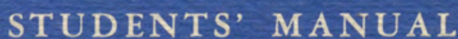
R.A. Marie



"THE COMPOUND MICROSCOPE"

STUDENTS' MANUAL

for

"The Compound Microscope"

Foreword

Living as we do in a productive society, most of us have come to take industrial and economic progress for granted. Directly and indirectly, the modern compound microscope is one of the most essential tools of this progress, an important factor in the continuing improvement in our standards of living.

The clothes we wear last longer, feel more comfortable and cost less because of the part played by the microscope in textile research. The abundance and quality of the food we eat stem from improvements in food production and refrigeration, industries in which microscopy is of prime importance.

This instrument is also a basic tool in developing new products of wood, plastic and other construction materials. The engineer who calculates stresses and strains of various metals is indebted to the microscope of his co-worker, the metallurgist.

The cars we ride in, the magazines we read, the "wonder drugs" that have scored major victories in the never-ending fight against disease—almost every essential and luxury of modern living is dependent, one way or another, upon industrial and scientific research, which would, to a great extent, be impossible without the modern compound microscope.

"The Compound Microscope"

A Motion Picture

—Synopsis—

The motion picture demonstrated construction, basic principles, and operation of the modern microscope.

The particular model you studied was a laboratory microscope with monocular tube. Of course, to satisfy the varying needs of today's highly specialized sciences and industries many different types of microscopes are necessary; research, metallurgical, stereoscopic, comparison, shop, and a wide range of other special-purpose microscopes. The model you saw in the motion picture is representative of all these various types. Differences that may exist are details of construction or operation; the basic principles are the same.

You saw how a lens is used to increase the apparent size of an object, and how magnification can be still further increased by introducing a second lens into the optical system.

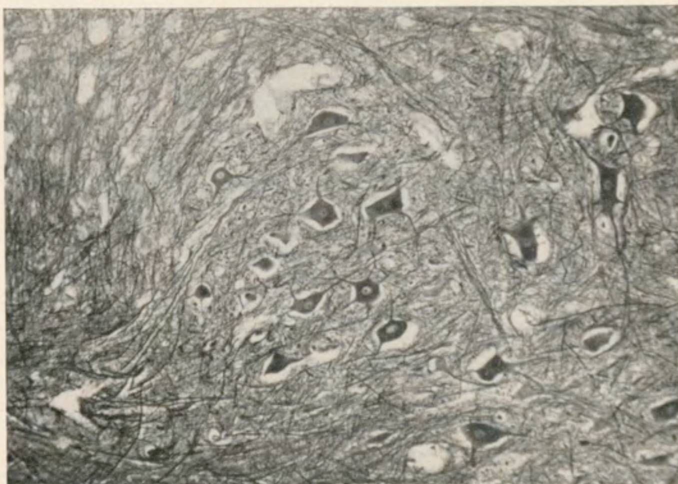
You saw various types of illuminators used to provide light of the proper beam size, color and intensity for the type of microscopical examination to be conducted.

You learned the proper procedure in focusing.

You saw typical uses of three objectives: the low power (10×) objective, to search the slide; the 43× objective, for higher magnification and sharp focus on smaller areas of the specimen; 97× objective, to obtain extremely sharp detail of still smaller areas of the specimen at still higher magnification.

You saw how resolving power and light intensity can be regulated, and how both these factors can effect image clarity.

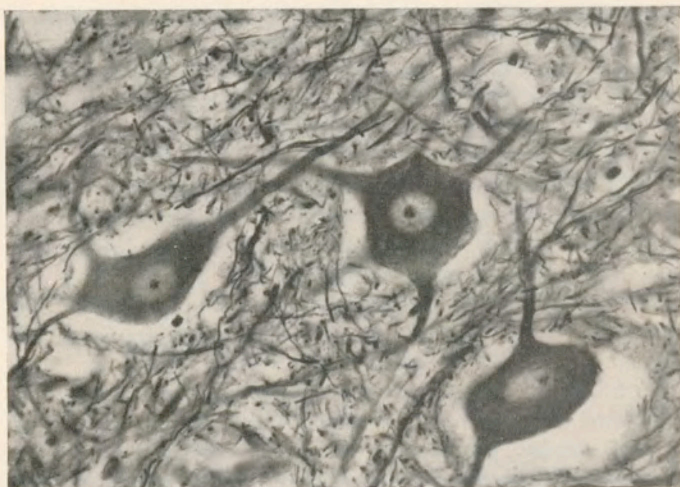
And, finally, you learned the basic rules of microscope care and cleanliness.

