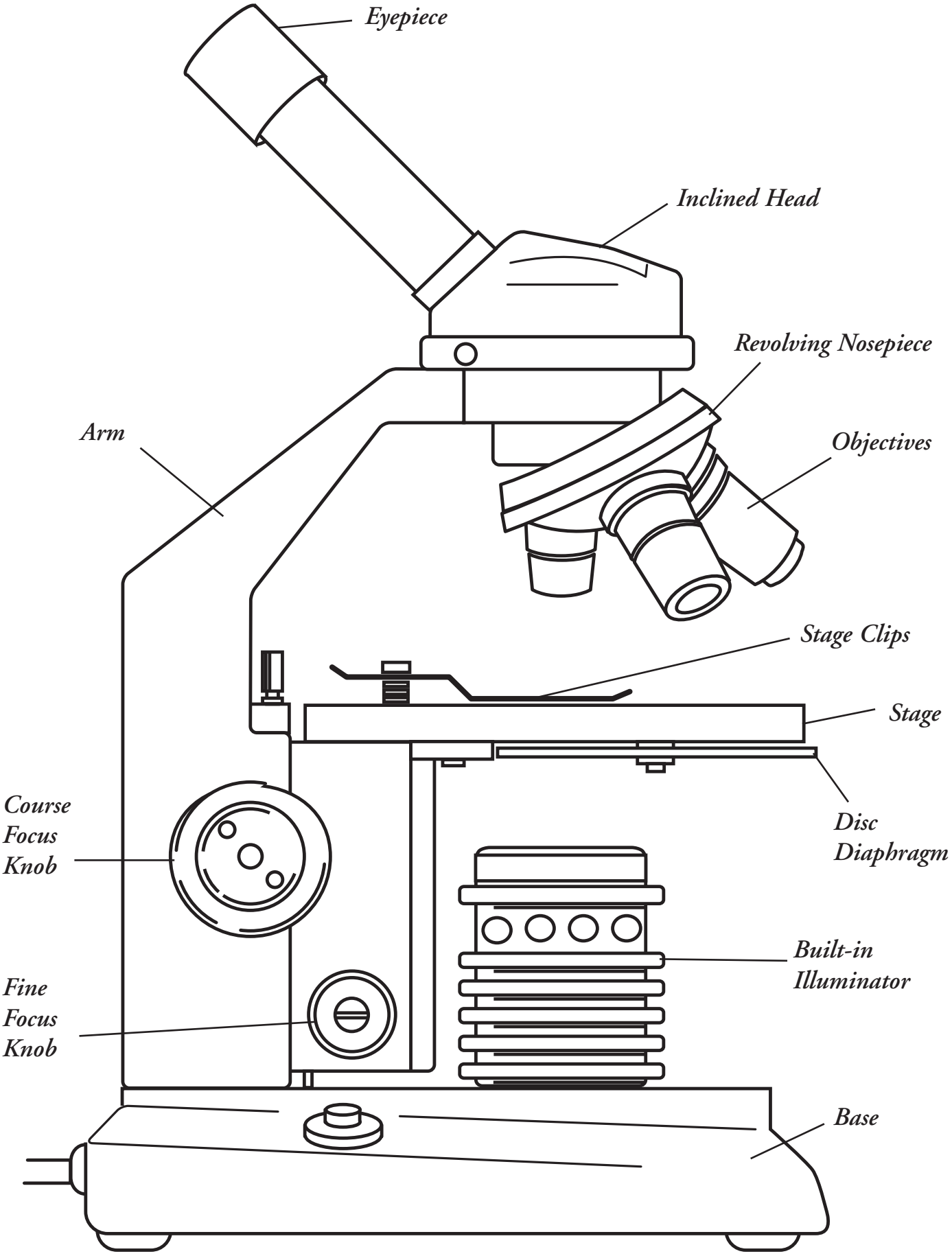
objective, lessening the amount the light bends in its passage from the illuminator to the lens system. In very precise microscopy, immersion oil is also used between the top lens of the condenser and the bottom of the slide, limiting stray bending even more.



The image, which is magnified by the objective lens, is further magnified by the eyepiece. The amount the image has been magnified is the product of the power of the objective and the power of the eyepiece. The most common power of eyepieces is 10x; the total magnification of any objective on a microscope with a 10x eyepiece is ten times the power of the objective. Most microscopes now come with widefield eyepieces. These are designed to give a bigger image and to allow you to see the image a centimeter or so above the eyepiece. This is usually more comfortable for viewing and also allows you to wear glasses while using the microscope. For some special applications a Huygenian eyepiece is used. This is an older style eyepiece, it has a smaller image size and you must put your eye quite close to the top lens to see the image.



