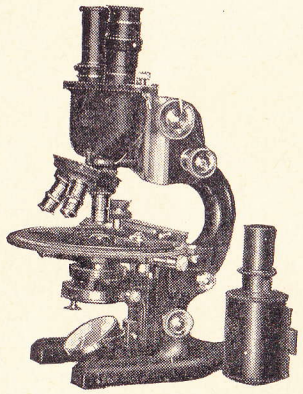
THE MICROSCOPE

CONSTRUCTION, USE AND CARE

PUBLISHED BY
SPENCER LENS COMPANY
BUFFALO, N.Y.



THE CONSTRUCTION, USE AND CARE *of the* MICROSCOPE

A brief outline of the mechanical and optical principles involved, with special reference to efficient manipulation

PUBLISHED BY
SPENCER LENS COMPANY
BUFFALO, N.Y.

FOREWORD

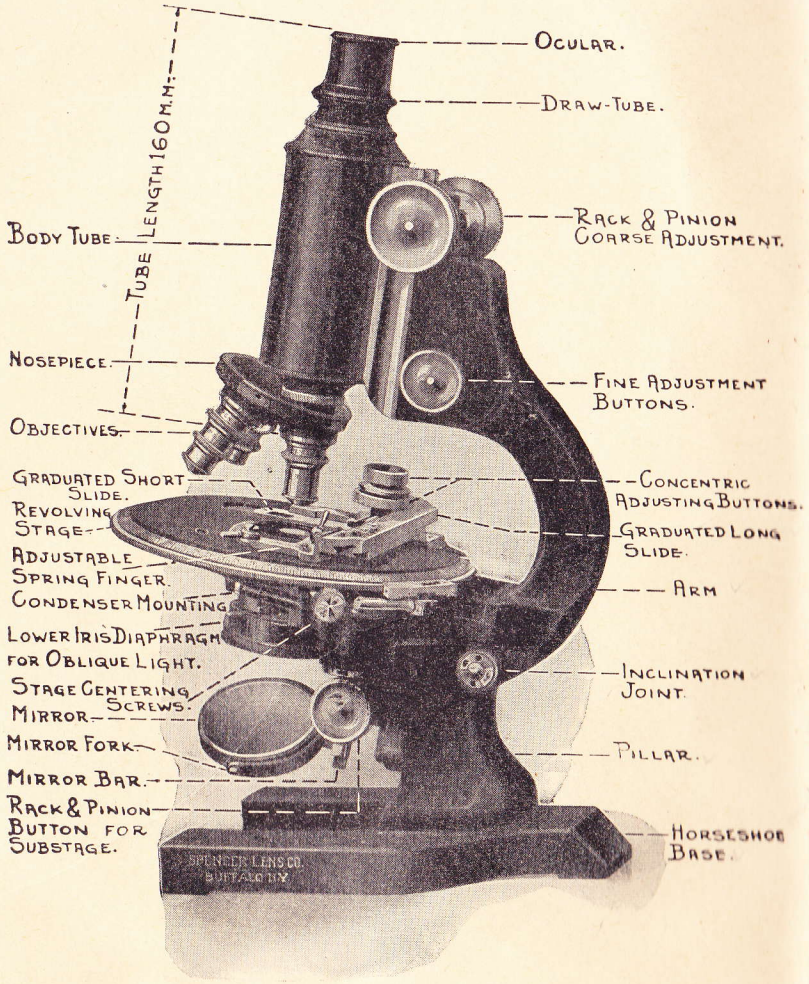
THE importance of a thorough knowledge of the microscope is more and more recognized as a necessity, antecedent to courses in which the instrument is used. Naturally the best work is done only after an intimate understanding of the use and relations of the different parts; and how to keep them in perfect working condition.

We are glad to respond to the demand for a short treatise on the construction, the care, and the use of the instrument—both mechanical and optical—going into detail only in so far as is necessary to make the instrument an efficient means for the study of the subject at hand.

Let it be remembered that no amount of direction will take the place of good judgment and careful, painstaking effort on your part, and that it is only the perfect adjustment of every part in relation to every other part which brings the best results. The neglect of one detail may destroy the virtues of all the others.

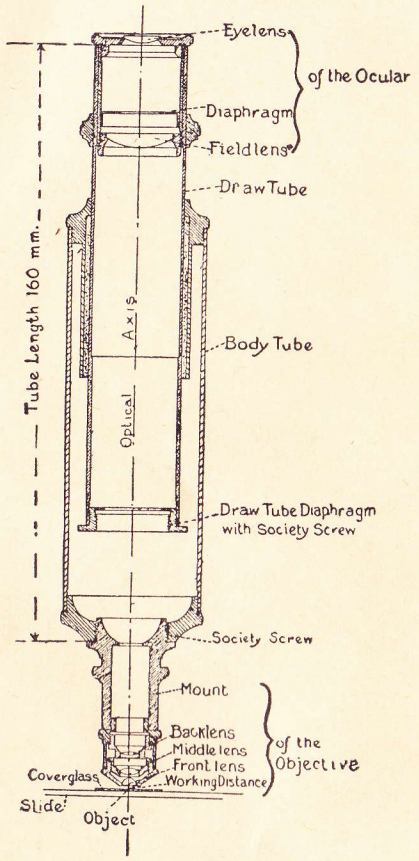
We desire that the use of the instrument may prove to be of great profit and great pleasure as well.

SPENCER LENS COMPANY



PART I

Construction and Care



EVERY microscope is regularly sent out from the factory securely packed in a case which becomes a safe abiding place for the instrument when not in use. After using it should always be returned to its case, or protected in some other way from dirt and dust and from direct sunlight. The case is always a safe vehicle for carrying the instrument about.

If any of the parts should become soiled, wipe them off with a soft cloth or *clean* chamois skin. If the soil does not remove

easily, breathe on the surface and rub gently. If this is not successful, try moistening with a little xylol, ether or chloroform, if indeed, water previously tried does not accomplish the end. Dry as soon as possible. *Never* use alcohol on lacquered parts unless you *know* the lacquer is alcohol proof. Fortunately nearly all lacquers now used are not soluble in alcohol. The black enamels which are more and more extensively used are not affected by any of the ordinary reagents.

The modern microscope is more compact, solid and rigidly built than those in use some years ago. It is better adapted to the serious purpose of modern laboratory methods. In all makes much careful engineering skill has been exercised to make a durable and efficient instrument.

Base and Pillar

Beginning with the base, stability is attained more by weight than by spread of the feet. Usually the base and pillar are one casting, although on some microscopes they are made separately and securely fastened together. There is no advantage either way.

Inclination Joint

Practically all microscopes are provided with inclination joints by which the body of the instrument is movably fastened to the top of the pillar so that it may be inclined to any angle which ease and convenience may dictate. The joint should work smoothly and freely and yet with sufficient friction to hold the body at any inclination.

There are several types of construction as illustrated. In all of them the pillar forms two parts of the hinge while the lower end of the arm is made to fit between. In all the best types the taper axle, or a modification,

is predominant. The necessary friction is obtained by drawing the cone into its bearing and compressing the sides of the pillar.

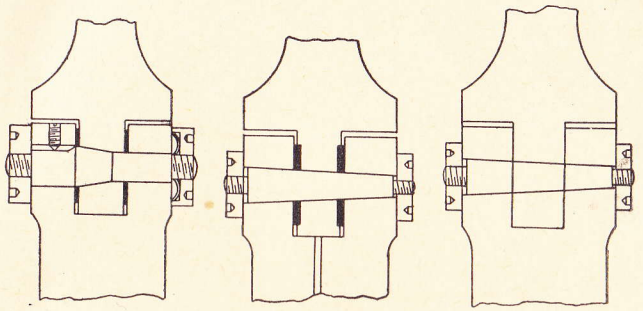


Fig. A *Fig. B* *Fig. C*

Once in a long while one of these joints may work loose so that the body will not remain at the desired angle. This may be remedied by tightening the nuts on the ends of the axis. These nuts are usually provided with two small holes for a "spanner wrench." Usually tightening the nut on the right side of the instrument draws the conical axis farther into its bearing. Sometimes it is necessary previously to slightly loosen the nut on the other side—tightening it again later. If a spanner is not convenient the nut can usually be turned with a pair of round nosed pliers. In any instance be careful not to mar the nut around the holes.

The Body

The body of the microscope is made up of the *Arm*, which on the best instruments is of brass or bronze, the *Intermediate Slide* whose bearings and movement on the arm are controlled by the *Fine Adjustment*,

and the *Body Tube*, whose bearings and movement on the intermediate slide are controlled by the *Coarse Adjustment.*

The Fine Adjustment

The fine adjustment is the vital part of the microscope stand. No high power work can be done without its proper functioning. One of the essentials is a perfectly fitting bearing which permits a free up and down movement in a line at 90° from the plane of the stage, with no lateral motion. The metal forming these bearing surfaces should be of such texture, the fitting so perfect, and the lubrication such that the movement always responds immediately to the least impulse. These bearings should be protected from dust and other foreign matter, and should be lubricated with oil which will not become gummy and sticky.

If for any reason these, or any other, bearing surfaces should become gummed, clean them off with xylol or chloroform, and re-lubricate with white vaseline. On all Spencer microscopes oil grooves are put in all bearing surfaces to hold a reserve supply of the lubricant. This insures a smooth movement which can not be accomplished in any other way.