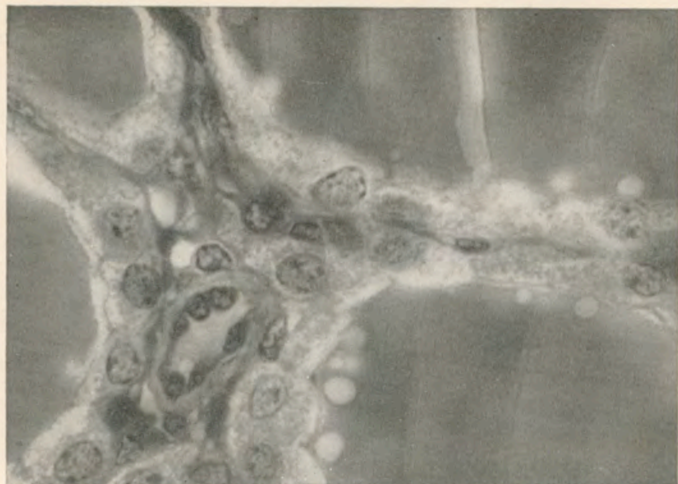


Note large field covered with 10 power objective. However, it shows little detail in nerve cells and fibers.

☆ *Nerve Cells and Fibers in Spinal Cord* ☆

With the 43 power objective, the field is much smaller. Detail is increased, due to higher N.A. and magnification of objective.



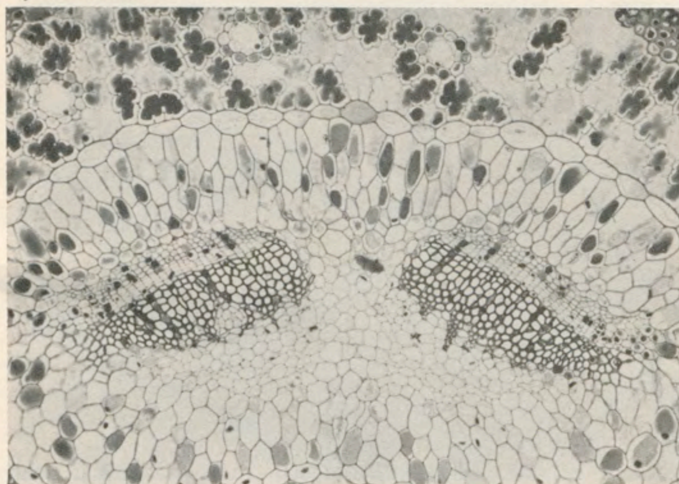


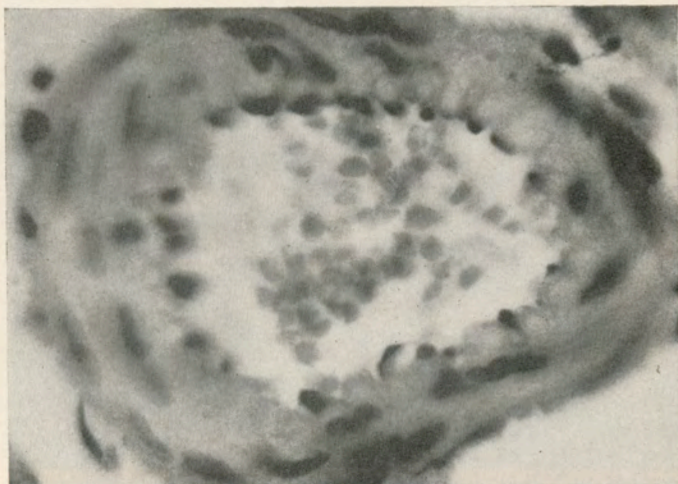
☆ *Normal Thyroid Section* ☆

Oil immersion objective provides maximum detail. Notice the well-defined nuclei (oval bodies) and the dark granular interior area.

☆ *Pine Needle Cross Section* ☆

The cell boundaries show very clearly in this type of specimen, though the 10 power objective is used.