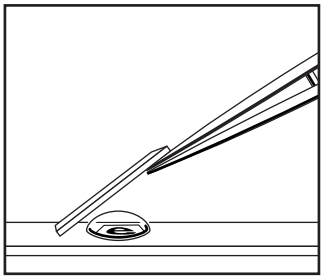
The focus of the image is controlled using the focus knobs. The focus knobs move the specimen slide and lenses closer together or further apart, either by moving the stage up and down or by moving the body tube, with the objectives and eyetube and eyepiece, up and down. Microscopes may have only a coarse focus knob, which moves over a distance of a centimeter or so, or they can have both coarse and fine focus. The range of movement of the fine focus control is only a matter of a millimeter or two. If a microscope has a fine focus control, those knobs can be entirely separate from the coarse focus, or they can be just outside the coarse focus knobs, on the same axis. That is called coaxial focusing. If a microscope has a 100x objective (1000x total magnification) it really must have a fine focus control, since the depth of field, the amount of the specimen from top to bottom that is in focus at any one time, is only a fraction of a millimeter with the 100x lens.


Eyepiece
Inclined Head
Revolving Nosepiece
Arm
Objectives
Stage Clips
Stage
Course Focus Knob
Disc Diaphragm
Built-in Illuminator
Fine Focus Knob
Base

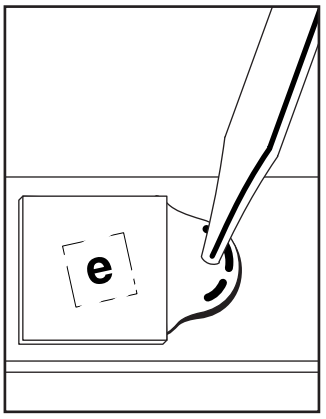
Using a Compound Microscope

Preparing a Slide

Before you use your microscope, you may want to learn how to make slides. A simple first exercise is making a letter e slide. There is a supply of the letter e for mounting in the back of this book. Begin by cutting out one letter.



Using forceps, place the e in the center of a glass slide. Add a drop of water from a dropper pipet, covering the paper completely. Carefully place one edge of a cover glass to the side of the water drop and the e. Lower the cover glass over the letter e, letting any air bubbles escape. If you end up with air bubbles trapped beneath the cover glass, you must remove them. Try putting gentle pressure on the center of the cover glass with the rubber bulb of the dropper pipet. If air bubbles are still present place a few drops of water along one edge of the cover glass. Take a piece of paper towel and place it against the other edge of the cover glass. Capillary action will wick water up into the toweling and draw the water drops from the other side under the cover glass. Hopefully, the air bubble will be drawn out. Be sure there is no excess water left on the top or to the sides of the cover glass when you are finished.



You have just prepared a temporary slide. Many other specimens can be prepared in a similar way. This kind of slide is temporary because eventually the water will evaporate. If your slide begins to dry out before you are finished viewing it, you can add more water without removing the cover glass. Place the end of the dropper pipet alongside the cover glass and slowly squeeze out a drop of water. The water drop will spread under the cover glass. Again, soak up any excess water from around or on top of the cover glass.

When you are finished with a slide, clean it in warm water that contains a drop of dish detergent. Hold the slide by its edges and rinse it under the faucet. To dry the slide, stand it on end on a dry paper towel, propping the upper end against a small jar. The water will drain off without leaving any marks on the slide. Cover glasses can be cleaned in the same way but must be handled very carefully since they are very thin and break easily. Hold them gently between your fingers by the edges so you don't leave fingerprints on them.

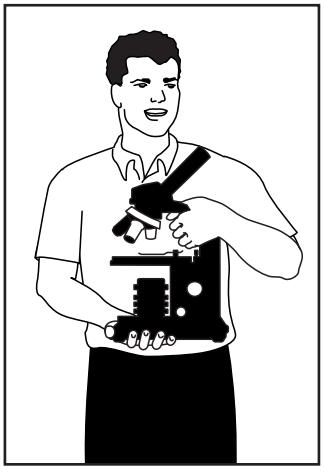
To prepare a permanent slide you follow the same method, but use Canada Balsam instead of water. Canada balsam cannot be mixed with water, or it turns cloudy. This is not a problem if you are preparing the slide of an e on dry paper, but almost all preparations from living organisms contain water. These must be carefully prepared by substituting another fluid for the water, before a permanent slide can be made. They also usually need to be stained, so the fine details show. There is a whole area of biology devoted to the preparation, viewing and photographing of microscope specimen slides called microtechnique. If you are interested in this, there are some books for beginners, such as *The Microscope and How to Use It* by Dr. George Stehli.

To make a permanent slide of an e, place a cut e on a slide as before. Place a drop of Canada balsam on the e. Apply a cover glass by placing one edge along side the preparation and carefully lowering it in place. You must be extra careful to avoid air bubbles, as Canada balsam is thick and air bubbles are hard to remove. Lay your prepared slide down somewhere where you won't have to touch it for two weeks. After two weeks you will find the cover glass is cemented to the slide with the e in between. The slide can now be stored vertically in a special slide box.