If, when working the fine adjustment up and down, while the eye is at the eyepiece, the object appears to move sidewise, the light coming from the mirror is not central, or there is a lateral movement in the fine adjustment bearings. If upon carefully centering the light the apparent movement of the object persists, the trouble is in the fine adjustment—either poor fitting, a decided lateral thrust in the mechanism producing the movement impulse—or both. It should not occur.

The mechanism for supplying the impulse must of necessity be of extreme accuracy, delicacy and durability. Nothing in mechanics will accomplish this as well as the micrometer screw with a sufficient number of threads in contact with its nut; especially when used in conjunction with the lever.

There are two classes of fine adjustments: the older with the micrometer threads perpendicular and the fine adjustment head at the top of the arm; and the newer with two fine adjustment heads, one on either side of the arm. The latter is the more convenient and is rapidly replacing the former. Of the former there are two distinct types: the one (Figure D) where the whole arm moves on a triangular pillar, and the other —later and better—where the intermediate slide moves on the arm actuated by a lever in connection with the micrometer screw (Figure E).

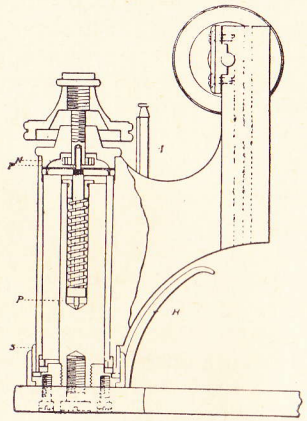


Fig. D

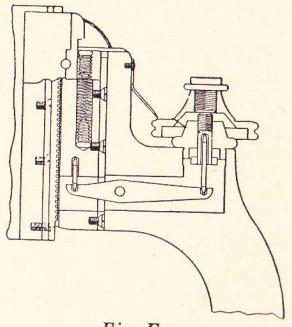


Fig. E

Stop nuts are provided at the end of the micrometer screw to prevent the fine threads being removed from their bearings. Sometimes these nuts are forced off, and the micrometer threads are removed. When this occurs be careful in replacing these very fine threads to see that they are started straight, and that they do not "run." Do not force the threads if they go at all hard. It will help to know that on most microscopes these threads are left handed. Care should also be taken to see that the little pin *P*, which fits loosely into the hollow end of the micrometer screw, is in position. Should it be lost it must be replaced. Should it fall into the mechanism it can be secured only by removing the nut *N* at the top of the arm. In some instances the pin drops out before the micrometer thread is entirely out of its bearings, and the defect is not noticed until the fine adjustment does not respond. As stated above remove the large nut at the top of the arm to replace the pin.

Most of the modern microscopes are of the second class where the two fine adjustment heads are one on either side of the arm. The mechanism here is slightly more complicated, hence the need for the best mechanical principles.

The bearings for the shaft connecting the two heads are on either side of the arm and should be in precise alignment for the free turning of the shaft. In no way is this accomplished as well as where the two bearings are in one continuous piece passing through the arm. Here as in the other class the micrometer in conjunction with the lever stands out as superior. Here there are a goodly number of threads always fully engaged through 360° of each thread, insuring a steady, regular and very durable lateral movement. When this steady

impulse is applied to the end of the longer arm of a bell crank lever, it generates a reduced upward thrust of the shorter arm to move the intermediate slide upward *without any tendency* to move it sidewise to create an apparent side movement of the object. The construction is such that the weight of the moving parts together with a compression spring continually keeps all of these parts in such contact that any possible lost motion is automatically taken up even though there may be the slightest wear through long continual use. See Figure F—or G, a somewhat simpler form.

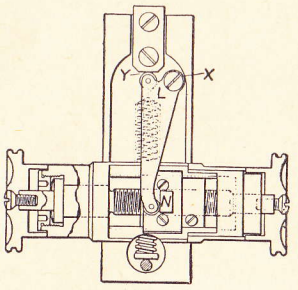


Fig. F

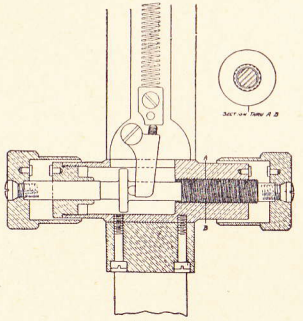


Fig. G

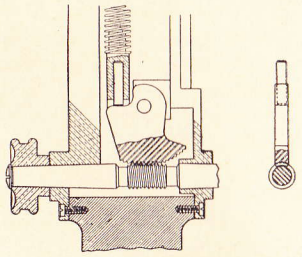


Fig. H

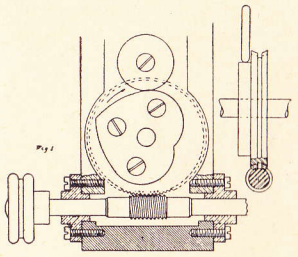


Fig. I

Figure H represents a fine adjustment ensemble quite similar to that of Figure G except that the micrometer thread is replaced by a worm-gear device. A segment of a gear takes the place of the nut and the long arm of the bell crank lever. The impulse from the short arm is upward, producing no lateral thrust as is also the case in Figures F and G. On all of the above described adjustments provision is made for automatically stopping the revolution of the shaft when the adjustment has reached the limit of its excursion, thus avoiding any injury to the threads or gears.

Figure I represents another worm-gear fine adjustment in which the complete gear circle is used. On this gear is fastened an eccentric heart shaped cam on the periphery of which a small roller is made to revolve. This roller is attached to the movable parts of the fine adjustment. When the gear revolves in the direction of the arrow as shown in the cut, one of the components of the movement of the heart shaped cam forces the roller upward, and will continue to do so until the apex of the heart passes under the roller when it will then move downward for 180° of the revolution. It has the advantage that the fine adjustment never "runs out."

An entirely new fine adjustment (see Figure J) has recently come out in which the necessary reduction is attained by means of a chain of spur gears similar to clock gears working in conjunction with a lever. It is very different from the types just described. It provides the direct upward thrust. The fine adjustment head is represented at F.B.

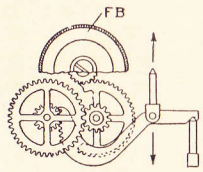


Fig. J

All standard microscopes are now made so that the positive impulse is always upward

against a slightly compressed spring. The mechanism allows the movable parts to descend by gravity and the force of the compression spring. Therefore in focussing down one is not so apt to cause damage if the front of the objective should come into contact with the cover glass. Avoid this contact if possible. If at all uncertain observe the proximity of the objective to the cover glass before looking into the eyepiece.

If when looking into the eyepiece no change of focus is noticed by turning the fine adjustment, it is quite possible that the objective is resting on the cover glass. It is possible that the fine adjustment may have "run out." It is always best to keep the fine adjustment about midway in its range. If the microscope is one provided with positive stops at the ends of its excursion, it will be necessary to place the mechanism somewhere near the midway point. If the microscope is one with the fine adjustment head at the top of the arm, the loose pin in the end of the micrometer screw may have been misplaced. See page 12.

The Coarse Adjustment

The Coarse Adjustment or rapid movement of the body tube is now affected on all microscopes by means of the diagonal rack and pinion. The bearing surfaces on the American and European instruments are quite different. Both are good. There is no advantage either way except for the oil grooves on Spencer microscopes. The bearings are very closely fitted. Any foreign matter on the surfaces seriously interferes.

Do not strain the teeth of the rack and pinion by forcing the bearings back and forth over one another when they are not clean. A little xylol or chloroform rubbed on the surface will clean them. Do not use emery or any

other abrasive. When the bearings are perfectly clean, oil them slightly with a good acid free lubricant (paraffin oil or watch oil). If the bearings become so loose that the tube will not stay in place, tighten the little screws at the back of the pinion box. All makers have a provision here for taking up lost motion and wear. Do not fill the teeth of the rack with paper, paraffin or any other foreign substance. If anything should accumulate in these teeth, clean them out.

The Body Tube

The body tube, together with the draw tube, is the support for the principal optics of the microscope. The objectives are located at the lower end of the tube and the eyepieces at the upper end. The graduated telescoping draw tube provides a means for varying the length of the tube (See page 7). It should move smoothly and easily. In pushing it in be careful not to push down the whole body tube to injure the specimen or the objective by bringing the objective violently into contact with the cover glass.

Binocular Body Tube

A comparatively new body tube has been introduced by which both eyes may be used while working with a single objective. These binocular body tubes are interchangeable with the single tube without disturbing the objectives. This is particularly true of the Spencer device by which either body when loosened may be lifted out of place and the other replaced without the least danger of disturbing the focus of the highest power objectives.

As illustrated on next page the light from the objective is divided by two cemented prisms immediately above

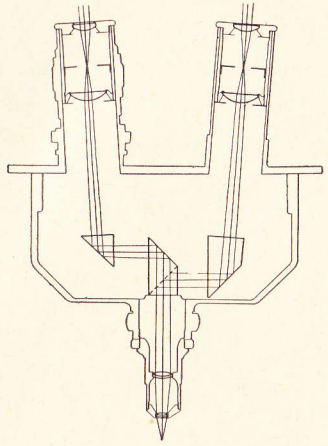


Fig. K

the objective. The 45° surface of one of these prisms is semi-platinized to reflect half the light to one side, and allow the other half to pass through to be reflected in the opposite direction by another 45° reflecting surface. These two beams of light are again reflected upward, each to its eyepiece, by prisms located directly below the eyepieces and moving with them. The eyepieces are movable to and from one another to accommodate different interpupillary distances. One of the eyepiece tubes is also variable as to length to adjust for a difference between the two eyes of the same person.

There are two distinct types of these binocular bodies: the one with the parallel eyepiece tubes and the other with the tubes slightly converging toward the objective. The argument for the parallel tubes seems to be that they are more restful for the eyes because "the eyes are at rest when looking at an object at infinite distance." Over against this is the stubborn fact that when using the parallel tubes many people have difficulty blending the two images into one picture as is regularly done in ordinary vision. Quite a number are able to do it after some practise. Some never accomplish it. In looking into the converging tubes there is no such trouble. Normally when looking at a near object the

lines of light converge from the eyes to the object. When looking at a microscope, or observing the placing of a slide on the stage, the eyes are converging. No adjustment is necessary when immediately looking *into* the microscope. The images appear as one. Again no readjustment is necessary in looking away from the microscope image to pick up a pencil to make notes.

The binocular body is a great advantage because it permits the use of both eyes, greatly relieving the strain on one eye when using the single tube, especially for long continuous observation. At the same time it presents a picture of the object not obtainable with the single tube. Some claim that the vision is stereoscopic; others assert that it is not truly stereoscopic. Be that as it may, all agree that the view is much more satisfactory, and more restful to the eyes for long continued work. Without doubt different layers in the structure of the object are much more easily differentiated.

The disadvantage of using the two eyepieces is the fact that the divided light will not present so brilliant a field with the same light source. Many, therefore, consider the single eyepiece better when a brighter field is demanded and when the exigencies require the most ciritical definition and resolution. Without doubt the two views of the same object are different and both make for a more intelligent interpretation. Therefore, most workers are willing to use both tubes even though the interchange is more or less cumbersome.

Combination Body Tube

To overcome this the Spencer Lens Company has recently designed a combination body tube providing

a quick and easy interchangeability between the single and double eyepieces. For the binocular vision both eyepieces are used in the usual way. Where the single eyepiece is desired the body is moved slightly to one side to bring the axis of one eyepiece coincident with that of the objective, duplicating the conditions of the single tube. All of the prisms and the other eyepiece are automatically moved out of the way. The con-

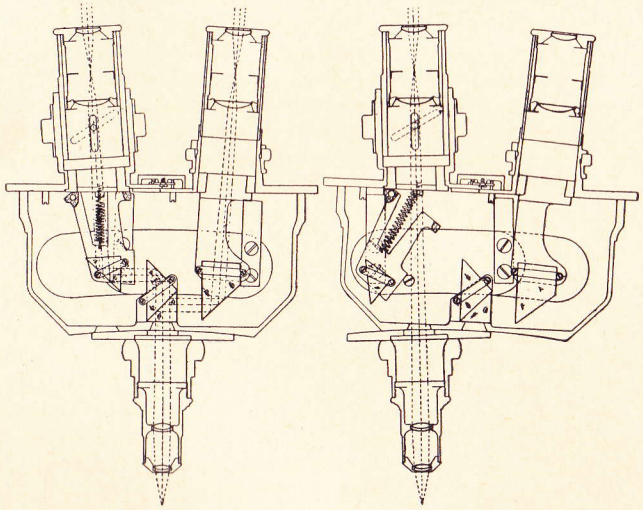


Fig. L *Fig. M*

venience of the device immediately appeals to the research worker. Figure L shows the position of the parts when both eyepieces are used. Figure M shows the same parts in the position assumed when but one eyepiece is in working position.

The Nosepiece

The body tubes of practically all microscopes are now so made that the nosepiece is a necessary concomitant to provide the proper tube length and to permit the objective being brought closs enough to the stage. The convenience of the quick interchange of objectives has become a necessity. The objectives sent out with all microscopes are made *parfocal* on the nosepiece: i. e., they are of such length that when one is in focus all the others on the nosepiece will be in focus within a slight turn of the fine adjustment when they are brought into the optical axis. The objectives are also centered so that an object in the center of the field of one will be close to the center of the field of all the others. Thus a low power objective may be used as a finder for the higher powers.

These conditions obtain *providing each objective is securely screwed into its particular place on the nosepiece*, which place is marked for it. If they are changed about, or if objectives from another microscope are used these desirable conditions are likely to be lost. Each objective is individually fitted by the maker to its particular place in the nosepiece. A change of tube length from the standard or a change of eyepiece will affect the parfocalization. Be sure to use the proper tube length and eyepiece.

The Stage

The stages of all microscopes are now covered with a layer of hard rubber or composition which is not permanently affected by the reagents, stains, etc., ordinarily used. Should the stage become soiled with balsam, immersion oil, or anything which water will not remove, it can be cleaned with a little xylol or

chloroform. If this has a tendency to turn the stage gray rub on a little heavy oil to restore the original black. If the gray color is of long standing, let the oil remain for a time; always wiping off before using the microscope.

All stages are either rectangular or circular. The rectangular are used in the great proportion of laboratory work because of their less cost and simplicity. The circular stages on the larger, the research microscopes, revolve around the optical axis. The centering screws make this center of revolution coincide with the optical axis. Many of the better microscopes are equipped with mechanical stages either as an integral part of the instrument:—on circular stages where they revolve with the stage, or as a detachable unit. The same precautions should be observed with reference to the care of the bearing surfaces, racks and pinions, etc., as has been advised for other similar parts. A careful reading of the verniers on any good mechanical stage will locate an object so that it can be found again easily even though the mechanical stage may have been removed from the microscope in the meantime.

With the best mechanical stages on the better microscopes the slide is held slightly above the upper surface of the microscope stage so that when the condenser is immersed the oil is not smeared over the surface of the stage when the slide is moved about. On the better Spencer microscopes is also another feature by which the slide is held down so that a thickened immersion fluid between the cover glass and the front of the objective can not cause the slide to follow the objective when it is focussed upward. The movements of all mechanical stages should be even, free and easy with no lost motion.

The Substage

The optics of the substage are the necessary complement to the optics above. Their importance is too little realized and the accurate manipulation of the same too little appreciated. Mechanically the substage must be capable of rigidly holding the axis of the condenser parallel to and coincident with the axis of the optical train above the stage. Centering screws furnish a means for accurately centering the condenser on all the best instruments. Sometimes the condensers are permanently centered by the maker. All the movements must be as smooth and delicate as any on the microscope. All the best microscopes are provided with a fine adjustment on the substage.

There are three types of Substages:

1. A simple substage ring fastened to the underside of the stage holds the condenser permanently in one position. Rarely is there any means for focussing. It is not very satisfactory and not much used.

2. The quick screw substage is very generally used and for ordinary work is reasonably satisfactory. The condenser is raised and lowered by a quick acting sextuple screw. The movement is smooth and fairly delicate. Care should be taken to see that the condenser mounting is squarely in the substage ring. If the threads of the screw become gummed or sticky " cut " the refuse with a little xylol or chloroform. When clean relubricate with a good oil and work the substage up and down until it works freely and smoothly. If the threads should be run entirely out of the nut do not force the threads if they do not start back easily. Try starting them in a new place. Remember there are

six threads each working best in its own thread in the nut. If the leaves of the iris diaphragms become rusted or gummed, clean them with xylol, and oil them thoroughly by opening and closing the diaphragm several times to evenly distribute the oil over the leaves. Should the leaves become bent or misplaced, submit them to the maker or a skilled workman.

3. The rack and pinion substage represents the ideal equipment. By it the optical parts are more definitely and accurately held in place, and the movable parts more freely and definitely manipulated. When using the best corrected condensers it is essential. A fine adjustment is also necessary for the best results.

On all the older forms the condenser and its mounting are held by friction in the substage ring, being put in from below. Usually this ring swings to one side in and out of the optical axis carrying the condenser with it. In many instances the iris beneath the condenser is fastened to another arm which swings out on the other side. Whereas this mechansim has been used for many years with satisfaction, it is not as good as the more modern equipment. The swinging arms are not usually sufficiently rigid to always bring the condenser and iris definitely back into the axis and hold the condenser there so that its optical axis is coincident with the axis of the optical train above.

Again with the above construction it is mechanically impossible to bring the iris to a position where it almost touches the large lens of the condenser—as it should.

The Spencer Microscopes are unique and alone in that on them the condenser with the iris in the proper

position, the provision for oblique light, and any other possible substage accessories are mounted in one complete unit, which unit slips in and out of a horse shoe, or fork shaped arm, it resting on a horizontal bearing on top of the very rigid arm. For convenience the arm

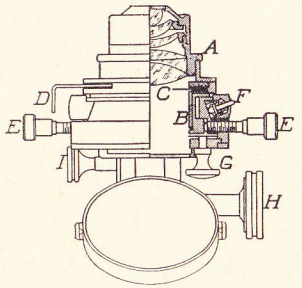


Fig. N

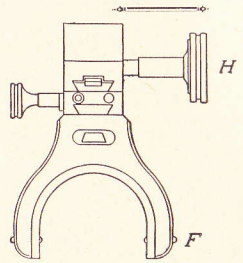


Fig. O

is open in front. This unit goes definitely into place, and when centered with the centering screws the whole optical train of the microscope is in perfect alignment.

The lower supporting ring fits into the fork and all the other parts are built up, one on top of the other, upon it. The whole ensemble is easily removed completely from the substage for cleaning or rearranging any of the parts. These features with the sturdy coarse adjustment and delicate fine adjustment make the ideal substage. If these bearings ever become dirty, clean them as previously directed for similar bearings.

The mirror is so mounted that it is independent of the movement of the substage. When the mirror is properly adjusted the focussing of the condenser does not interfere with the direction of the light.