SAFETY NOTE

If you are going to try preparing your own slides, you must be very careful in cutting sections. The material you view must be very thin and this is often done by cutting it, either with a single edge razor or a microtome. A microtome is an instrument designed to cut thin sections. It has a device to hold and move the sample and a holder for a blade or knife. A microtome helps you achieve more even sections and can be safer to use, but any method will involve very sharp blades and should be used under adult supervision and with great care. If you cut yourself, wash the cut thoroughly with soap and water and use a disinfectant. Clean all your tools when you are through with them and store them out of reach of any younger children who might be around your work area.

Setting Up Your Microscope



- Prepare a cleared area to work with your microscope.

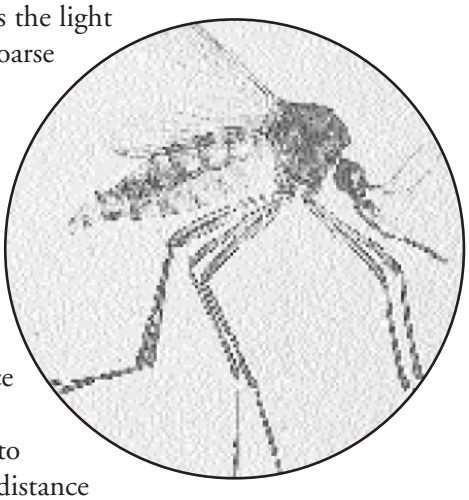
- Bring your microscope from where it is stored. When you carry your microscope, remember to always keep it upright and use two hands, one on the base and one on the arm.

- Set up your microscope so that it is comfortable to look into the eyepiece. Plug in the illuminator, if it has one. If you are using a mirror, use the concave (curved) side for indoor light and the flat side if you are near a window and using sunlight (caution: do not use direct sunlight, it can damage your eyes). Position the mirror so the maximum amount of light is reflected up to the eyepiece. Remember not to move the microscope or the mirror once you get good illumination.

Viewing a Prepared Slide

- Look just under the stage of your microscope. You will have either a disc diaphragm or a mounted lens system (Abbe condenser) with an iris diaphragm below it. Set the disc diaphragm to the second smallest opening or the iris diaphragm so the opening is about a quarter of the way open. This is generally a good amount of light to start with. If your illuminator has a dimmer switch, start with the light turned all the way up, or as far as is comfortable when you look into the eyepiece.

- Turn the nosepiece so the lowest power lens is in viewing position. Move the coarse focus of your microscope so the objective is as far from the stage as possible (if you have fine focus, it is best to start with it in the middle of its range of movement). Now take your slide and place it under the stage clips or in the holder of the mechanical stage if you have one. Position the slide so the specimen is centered over the opening in the stage that allows the light up. Now rack the coarse focus until the lens and stage are as close together as possible (if the lens comes in contact with the slide, stop and get help). Look through the eyepiece while you use the coarse focus knobs to slowly increase the distance between the objective and slide until you can see the specimen. This should happen close to the starting position. Carefully adjust the coarse focus until the image is as clear as you can get it. If you have fine focus, you can use it for the final adjustment. Try adjusting your diaphragm to obtain the best lighting for the specimen. Be careful not to use so much light that you "wash out" the fine details.



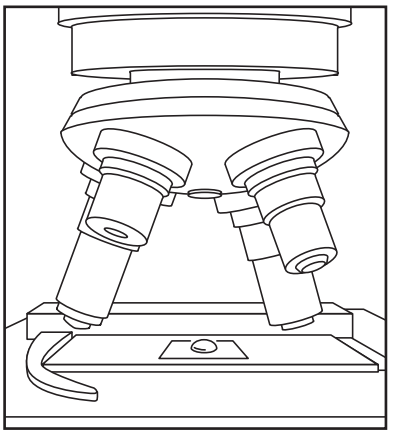
- Move the slide slightly on the stage. Notice that the image moves in the opposite direction. The image is also upside down and reversed from the object. This is easy to see if you are looking at the letter e.

- With the image in clear focus and centered in the field of view, move the nosepiece to the 10x objective. The image should be centered and should require only minor focusing adjustments to bring it in clearly. If you don't see any image, turn the nosepiece back to the lower power. Check to see that the object is in the center of the field of view. As you increase power, you decrease the size of the field of view proportionally. That means in switching from the 4x lens to the 10x

lens, you will only see 40% of the area you could see under the 4x lens, and switching from the 10x lens to the 40x lens, you will see only 25% of what you could see under the 10x lens. If your microscope has fine focus, only the fine focus should be used or needed on any but the lowest power. Don't try to "find" the image by using the coarse focus. Ask your teacher for help. Once you have the image sharply focused and centered under 10x, you can try the high power lens.