PART II

General Theory of Microscope Optics

Ray Paths—Axial Rays

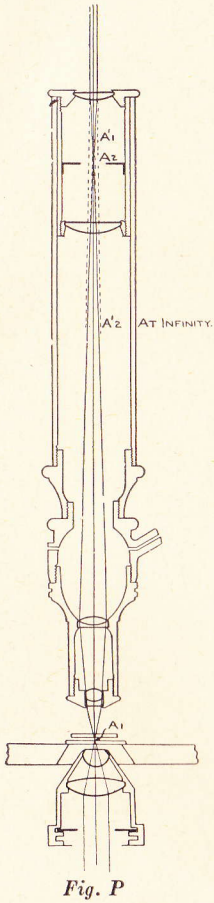


Fig. P

A MICROSCOPE objective forms an image that may be considered as made up of an infinite number of points. A study of two of these points will give a good idea of how the objective functions to form this image. The first point to be considered is the image of the point where the axis of the objective intersects the object. A bundle of rays proceeding from this point is shown in Figure P. These rays would be brought to a focus at A_1' in the image plane of the objective, if the field lens of the eyepiece did not intervene. The field lens places this image at A_2 which is in the plane of the diaphragm and the focal plane of the eye lens of the eyepiece. The eye lens forms an image of this point at infinity which can be observed by an eye which is completely relaxed. A small movement of the fine adjustment will shift the whole optical system so that the final image is not formed at infinity. If such is the case the observer's eye is put under a constant strain to make up for the lack

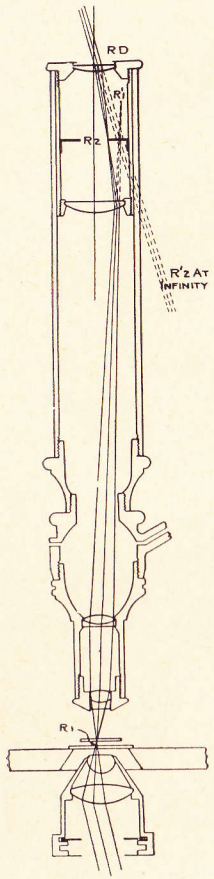


Fig. R

of proper focus of the instrument. This should be avoided. It is not always easy to know that the instrument is properly focussed. To test for this, gaze vacantly out of a window at a distant object and then look quickly into the microscope. If the image is blurred at the first instant and gradually clears, the instrument is not properly focussed and it should be refocussed.

Ray Paths—Field Rays

The second point to be considered is the image of a point at the extreme edge of the field. An examination of Figure R shows that in the bundle of rays proceeding from this object point there is no symmetry about a central ray such as existed in the bundle shown in Figure P. There is, however, one ray of this bundle that has special significance because it proceeds through the objective without any angular deviation. This ray is called the principal ray and is shown as the central ray of the bundle. It is directed at what may be called the optical center of the objective and from there toward the point R_1' where all the other rays of this bundle would cross it if it

were not for the field lens of the eyepiece. The image point R_1' lies in the same plane as the point A_1'. The field lens places this image at R_2 which is at the edge of the diaphragm and in the same plane. The eye lens of the eyepiece deviates the principal ray so that it crosses the axis at the point *R D* which is variously called the Ramsden disc, exit pupil, or eyepoint. It is the point at which the pupil of the eye should be placed to be able to see the whole field. The other rays of the bundle surrounding the principal ray are made parallel to it by the eye lens, if the microscope is correctly focussed, and therefore form an image R_2' of R_2 at infinity.

Magnification—Objective

It is easy to see that the image point R_1' is very much farther from the axis of the microscope than is the object point R_1. Thus the objective forms an image which covers considerably more area than does the object. The magnification of the objective alone is the ratio of the distance of R_1' from the axis to the distance of R_1 from the axis. A reference to the figure will show that the magnification of the objective is dependent on the distance of the optical center of the objective from the object and also from the image. The distance of the optical center of the objective from the image is determined by the tube length. With the tube length standardized the distance of the optical center of the objective from the object is determined by the focus of the objective only. With these data the magnification of all our objectives is computed for a standardized tube length of 160mm. It is engraved on the boots of the objectives together with their focii and numerical apertures.

Magnification—Eyepiece

The eyepiece magnifies the image formed by the objective on the principle of the simple microscope. An object ten feet away appears to an observer to be a certain size. If the distance be reduced to five feet it appears to be twice the size. The nearer to the observer it is the larger it appears. But nature has put a limit on the accommodation of the human eye such that it can not easily focus on an object that is closer than ten inches from the eye. If we wish to see an object on a scale larger than it appears at ten inches from the eye it is necessary to place a lens between the object and the eye to assist the accommodation. If this lens has a focus of two inches the object can be placed two inches from the eye and be seen as though it were at infinity. In other words the lens does the work that the eye can not do, and allows the eye to see the object with no effort at accommodation. The object being only two inches from the eye appears to be five times as large as though it were viewed at the nearest possible distance by the unaided eye. Thus the lens is said to have a magnification of five. It is on this basis that our eyepieces are computed and marked for magnification.

Magnification—Compound Microscope

The total magnification of a compound microscope is the product of the magnification of the objective and eyepiece. Thus if an oil immersion objective and 10x eyepiece is used the total magnification is 980, found by multiplying 98, the magnification of the objective alone, by 10, the magnification of the eyepiece.

Definition—Spherical Aberration

In explaining the paths of rays through a microscope objective, it was assumed that the rays crossed at a *point* to form an image. This is never strictly true and is approximated only when the correction for spherical aberration is good. If the correction for spherical aberration is perfect, the rays of a bundle are so directed that they would cross at a point if it were not for the fact that light is a wave motion. Due to this fact they do not cross at an exact point, but jostle each other near the crossing point in such a way that the otherwise point image is spread out into a small disc of measurable dimensions. Furthermore, this central disc is surrounded by a series of concentric rings of alternate light and dark. This whole pattern is called a diffraction pattern. When the correction is good these surrounding rings are faint and diminish in width so rapidly from the center out, that only one or two of them can be seen by the most critical test. If, however, the correction is poor the rings are bright and broad. The central disc remains the same size, but a large portion of the light that should be concentrated in this disc is lost to the surrounding rings. Since the central disc is the only portion of the whole pattern that goes to make up the image, this means that the image will be faint and that the lost light will be spread over the surrounding area and tend to haze the image of nearby points. Thus contrast in the image is destroyed and it appears hazy and washed out. This is the true interpretation of definition. It is only a matter of contrast. The proportion of light concentrated in the central disc determines the quality of the definition.

Definition—Chromatic Aberration

The general features of these diffraction patterns will vary with the correction for color. If the axial correction is not good the rings will occur at different points along the axis for different colors. Their size and relative concentration of light in the central disc will also vary with color if the so-called chromatic variation of spherical aberration is not corrected. It is evident that the contrast which determines the definition can be lost quite as easily by a poor correction for color as by a poor correction for spherical aberration. It is not possible to obtain a perfect correction for color because of the limitations of manufacture of optical glass. The usual type of lens is corrected for two colors on the axis and spherical aberration for one color, and a close approach to a correction for spherical aberration for the other colors. Achromatic objectives have such a correction. The lack of perfect color correction is evidenced by a small fringe of color around a dark object, especially when oblique light is used. It is possible with the aid of the newest glasses and the mineral fluorite to correct for three colors on the axis and to correct the spherical aberration for two colors. Such a combination must of necessity be very complicated. It is termed an apochromatic objective and is the finest type obtainable. With such a lens no color fringe is noticeable except under the most trying of conditions and even then it is faint and narrow. The apochromatic objectives are extensively used for micro-photography because of their more perfect color correction. It forms an image that is extremely pleasing to the eye in its crispness of detail and faithfulness of reproduction of color.

Resolving Power—What It Is

There is a conception among some users of the microscope that magnification and definition are of paramount importance. This is not strictly true, for there is another requirement that is more important than either of these. This is resolving power, the ability to pick out and recognize fine detail. As an example suppose we consider a piece of fine cloth having a pattern woven in it. Suppose that this cloth is held at such a distance from the eye that the pattern is not distinguishable. A slight magnification will allow the pattern to be seen clearly and easily. Now suppose we magnify it a thousand times, and that the definition is good enough so that the pattern is seen clearly and distinctly, but on a very much larger scale. On first consideration this may seem sufficient. On further thought, however, it appears that at a thousand magnification the threads of which the cloth is woven should appear to be more than an inch broad, and the spaces between them sufficiently wide to be seen easily. The ability to see these fine details in the object is called resolving power. However, the image may show no indication of a thread structure due to insufficient resolving power. Thus the optical system, having good definition, and more than enough magnification, fails completely to give a true interpretation of the object. This illustration is given to show that there is no connection between resolving power and magnification or definition. It is a thing quite apart from other properties of an optical system and for the finest microscope work is by far the most important.

Resolving Power—Oil Immersion Objectives

A quantity called the numerical aperture is the measure of the resolving power of a microscope objective.

The numerical aperture is expressed by the equation

$$NA = n \sin a$$

where n is the lowest index of refraction between the object and the objective, and a is the angle between the extreme ray of the axial bundle and the axis, shown in Figure P. This expression that is arbitrarily called the numerical aperture is derived from a mathematical study of resolving power. It is beyond the scope of this book to give the derivation here. The reason why the lowest index of refraction between the object and the front lens of the objective enters into the expression will be touched on under the heading of "Resolving Power from the Standpoint of the Diffraction Theory."* It is at once apparent that in order to secure a high resolving power, it is necessary to design the objective to take in a large angle. There are, however, practical limits beyond which it is not advisable to go. This angle can not be much more than 60°. For 60° the numerical aperture would be .86 if the lowest index medium between the object and the objective were air having an index of refraction of one. If, however, the objective were designed to have oil of an index of refraction of 1.52 between the object and the objective, the numerical aperture would be .86.1.52=1.30. This is the theory that led to the design of the oil immersion objectives. The use of the oil immersion principle multiplies the highest numerical aperture otherwise obtainable by 1.52, the index of the oil. This is only true, however, if a condenser of at least the same numerical aperture as the objective is used, and if an oil contact *is used between the condenser and the slide.*

Necessity of Immersing Condenser

Figure S shows the top lens of a condenser and the front

* See page 34

lens of an oil immersion objective with the slide and cover glass between. The ray marked p--p is shown traversing the system with oil contact between the condenser and the slide, and between the front lens of the objective and the cover glass. It is assumed that the object is embedded in a medium having the same index as the slide and the cover glass. It is seen that the ray goes through the system without any deviation between the two curved surfaces, due to the fact that the oil has the same index as the slide, cover glass and the two lenses under consideration. Let the angle of this ray to the axis be a_p. Then its numerical aperture is

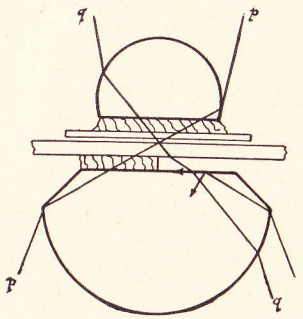


Fig. S

$$NA_p = n \sin a_p = 1.30$$

The ray marked q-q is shown traversing the system without an oil contact between the condenser and the slide. This ray is deviated by the upper surface of the condenser and the lower surface of the slide in such a way that it traverses the slide, cover glass, oil contact and front lens of the objective parallel to its direction in the upper lens of the condenser. If this ray struck the upper surface of the condenser at the same angle to the axis as the ray p-p, it would be totally reflected by the upper surface and would not leave the lens. The largest possible angle to the axis for the ray to leave the upper surface of the condenser is determined by the equation

$$\sin a_q = 1/n.$$

Thus the largest numerical aperture possible under these conditions is

$$NA_q = n \sin a_q = n/n = 1.0$$

It is evident that a large part of the benefit of having an oil contact between the object and the objective is lost by not having an oil contact between the condenser and the slide, even though it is retained between the cover glass and the objective.

Resolving Power—From Standpoint of Diffraction Theory

The size of the central disc of the diffraction pattern formed by a microscope objective determines its resolving power. If the centers of two discs representing the images of two points lying very close together, are separated by only a small part of the diameter of either, they will not appear to be separated but will blend together and appear as one large spot. It is assumed that when the center of one disc lies on the circumference of the other, they will just begin to appear as separated. The size of the central disc formed by any objective is determined only by the wave length of the light used for illumination, and its numerical aperture. The greater the numerical aperture the smaller will be the central disc, and consequently two discs can lie closer together and still be seen as separated. It has already been pointed out that the size of the central disc does not vary with the corrections

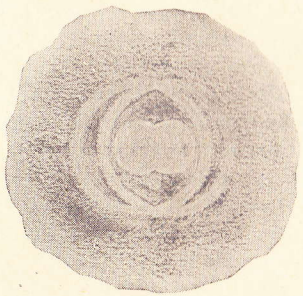


Fig. 8

which determine the quality of the definition. Evidently then, the resolving power does not depend in any way on the definition.

The reason why the quantity *n* enters into the expression for numerical aperture again involves the fact that light is a wave motion. When the rays forming a wide angle cone impinge on an object, they are jostled by a process called diffraction into groups with a separation between them. The sum total of these groups lies in a cone of larger angle than the original illuminating cone. In other words the light is fanned out into a wider cone by the process of diffraction, but with an uneven distribution of light through this new cone. For an objective to show all of the detail in an object it must take in all of these groups in this cone. The more of these groups it takes in, the more truly will it depict the object by showing more of the detail in the object. This is simply another way of saying that the resolving power will be greater. It is easy to see that the resolving power will be increased if the jostling of the rays around the object can be decreased, thereby decreasing the fanning out of the cone and allowing the objective to take in more of it. It so happens that the jostling is less for the shorter wave lengths, as would be expected by a little thought. The wave length of light is changed when it enters a medium of different index of refraction. It is reduced in the ratio of the index. Light having a wave length of .00056mm in air has a wave length of .000368mm. in a medium having an index of 1.52. This is the physical reason why a high index medium between the object and the objective will increase the resolving power of the objective. The high index medium decreases the wave length of the light traversing it, thereby decreasing the fanning out due

to diffraction around the object; and therefore allows an objective of a given angle to take in more of this diffraction pattern. The objective can then form an image that includes more of the fine detail of the object, which means that its resolving power is greater. It should be remembered that this high index medium must lie in an unbroken chain between the object and the objective, to prevent the fanning out before the light enters the objective. The above line of reasoning also serves to explain why the use of short wave lengths such as the ultra violet increases the resolving power.

The shortest distance between two objects that can be shown as separated by a microscope objective used with a condenser that is properly immersed, is given by the expression λ/NA, where λ is the wave length of light used for illuminating the object. Without a condenser this becomes λ/NA. The wave length of the brightest part of the spectrum is about .00056mm. This is the value used in the following table.

Numerical Aperture	*Limit of Resolution Without condenser*	*Limit of Resolution With Condenser*
.10	.0056 mm.	.0028 mm.
.25	.00224 mm.	.00112 mm.
.50	.00112 mm.	.00056 mm.
.66	.00085 mm.	.000425 mm.
.85	.00066 mm.	.000330 mm.
.95	.00059 mm.	.000295 mm.
1.00	.00056 mm.	.000280 mm.
1.25	.00045 mm.	.000225 mm.
1.30	.00043 mm.	.000215 mm.

The above table gives the smallest distance between two objects that can be seen as separate objects when viewed under the conditions indicated. It should be

noted that this distance is exactly halved when a condenser is used. This emphasizes the necessity of using a condenser when high resolving power is required. This limit of resolution is not dependent on the magnification providing sufficient magnification is used. For instance, no amount of magnification will show the separation between two objects that are separated by .0003 mm. unless an objective of at least .95 numerical aperture is used, with a condenser.

Proper Magnification

It is not necessary to use a magnification greater than enough to depict clearly to the eye the finest detail resolvable by the objective. A greater magnification will show no more detail and will materially decrease the illumination. It will also tend to decrease the contrast of the image, so that the ease with which an object can be studied will be decreased rather than increased by increasing the magnification above a certain advisable maximum. The field and the depth of focus both decrease with an increase of magnification. The magnification should, therefore, be as low as possible. The maximum useful magnification can be computed by dividing .3 mm. by the limit of resolution of the objective used, given in the above table. Thus for an objective having a numerical aperture of 1.30 and a limit of resolution of .000215, the greatest total magnification that should be used is 1400. This indicates a 15x eyepiece with a 1.8 mm. focus objective. This represents the highest magnification that should ever be used except in very unusual cases. For most work the proper magnification is much less.

Depth of Focus

Assuming the same quality of correction and the same magnification, the illumination varies as the square of

the numerical aperture. The depth of focus or penetration varies inversely as the numerical aperture. If the numerical aperture is doubled the illumination is quadrupled and the depth of focus is halved. For any given numerical aperture and magnification the depth of focus can not be altered except by destroying the definition. Strictly speaking this does not result in an increase in depth of focus but rather in an increase in the indefiniteness of focus. Lenses having the same numerical aperture should never be compared for depth of focus except to the detriment of the one having the greater depth. If depth of focus is a valuable asset for the work under consideration, resolving power should be sacrificed to obtain it. It is for this reason that we list lenses having the same focus but different numerical apertures.

Working Distance

Working distance is the free distance between the cover glass and the objective when the latter is focussed. It decreases generally with increasing power and numerical aperture of the objective. Of two lenses with the same focal distance, the one with the higher N. A. will have the shorter working distance. The working distance also depends on the mounting of the front lens. If the lens has a prominent mounting projecting beyond its surface the working distance is lessened thereby.

PART III

The Use of the Microscope

Equipment

A MICROSCOPE equipment may be very elaborate, and some work requires such an outfit, but every microscope to be efficient should be provided with at least two objectives, and preferably two oculars. If but one ocular is bought the 10X or 1″ is best. If two are provided, the 6X and 10X (2″ and 1″) are preferable. For ordinary biological, histological and pathological work the most desirable and most universally used objectives are the 16mm. (2-3″) and 4mm. (1-6″). In some cases where a higher power is desired a 3mm. (1-8″) is used. For counting blood corpuscles a narrow angled 4mm. (1-6″) with a long working distance for working through the thick cover of the blood counter is used. For entomological work and other low power work a 40mm. (1¾″) is very desirable.

If bacteriological or special cytological work, where high resolving power is required, is to be done, an immersion objective is indispensable. The 1.8mm. (1-12) is most used. For extreme work the 1.5mm. (1-16) is called into use. The high resolving power required in this work demands the use of a condenser. These are of two great classes, the achromatic and non-achromatic. Of the latter class the Abbe condenser is the one most commonly supplied. This condenser is capable of very good work and is commendable for its cheapness. The best work can only be done with a condenser that is corrected to a perfection that approaches that of the objective. The achromatic condensers that are

also aplanatic are in this class. The best of these have a short focus which means that they are unusually small in diameter.