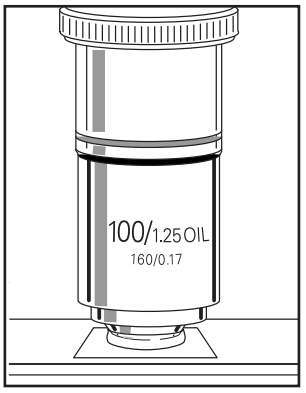
- Turn the nosepiece clockwise once more to bring the 40x objective into viewing position. Again adjust the area of interest to the center of the slide and bring it into sharp focus using the fine focus knob. If you are using a wet mount, be sure you have wicked away enough liquid so the lens of the 40x objective will not touch the liquid. This lens may not be sealed and water might get inside between the lenses, making it necessary to have the lens disassembled in order to clean it. As you increase power, you may have to increase the light, because the amount of light entering an objective decreases as its magnifying power increases.

- If you have a 100x objective, it is an oil immersion objective. Make sure your specimen is in sharp focus and the area of interest is centered in the field of view using the 40x objective before you change to the 100x objective. Swing the 40x objective clear of the light path. Drop one drop of immersion oil (such as our type A immersion oil) on the slide where the light is. Then swing in the 100x objective.



If you have done this correctly, the oil should form a little column from the cover glass up to the objective lens. Focus slightly with the fine focus knob, as needed. When you are done using the oil immersion lens, use lens paper and lens cleaning solution to clean the oil from the lens. Do not let the oil dry. Also clean the slide. Be sure you have the area of interest in place before you apply oil. If you need to go back to the 40x objective, you will have to stop and clean the oil off the slide, because the 40x objective may not be sealed, allowing it to touch the oil might damage it.

- On either 40x or 100x the part of the specimen in sharp view from top to bottom is likely to be less than the thickness of the specimen. To see all of the detail of the slide, it is best to focus up and down through the specimen using the fine focus.



- You will see the most detail if you experiment with the lighting and move the fine focus up and down as you view a specimen.

Stereomicroscopes

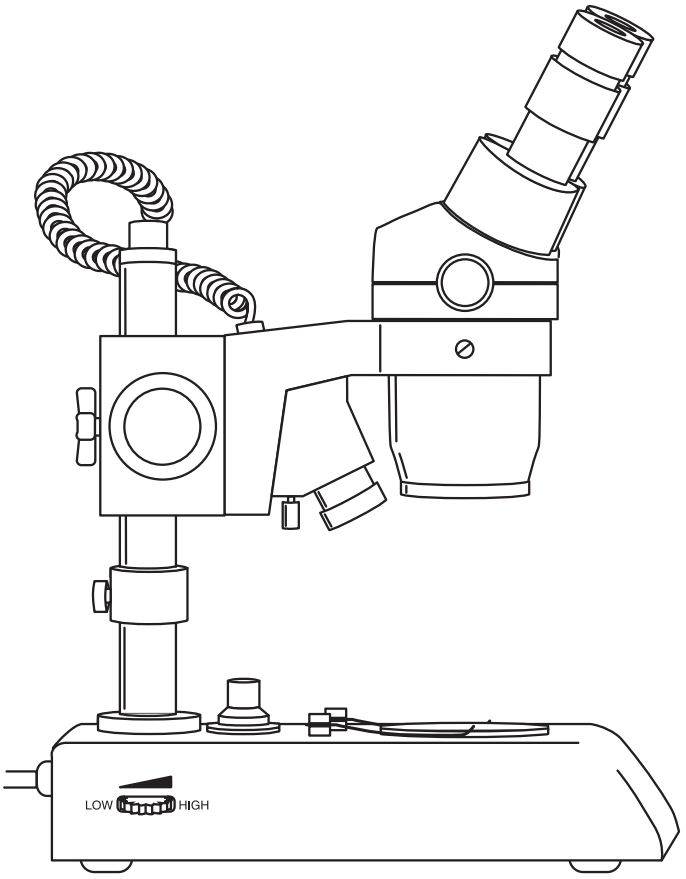
Parts of a Stereomicroscope

A stereomicroscope is actually two separate optical systems, one for each eye. They are aligned so they focus together and are centered at the same point, but they are not exactly alike. Each system is aimed at a slightly different angle, so you get a single three-dimensional image like when using your unaided eyes to look at an object. Your brain combines the two images from the eyepieces of a stereomicroscope the same way it combines the two images from your eyes, to produce a single three-dimensional image. This three-dimensional effect is greatest when using the lowest magnification, because then the depth of field (the amount of the specimen from top to bottom that is in focus) is greatest. The image seen in a stereomicroscope is also oriented the same way as the object, and moves in the same direction as the object. This makes it much easier to manipulate the specimen, such as when you are doing a dissection.

The stereomicroscope has a platform for placing the specimen that is combined with the base of the instrument. It may use just the room light for illumination, or it may have one or two lights built in. For each magnification power it must have two objectives and two eyepieces. Finally it must have a way to move the lenses closer to or away from the specimen to focus the image.

The simplest design has a round white disc in the base where the specimen is placed. That stage plate may have white on one side and black on the other, so the user can choose the background that shows the object best. The most basic illumination for a stereomicroscope is an incident illuminator. This is a light that shines down on the specimen. Other models also have an in-base illuminator. These models have a frosted glass stage plate to let the light shine up through the specimen. Sometimes you can use both the incident and in-base lights together, which is helpful for very irregular objects.