For the most critical work apochromatic objectives should be used. The superiority of these over the achromatic objectives has already been pointed out and need not be repeated here. The fact that the apochromatic objectives generally have a higher numerical aperture than the corresponding achromatic objectives also recommends their use for critical work.

Position

In choosing a place to work one should select a comfortable position where he can obtain the best light available and have room for his microscope and necessary accessories and reagents. There is some controversy in regard to the using of the inclination joint. There is no harm in using it if it is more comfortable to do so. If one is working with fresh mounts or fluids, the horizontal stage is necessary. Because such preparations are so often used in the laboratory, it is best for one to train one's self to use the microscope with the tube in the perpendicular position and make it a rule to keep it in that position.

Make it a rule to work with both eyes open, and, if possible, use either eye interchangeably. A very little practise will enable one to do so. By paying attention to this and proper lighting there is no reason why any reasonable amount of work with the microscope should injure the eyes. The new binocular microscope obviates this difficulty.

Light

The best light is obtained from white clouds, although some authorities claim that the light from the blue

sky is best. Avoid the use of direct sunlight. If the room is so situated that the sun shines in, use white shades to modify the light. If possible, select a window which is free from cross bars, wire nettings, etc., and which is some distance from swaying branches of trees.

For long continued work on any one subject artificial light has one advantage over daylight in that it is constant in quality and intensity. The best artificial light is a tungsten burner with " Daylight " glass between it and the microscope. A fair substitute is a Welsbach burner or a small arc lamp. A whitened incandescent lamp is good. Ordinary daylight can be used very successfully. In using artificial light it is best to use a bull's eye condenser between it and the mirror. It is also best, wherever possible, to use a blue glass between the light source and the specimen. Some workers make a glass globe filled with ammonium copper sulphate solution serve the purpose of both the condenser and the blue glass. It is so mounted in a shade as to exclude all other light from the microscope. An eye shade, or some shade cutting off all light from the microscope, excepting that which strikes the mirror, is often desirable.

The lamps designed for microscope illumination are strongly recommended for long continued work with the microscope. They are provided with a " Daylight " screen that gives an ideal tone to the light and are so constructed that no stray light reaches the eye of the worker. With their use ideal illumination conditions can be obtained and maintained for any length of time.

Focussing

After seeing that an objective (low power) and an ocular are in place, put a transparent or semi-trans-

parent specimen on the stage, swing the mirror bar to the median line, take hold of the edge of the mirror and adjust it so as to illuminate the object as evenly as may be judged by looking directly at it.

Focus the body tube down by means of the coarse adjustment until the objective nearly touches the cover glass, being careful not to touch it. Then, with the eye at the eyepiece, focus up carefully with the coarse adjustment until the specimen comes plainly into view. Be careful not to pass by this focal point without noticing it. This is likely to occur if the light be too intense and the specimen thin and transparent.

When the object is brought fairly well into focus by means of the coarse adjustment, use the fine adjustment to obtain the sharpest focus to bring out details. Do not expect too great a range in the fine adjustment. It is even more dangerous to focus down to any extent with the fine adjustment than with the coarse adjustment, because any impact of the front of the objective on the cover can not be felt easily, unless the fine adjustment is a modern one. While moving the specimen about to observe different parts of it, it will be necessary to continually work the fine adjustment to keep the object in focus. It is always well to move the specimen when trying to get a focus, for without the movement one may be trying to focus upon a point where there is no object, and, again, the moving object is more apt to be noticed as the lens comes into focus.

It will be noticed during this movement that the microscope reverses the image, and that the specimen seems to move in the direction opposite to that in which it is moved. This, along with the fact that the microscope

magnifies the movement as well as the specimen, is perplexing at first, and makes it difficult to move the specimen just where it is wanted, and no farther. With practise comes the delicacy of movement which enables one to put the specimen just where he wants.

The beginner should always use the low power objectives and oculars first. The low power objectives have longer working distances and are not so apt to be injured. They always show a larger portion of the specimen and thus give one a better idea of the general contour. After obtaining this general idea the higher powers can be used to bring out greater detail in any particular part. If the objectives are parfocalized and centered on a nosepiece, the change of objectives is made by simply turning one objective out of the optical axis and the other into it without the necessity of re-focussing (except for a slight turn of the fine adjustment) and again hunting up the particular spot desired, for if this spot is in the center of the field of the low power it will be somewhere in the field of the higher power. It is too much to ask of the maker that the lenses be made absolutely parfocal and centered. The delicacy of the centering can be appreciated when the magnification and the extremely small portion examined are considered. When the objectives are not thus fitted to the nosepiece, re-focussing and again hunting up the object are necessary. In doing so we repeat the caution to always focus up before turning the nosepiece. When no revolving nosepiece is used, the change of objectives means the unscrewing of one and the screwing of the other into its place and re-focussing as before.

Illumination Without Substage Condenser
Central Light

It has been necessary in the foregoing paragraphs to secure some light upon the specimen, but no directions have been given as to the proper illumination of the same. Accuracy of results depends upon correct illumination more than any other one thing. A vast majority of all microscopic work is done by light transmitted through transparent or semi-transparent objects. We will at present consider only such objects. The matter of illuminating opaque objects will be taken up later. The mirror is placed below the stage as a convenient means of reflecting the light through the object into the objective. It is plane on one side and concave on the other. The concave mirror is always used when the substage condenser is not used, except in the case of very low power objectives, when it is best to use the plane mirror.

When the light is thrown upon the specimen, and the objectives focussed as previously directed, remove the ocular and look into the tube at the back lens of the objective. With the medium and higher power (16mm. and above) objectives the minimized image of the mirror with its mounting will be seen. Swing the mirror bar to the median line and as nearly as possible arrange the mirror so that its mounting will be concentric with the periphery of the back lens of the objective. All of the better microscopes are made with a " center stop " indicating when the mirror bar is in a line parallel with the optical axis of the microscope. This is done because central, or axial, light gives a symmetrical illumination, which is best for observing the large proportion of transparent objects. This in itself does not insure axial

light. The mirror must be so turned that the rays of light, or the axis of the cone of light, reflected from it enter the objective parallel to its axis. This can not always be done. Other considerations are more important than exact central light. In working with daylight, reflections from trees, window sash, etc., are apt to be seen on the mirror. If the whole microscope can not be so shifted as to clear the mirror of these reflections the mirror itself should be turned so that, if possible, no images will appear upon it.

If artificial light is used, the mirror should be so turned that the image of the light is seen in the center of it. The more nearly this image covers the mirror the better. If a bull's eye condenser is at hand, so place it between the light source and the object that a sharp image of the light source will be seen in the center of the back lens of the objective.

If the above rules are followed it will be found upon replacing the eyepiece that the field is evenly illuminated. It may be necessary to vary the width of the cone and the quantity of light, by use of the diaphragm, which is always placed on all the better microscopes, as nearly as possible even with the top surface of the stage. "When no condenser is used, the size of the opening in the diaphragm should be about that of the front lens of the objective. For some objects and some objectives this rule may be quite widely departed from; one must learn by trial."* The concave mirror acts as a lens and has a focus like a lens.

It may be found that, in focussing up and down, the image shifts slightly from right to left, or to and fro. This may possibly be due to an imperfection in the microscope, but if the instrument is in good repair,

*The Microscope-Gage

and from any one of the reputable makers, the chances are more than likely that the shifting is due to oblique light, even though the mirror bar may be in the median line. This is even more apparent with a condenser than without it. Manufacturers are often condemned because of a mistaken idea that the mirror bar in this position means axial light. A slight turn of the mirror will stop the shifting and give axial illumination. When there is no lateral motion in focussing, the light is centered.

Oblique Light

Some objects, such as diatoms, rulings, etc., are better defined when oblique light is used. This is accomplished without the condenser by swinging the mirror out of the optical axis, and so turning it as to throw as much light as possible upon the object. When the ocular is removed, the image of the mirror will be seen at one side of the center of the back lens of the objective. When focussing, a decided lateral motion of the object will be noticed.

Illumination With Substage Condenser
Central Light

All of the better microscopes are provided with a condenser fitted beneath the stage, which brings light from a distant source to a focus on the object. With the lowest powers a condenser is not needed, but for the medium and high powers the condenser not only furnishes the amount of light needed, but provides an easy means of providing each objective with a cone of light suitable to its aperature.

The plane mirror should always be used with a condenser except when the source is quite near the microscope. After removing the ocular, turn the mirror

so that the back of the objective is fully and evenly illuminated, and, if possible, free from any images of trees, window sashes, etc. If these images can not be dispelled by turning the mirror use the concave mirror. Slightly lowering the condenser will also accomplish the same end but there is an objection to doing this unless it is necessary. The condenser does its best work only when the source of light is focussed sharply on the object. If the open sky is used as a source, any intervening objects such as trees or window bars will throw a shadow on the object. Lowering the condenser to remove the shadows destroys the proper focus of the condenser. The operator must decide for himself as to whether he gets better results with the sharp focus and the images or without either. If he decides to retain the images he can get best results by turning the mirror so that they are as symmetrically distributed over the back of the objective as possible.

To secure central illumination it is highly important that the axis of the condenser should coincide with the axis of the objective. All good microscopes are constructed so that the center of the diaphragm lies on the axis of the condenser. The centering of the condenser can, therefore, be tested by observing the centering of the diaphragm. When the condenser is properly focussed the image of the diaphragm appears in the back of the objective. Close the diaphragm until its edge can just be seen through the back lens of the objective. It should, of course, appear to be centered with the rim of the objective. The more expensive microscopes have screws by means of which the centering can be accomplished. In the cheaper microscopes this centering is done in the factory and can not be altered.