The light reflected from, or passing through the specimen goes directly into the paired objectives. The objectives usually magnify the object one to four times. Sometimes there is a lens that can be added over the objectives to magnify the image two times. On other models there are two sets of objectives mounted in a nosepiece that turns to bring one set or the other in viewing position. These sets of objectives are parfocal, so the object remains in



focus when you switch objectives. A more expensive arrangement is to have objectives with lenses that move in and out to change the image size continuously over a range. These are referred to as zoom stereomicroscopes.

All of these types then pass the light through eyetubes to eyepieces. 10x and 15x are common powers for stereomicroscope eyepieces. The total magnification you find in stereomicroscopes is much lower than for compound microscopes, and usually ranges from 10 times to 60 times the size of the object.

The eyetubes of a stereomicroscope can move together and apart. You have to adjust them so each of your eyes looks straight down the eyetube, or you won't see the three-dimensional image. Many stereomicroscopes also have one or both eyetubes that will twist and get longer or shorter. This is called a diopter adjustment and is used to focus the eyetubes separately, in case your vision is different from one eye to the other.

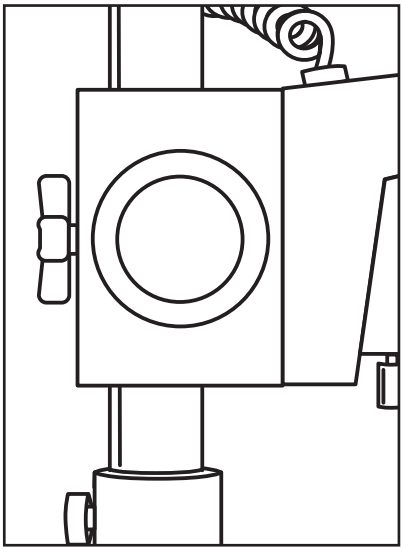
Since stereomicroscopes magnify the image less, they have only one set of focus knobs. These move a gear that raises and lowers the body of the microscope, including the objectives and eyepieces. On many models you can also raise and lower the body with a clamp on a post. This movement sets the proper height of the body as it relates to specimen size. It is not used for focusing.

Stereomicroscopes have a set distance from the lens at which the object is in focus. This is the working distance. More expensive optical systems have longer working distances, which gives you more room between the objectives and the specimen to work with the specimen, such as when you are dissecting an organism. If a stereomicroscope has two sets of objectives, the working distance will always be less for the more powerful objectives.

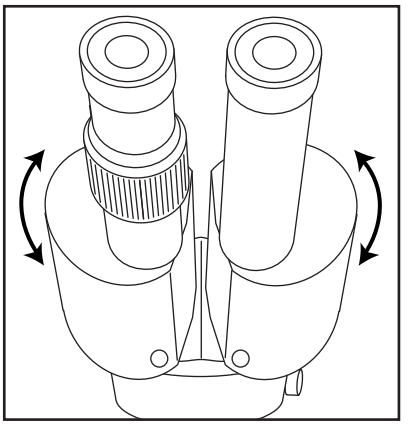
Using a Stereomicroscope

- Place the specimen you are viewing in the center of the stage. If you have a microscope with a body clamped to a post, quickly run through the range of focus to make sure there is a point where you can see the image. If not, let your teacher know that the body height needs adjusting.

- Body height adjustment: If your microscope has a retaining ring below the body clamp, make sure that it is in place and tight before adjusting the body clamp. In general, you will need to move the body up for very thick specimens and down for very thin ones. Adjust the retaining ring first. Then loosen the clamp that holds the body on the post, while holding the weight of the body and head in your other hand. Be sure you are not touching the objectives. Move the body up or down as needed. Moving it 3 to 5 cm should be sufficient. Make sure the objectives are centered over the stage and tighten the clamp. Do a quick run through the range of focus again, to make sure the position is correct.

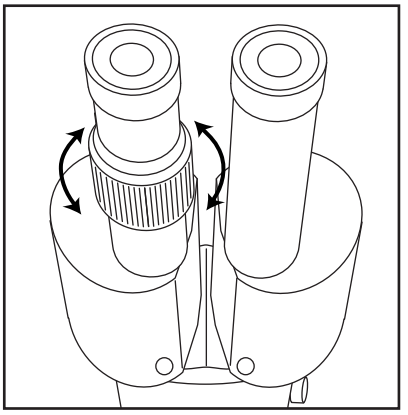


- Move the focus knob until the specimen comes into view. Do not worry yet if you can see through both eyes.

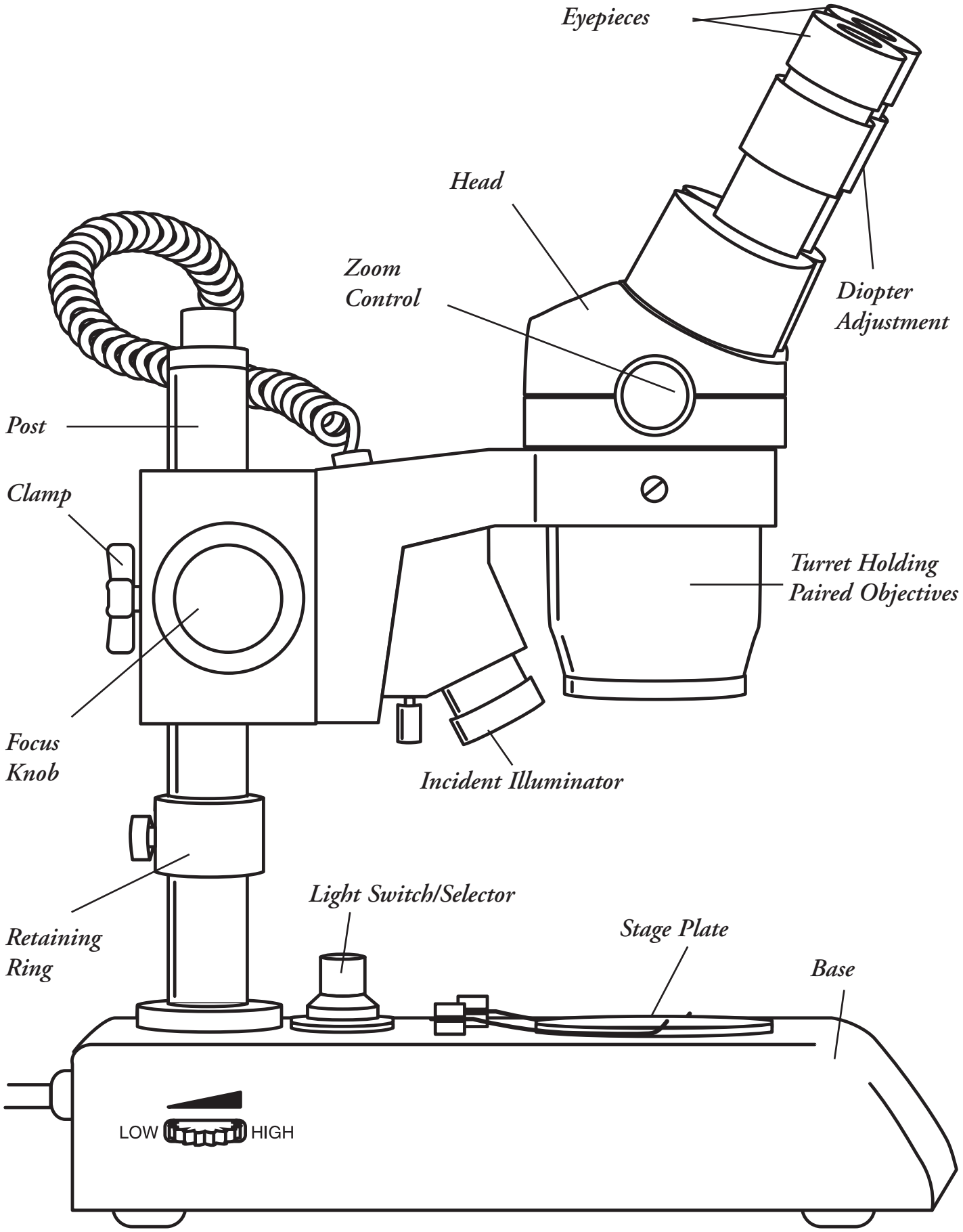


- Once a part of the specimen is in focus, grasp both eyetubes and gently move them towards or away from each other until you see a single three dimensional image with both eyes. Be patient. This is an important step and will reward you with both a better image and more comfortable viewing.

- If your stereomicroscope has a knurled ring on one of the eyetubes, that is a diopter adjustment and is used to adjust the focus to match your eyesight. Close the eye using the eyetube with the diopter adjustment. Focus one feature of the specimen as sharply as you can for the other eye using the focus knobs. Then close the second eye and focus the specimen sharply for the eye using the eyetube with the diopter adjustment by turning the diopter adjustment only. Now the image should be in sharp focus for both eyes. The diopter adjustment only needs to be set once for each viewing session.



- If your stereomicroscope has a reversible black and white stage plate or in-base as well as incident illumination, you can experiment with different combinations of background and lighting to get the optimal image for a given specimen.