When the condenser is properly focussed and centered the correct cone of light can be secured by opening or closing the diaphragm below the condenser. A good general rule is to close the diaphragm so that in looking at the back lens of the objective, the diaphragm opening which can be plainly seen, appears to be about half the diameter of the back lens of the objective when it is in focus. Then with the ocular in place change the opening to give the best results. The thinner the tissues and the greater the contrasts, the larger is the cone of light which may be used. Thicker tissues and those with less contrast require a narrower cone, gaining thereby greater depth of sharpness (penetration). The narrower the cone, the flatter the field appears. Very few objects permit of a cone which fills the back lens of the objective and in no case should the diameter of the iris diaphragm appear to be larger than the diameter of the back lens when the ocular is removed.

When objectives of over 1.0 N.A. (immersion objectives) are used, the full aperture of the condenser can not be utilized without immersing it, i. e., placing a drop of oil between it and the lower surface of the slide. This is seldom practised in general laboratory work on account of its inconvenience, but it is necessary to the most critical work.

Artificial Light

All of the difficulties incident to the use of daylight, noted above, will be eliminated by the use of artificial light. The same general rules as those already given apply to the use of artificial light with a microscope having a condenser. It is, however, necessary to pay much more attention to the proper adjustment of the light to the microscope when a condenser is used. The center of the source should be carefully centered in

the back of the objective by twisting the mirror after the mirror bar has been swung to the median line. If the source of light is a ground glass or Daylite glass, as is the case in most of the lamps especially designed for microscope illumination, this glass should be so placed that a line perpendicular to the glass at its center will pass through the center of the mirror. This is necessary to insure even intensity over the whole field. After this is done the condenser should be focussed so that an image of the glass is formed in or very near the plane of the object. This can be checked easily by using a low power objective. If the lamp used is not supplied with a blue glass or Daylite glass, the blue glass supplied with the microscope should be slipped into its ring below the condenser.

If these directions are faithfully followed it will be found that artificial illumination is more satisfactory than daylight illumination from every standpoint. The conditions can be maintained or duplicated at will. It brings out all of the detail of the object that can be interpreted by the objective. It is pleasing to the eye and can be used for longer periods than the ever-changing daylight without eye fatigue.

Oblique Light

It has been pointed out before that the use of oblique light aids in the interpretation of an object. This is accomplished by an increase in resolving power attendant on its use. By the aid of oblique light details of an object can be brought out that can not be seen with central illumination. It should be remembered, however, that this increase in resolving power is in one azimuth only. For instance, if a diatom that can not be resolved with direct illumination is examined

with oblique light, the cross hatching of which it is composed will appear as a series of lines all running in one direction. If the azimuth of the obliquity is turned through 90° a similar set of lines at right angles to the first set will be seen. It is thus seen that a true idea of the object can not be obtained except by adding together its appearance for all azimuths of obliquity.

The low and medium priced microscopes are not provided with any means of securing oblique light. It can be obtained by swinging the mirror or by inserting a card below the condenser to cut off the light from one side. The latter method is to be preferred. The more expensive microscopes are provided with some method of securing oblique light, usually by decentering the diaphragm. This obliquity can be obtained in any azimuth on most of these microscopes by turning the moving member about the axis of the condenser.

Illumination—Opaque

There are some objects which can not be made transparent and must be examined by reflected light. When low powers are used and the mirror brought above the stage the concave mirror is sometimes sufficient. The bull's eye condenser gives better results. In using it some care must be exercised to light the object and at the same time cast as few shadows as possible.

Where high powers, with consequent short working distances, are necessary, light must be thrown down through the objective by a prism placed in the objective mount or above it and reflected back again to the eye. Such a device is called a vertical illuminator. There are many such devices on the market. The best of them have a light source built into them so that the adjustment of the light, which is always troublesome, is made easier.

The Objectives In Use

Cleaning the Objectives

To get the best results with a microscope objective it is absolutely necessary that it should be clean. The performance of a high power objective can be ruined by dirt of any kind, especially on the surface nearest the cover glass. Dirt scatters the light and causes an appearance similar to that of a poorly corrected objective. Finger marks are particularly harmful to the definition.

If the lenses are dirty they should be wiped gently with Japanese lens paper, which can be obtained from any dealer in microscopical supplies. It is so cheap that one can hardly afford to use anything else on his lenses, especially the objectives. If the lens paper is not obtainable, a soft old linen handkerchief is best, *providing it is clean.* Avoid chamois skin. The natural oils in it soil the surface of the lens, and its aptitude to catch and hold dirt makes it unsafe.

If the front lens of an objective becomes soiled so that gently wiping will not clean it, breathe upon it and then wipe gently with lens paper or some soft linen. If this does not remove the soil, moisten the paper with Xylol or chloroform, being careful not to use too much. Although the necessity of using these reagents is unfortunate, it is better to use them and wipe the lens gently than to apply too much friction.

An immersion objective should always be cleaned immediately after using. It can then be cleaned by gently wiping with a piece of lens paper. If the oil is allowed to dry, xylol or chloroform must be used to clean the lens. The oil collects dust and grit, which are apt to scratch the lens.

If any dust settles on the back lens of the objective it is best removed by a camel's hair brush. An eyepiece should always be left in the tube to keep dust from settling into the objective on the lower end.

Never attempt to take an objective apart. If it has any ailment serious enough for that, it is serious enough to go to the maker.

Focussing the Immersion Objective

The directions for focussing previously given must be carried out to a nicety when an oil immersion objective is used. The extremely short working distance of these objectives makes it highly important to focus up rather than down. A drop of oil can be placed either on the front of the objective or on the cover glass. The former is preferable when convenient. As soon as the body tube is racked down so that there is oil contact between the front of the objective and the cover glass, it is wise to try focussing up with the fine adjustment. In most cases it will be found that the image comes into focus very soon. If not, try focussing down again with the coarse adjustment, and this time allow a little more bulge of the oil around the front of the objective. A little practise will allow you to gain sufficient skill to place the objective below the focus with the coarse adjustment without allowing it to come in contact with the cover glass. Should this occur it will probably be found that there are bubbles in the immersion oil. These can be seen by removing the eyepiece and looking at the back lens of the objective. These bubbles must be removed before doing any work with the objective. Generally it is best to wipe all oil from the objective and cover glass and start over. The necessity of immersing the condenser when using an oil immersion

objective has already been dealt with at length. It may not be amiss, however, to mention it again.

Tube Length and Cover Glass

All objectives are corrected to a certain tube length (160mm. by most makers—Leitz 170mm.) and all objectives in fixed mounts of over .70 N.A. are corrected to a definite thickness of cover glass as well. (Zeiss, .15mm.-.20mm.; Leitz, .17mm.; Bausch & Lomb and Spencer .18mm.). These objectives give their best results only when used with the cover glass and tube length for which they are corrected. As indicated the tube length extends from the eye lens of the eyepiece to the end of the tube into which the objective or nosepiece is screwed. If a nosepiece is used, the draw tube must be correspondingly shortened. If the cover glass is thinner than that for which the objective is corrected, the tube must be lengthened to obtain best results; if thicker, shortened. The most expensvie objectives are provided with adjustable mounts by which the distances between the lens systems may be changed to compensate for difference of thickness of cover. They are successfully used only in the hands of an expert. One of them out of adjustment is worse than an ordinary objective.

Testing Objectives

Under the heading of General Theory of Microscope Optics the definition of a microscope objective was explained from the standpoint of a physical interpretation. It is impossible, however, to demonstrate the phenomena used in that explanation without special apparatus. It is possible to gain a good general idea of the performance of an objective by using any slide that is made up of dark opaque objects having definite

outlines. The well known Abbe test plate is prepared especially for this purpose and makes an ideal test object.

Color Correction

A good idea of the color correction can be gained by observing the edges of opaque objects near the center of the field. These will be bordered by a faint fringe of color when achromatic objectives are used. Oblique light will bring out this color fringe very noticeably. When oblique light is used it will be noticed that the color fringe is a light green on one side of the object and reddish purple on the other side, when the objective is well corrected for color. These color fringes are the secondary spectrum, or irreducible minimum of chromatic aberration caused by the fact that the compensation of one kind of glass for the color introduced by another is not perfect over the whole spectrum. The use of fluorite and a more complicated construction in the apochromatic objectives greatly reduces this color fringe. No color fringe should be noticed with central light and only the faintest trace with oblique light.

Spherical Aberration

If the correction for spherical aberration is good the edges of an opaque object should be distinct and crisp. They should come into focus with a definiteness that allows of no uncertainty as to the best focus. The edges of the object should appear equally out of focus for equal movements of the fine adjustment either above or below the true focal point. When oblique light is used the edges of the object should appear fairly distinct except for the color fringe. If the spherical aberration is poorly corrected, these edges will be bordered

by a fringe of turbid grey as well as the usual color fringe when oblique light is used.

A general haziness that causes a washed out appearance to the image under all conditions is almost always caused by dirt. It is easy to locate by removing the eyepiece and looking through the back of the objective, and must be removed before the objective will give proper results.