Eyepieces
Head
Zoom Control
Diopter Adjustment
Post
Clamp
Turret Holding Paired Objectives
Focus Knob
Incident Illuminator
Light Switch/Selector
Retaining Ring
Stage Plate
Base
LOW
HIGH

Glossary

Abbe condenser: a condenser invented by Erst Abbe, a German physicist. It consists of two lens elements, the top element being removable for low power observation or photography.

achromatic objectives: objectives designed to bring the red and blue parts of the visible light spectrum into the same focus while bringing green and other colors into a shorter focus.

arm: the main vertical section supporting a microscope.

coaxial coarse and fine focus: having both the coarse and fine focus operate on a single axis, so the knobs are concentric (usually with the coarse focus being the larger of the two knobs on the same side).

coarse focus adjustment knob: the control used to move the objective lens or stage up and down during initial focusing on a specimen. The amount of movement of the stage or lens is greater in proportion to the movement of the knob than for the fine focus adjustment knob.

compound microscope: an instrument fitted with objective and ocular (eyepiece) lenses and used for viewing small objects.

condenser: a mirror, lens or combination of lenses located under the microscope stage; used to gather light and direct it onto the object being viewed.

depth of field: the vertical distance that can be sharply focused on a specimen. As the power of magnification increases, the depth of field decreases for any given microscope.

disc diaphragm: A disc with five or six different sized holes fitted under the stage of a microscope. As the disc is turned, one after another of the holes swings in place beneath the opening on the stage for the light. It controls the amount of light that passes up through the specimen slide.

eyepiece (ocular): the lens system closest to the eye. It is the component of the compound microscope that magnifies the primary image of the objective.

field of view: the area visible through the microscope. As the power of the objective increases, the size of the field of view decreases for any given microscope.

fine focus objective knob: the control used to bring the specimen into sharp focus by moving the objective or stage up and down in very small increments.

high power objective: a lens with greater magnification ability.

iris diaphragm: a device under the microscope stage that uses a series of metal leaves that work together to control the size of the opening through which light passes into the condenser.

low power objective: lens with lower magnification ability.

magnification: the number of times that a microscopic image appears larger that the original specimen.

mechanical stage: a device to hold the specimen slide and move it to bring any part into the optical path or field of view.

nosepiece: the rotating device that holds the objective lenses.

objective: lens closest to the object being magnified.

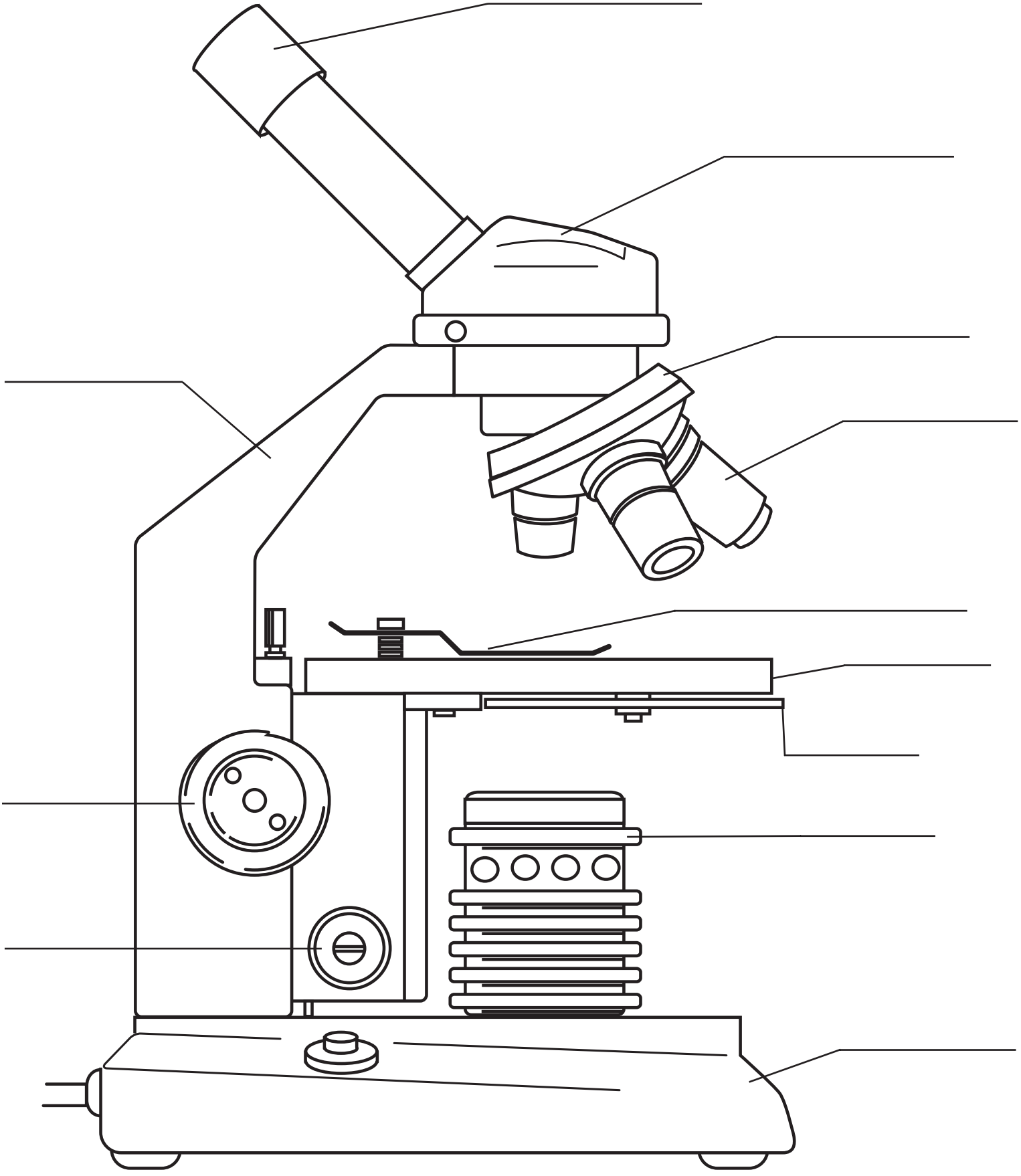
oil immersion objective: an objective lens system used for obtaining very high magnification. In use, the objective lens is immersed in a drop of oil that is in contact with the cover glass on the slide.