Flatness of Field

It is impossible to attain true flatness of field in a microscope objective. Except for a correction for the error known as coma it is impossible to design the objective to include a wide angle and fine definition in the center of the field with flatness of field. The only demand that should be made of an objective is a correction for coma. To test for coma bring the edges of the field into the best possible focus. When this is done it will be noticed that the center is not in focus. This can not be avoided in the design and no objective should be condemned because of it. It is reasonable, however, to expect that the definition at the edges of the field when they are brought into focus should be fairly good. If the correction for coma is poor it will be impossible to secure any definition at the edges of the field no matter how the focus is changed. Objectives vary greatly in this respect. None of them gives as good definition at the edges as at the center of the field, and this difference is more pronounced in the lenses having a high numerical aperature, but it should be possible by refocussing to gain a fairly good idea of the nature of an object at the edge of the field. It should then be moved to the center of the field for more critical study.

The Oculars

It should be pointed out here that an objective can give good definition for only one tube length and cover glass thickness. In making a critical test of an objective great care should be exercised to keep these dimensions correct. The correct tube length for our objectives is 160mm., and the cover glass thickness should be .18mm. Lengthening the tube length will increase the magnification at the expense of the definition. Using a higher power objective or eyepiece is greatly to be preferred.

A certain magnification by the ocular will be necessary and sufficient, to bring out all the detail in the image which can be secured from the numerical aperture of the objective. If we use a higher ocular we lose depth of sharpness and size of field, since they are both inversely proportional to the magnification. We also lose illumination, which varies inversely as the square of the magnification.

We, therefore, get the greatest effectiveness out of an objective—the largest field, the greatest penetration and the best illumination—by using the lowest magnification which makes all the detail in the image visible. If we increase the magnification beyond this point we do so at the expense of other good qualities.

A rule for determining the maximum useful magnification of the eyepiece has been given under the heading of General Theory of Microscope Optics.

Dirt on the lenses of the eyepiece will cause spots in the field that are more annoying than harmful. They will, of course, revolve with the eyepiece. Sometimes dirt on the lenses of the condenser will cause spots in

the field that closely resemble those caused by dirt in the eyepiece.

Final Hints

Sometimes the worker may have faithfully carried out all the directions heretofore given and been assured that his lenses possess the above named qualities as they ought, yet be unable to obtain the desired results. He may be working with a water mount and his dry objective become "immersed" in some water which has worked to the top of the cover glass. His objective may be dirty from a previous "immersion." The field may be dim or hazy, due to dirt on the back of the objective or a film on the inner surfaces of the lenses of the ocular, or because of moisture settling on the lenses because they have just been brought from a cold into a warm room. If these objects revolve when the ocular is revolved they are evidently on one of the lenses of the ocular. He may see great streaks on his field, which are due to his own eyelashes, or he may see small moving bodies floating across the field. The muscae volitantes, as these last named bodies are called, are little specks or shreds in the vitreous humor of the eye which can not be removed, but which can easily be disregarded.

Most of these troubles can be located by removing the eyepiece and looking through the back of the objective. In fact, this should always be done when anything is wrong with the performance of the microscope. It will give at once information as to causes of trouble that may require considerable time to locate any other way.

In water mounts and fresh balsam mounts one is apt to find air bubbles. To be sure that the object is an

air bubble, focus up with central light. The bright spot in the center will become clearer while the edge will become darker. With oblique light the bright spot will be thrown to one side. In studying water, blood or any fluid, always cover the drop with a cover glass. The objectives are corrected for rays passing through media with parallel surfaces. If such a mount is not kept horizontal, currents will be set up, due to gravitation, and they will be seen with a magnified velocity seemingly running up hill.

The fact that the microscope reverses every movement and magnifies it may be mentioned again.
Beside any movement due to currents there is sometimes a peculiar indefinite to and fro movement of particles from one position to another. This is called Brownian movement.

In studying sections a true idea of the structure of the tissue can only be obtained by moving the slide about to bring different parts into the optical axis, and by focussing with the fine adjustment to bring different levels, or optical planes, successively into view. Where serial sections are used each section must be studied in relation to its neighbors.

Sometimes sections which are freshly mounted in balsam appear cloudy and indistinct. This is because of failure to thoroughly dehydrate the specimen before putting it into the balsam. But this brings us into the realm of laboratory technique which is beyond the scope of this little volume.

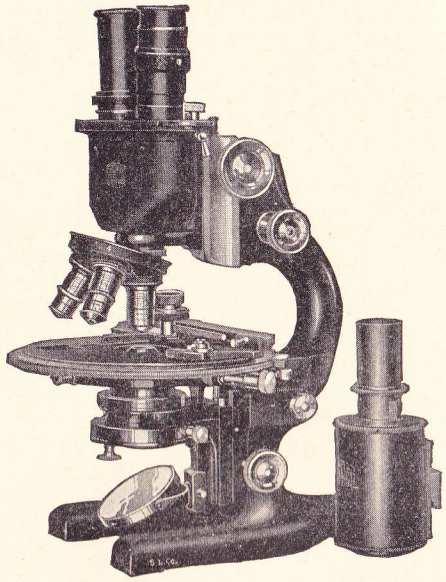
MAGNIFICATION TABLE

The magnification of any combination of objectives and oculars may be obtained by multiplying the magnification of the objective by the magnification of the ocular.

The magnifications of the objectives are as follows:

Focus	*Magnification*
48 mm.	2.2
40 mm.	2.8
32 mm.	4.0
16 mm.	10.0
4 mm.	44.0
3 mm.	60.0
1.8 mm.	95.0
1.5 mm.	113.0

For instance:—the magnification of the 4mm. objective (magnification 44) when used with the 6X ocular is (6 x 44) 264.

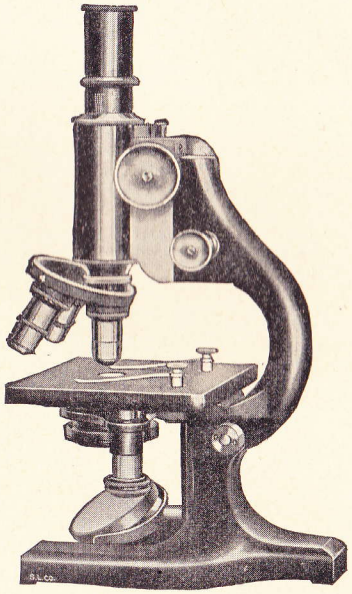


SPENCER BINOCULAR RESEARCH

MICROSCOPE NO. 7

WITH

FORK-TYPE SUBSTAGE

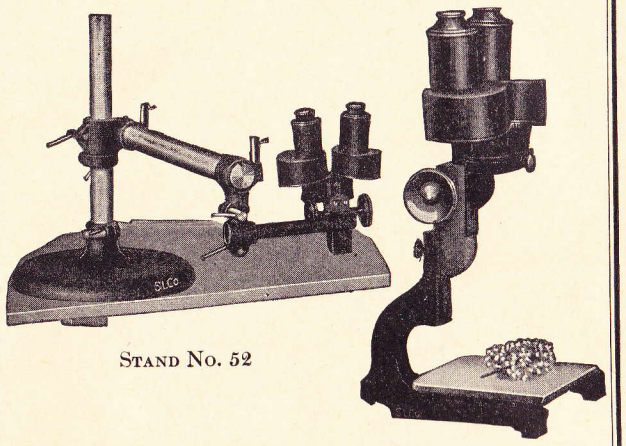


SPENCER MICROSCOPE NO. 44

WITH

SUBSTAGE OMITTED

NO. 64



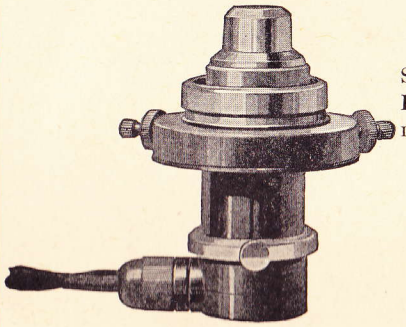
STAND NO. 52

STAND NO. 51

SPENCER UNIVERSAL BINOCULAR MICROSCOPE

LOW MAGNIFICATION WIDE FIELD

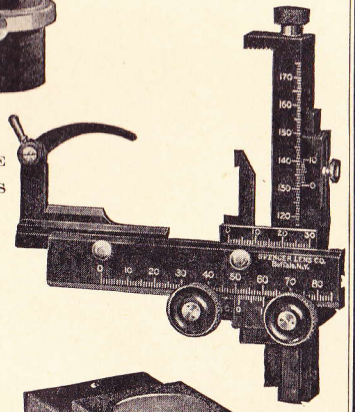
Supplied With Several Types of Stands

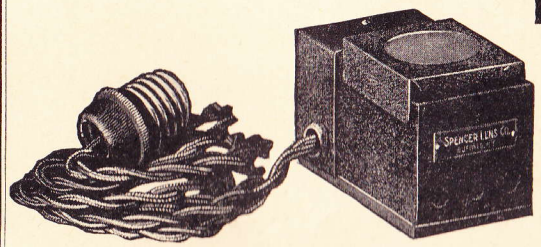


SPENCER ELECTRIC DARK-FIELD ILLUMINATOR NO. 330

SPENCER ATTACHABLE MECHANICAL STAGES NOS. 485 AND 490

Attachable to any Square Stage Microscope.





SPENCER MINEATURE MICROSCOPE LAMP NO. 385

List of Some of the Products
Manufactured by

SPENCER LENS COMPANY

- Microscopes
- Microtomes
- Delineascopes
- Photo-Micro Apparatus
- Spectroscopes
- Spectrometers
- Colorimeters
- Refractrometers
- Goniometers
- Haemacytometers
- Haemaglobinometers
- Optical Glass
- Telescope Discs and Lenses
- Prisms
- Magnifiers
- Lenses
- Dissecting Instruments