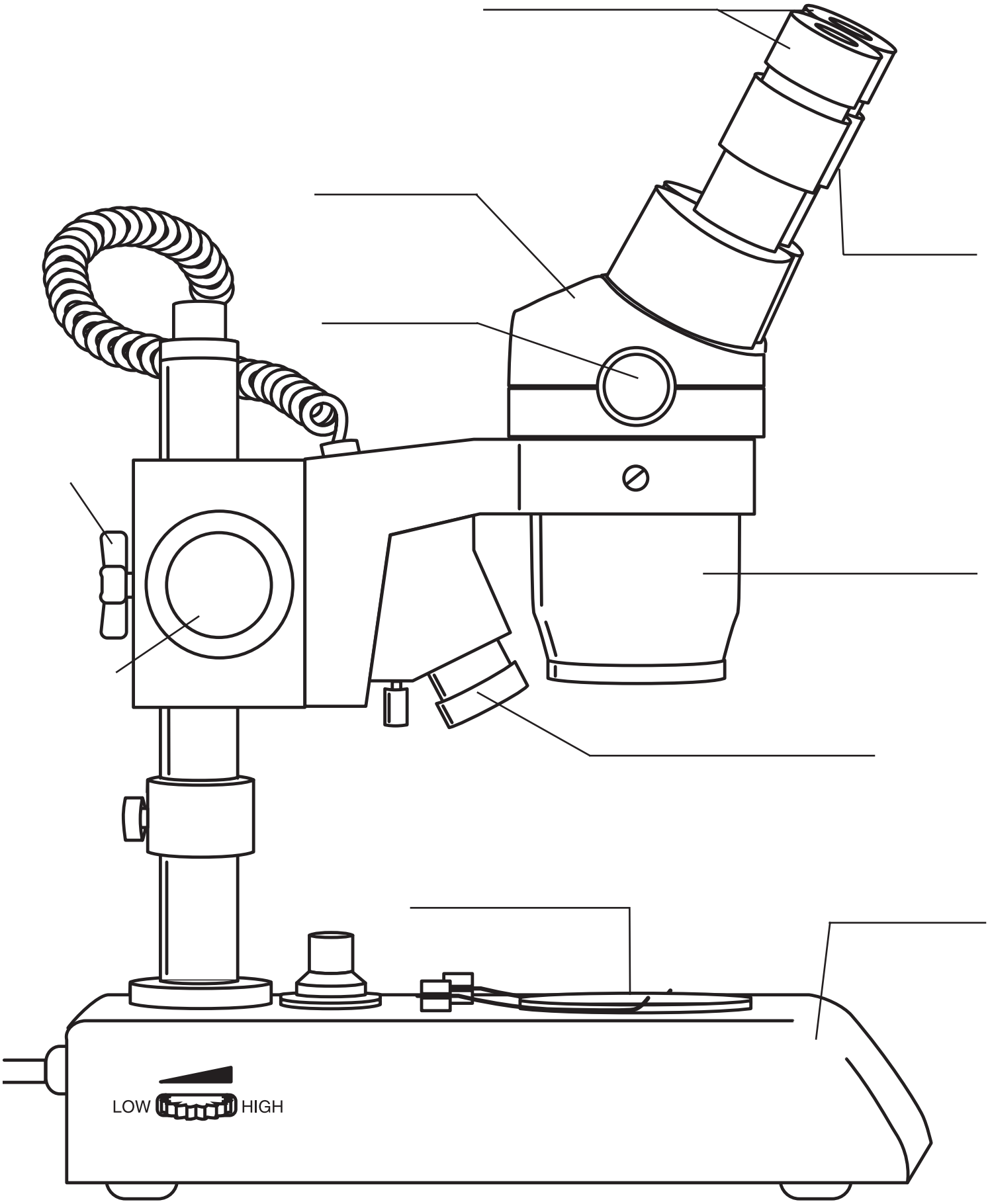
parfocal lenses: lenses that remain in focus when the magnification is changed.

resolution: the ability of a lens to distinguish and separate one tiny structure from another.

wide field eyepiece: an eyepiece with an entrance lens larger that on most types of eyepieces; it lends itself to varying eyepoint requirements.

working distance: the distance between the front end of the objective and the specimen, when the specimen is in sharp focus.




LOW
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