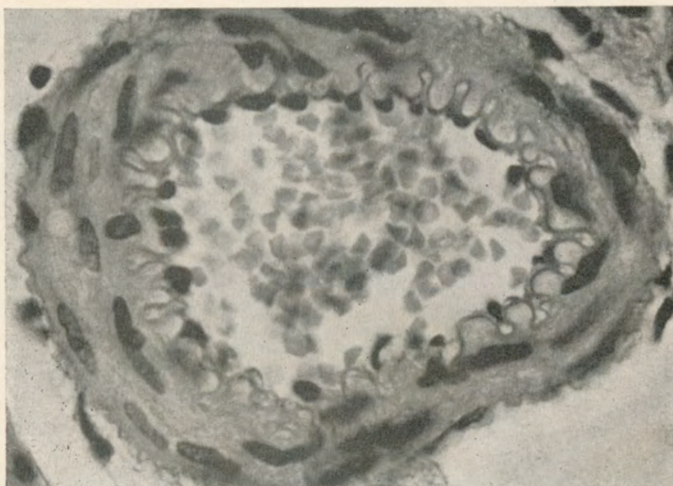


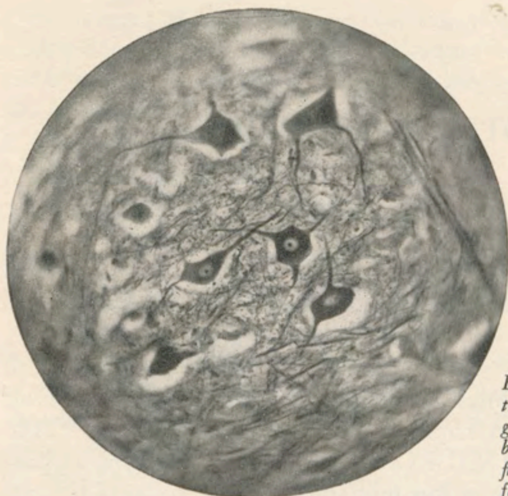


Focal length and numerical aperture of condenser and objective are equal.

☆ ***Blood Vessel Cross Section*** ☆

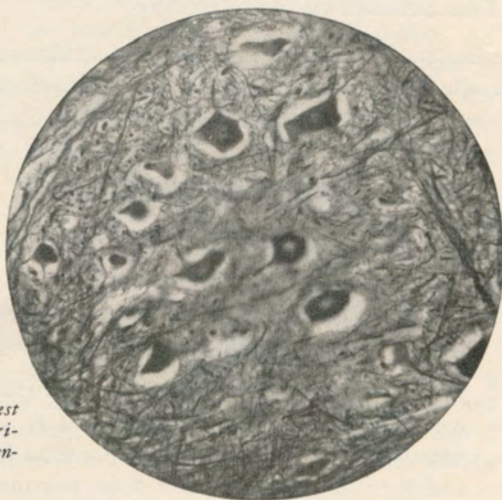
The substage diaphragm has been partially closed. Note, especially, the increased detail of the elastic fiber (corrugations at inner wall) and the dark smooth muscle nuclei in the wall.





Exact focus is at central portion of field, giving good detail of both nerve cells and fibers. Edge out of focus.

☆ *Curvature of Field* ☆



Refocused for best definition at periphery of field. Center out of focus.

STUDENTS' MANUAL

for

"The Compound Microscope"

This study guide is designed to supplement the basic information you acquired from the motion picture, "THE COMPOUND MICROSCOPE," and to help you acquire skill in your use of this fundamental instrument of science.

The Science of Microscopy

Microscopy is the skillful use of the microscope in a thorough and systematic manner for the investigation of matter too small to be seen by the unaided eye.

Successful Microscopy Requires:

1. The use of a microscope mechanically and optically suited to the work at hand;
2. A knowledge of the basic principles of the microscope and of its optical system;
3. Skill in the use and care of the microscope;
4. An understanding of the nature of the material to be investigated, the method of preparation for examination, and the illumination required for adequate observation.

Obviously, then, there may be many variable factors which must be controlled for successful microscopy. In order to accomplish this it is essential that you understand the relationship between:

1. Magnification;
2. Resolving power; and
3. Illumination.

MAGNIFICATION

Magnification is an apparent increase in the size of an object.

Placing a convex lens between the eye and any object will result in magnification—an increase in the apparent size—of that object. But certain other changes also take place in the relationship between the eye, the lens and the object. An understanding of these changes is tremendously important for effective use of the microscope.

Light passing through the lens is refracted, determining the angle at which it enters the eye. The greater the curvature of the lens surface, the greater the angle of refraction, and the larger the object appears to be. Therefore, the greater the curvature of the lens, the higher the lens power.

Magnifying power of a single lens can be roughly calculated by using the following formula in which f is focal length in inches, the approximate distance between the lens and the object when the object is in sharp focus:

$$\frac{10}{f} = \times \text{ (Magnifying power).}$$

As can be seen from the formula, the greater the focal length, the lower the magnifying power. Conversely, the higher the magnifying power of the lens, the shorter the focal length.

The area of the object which can be examined through the lens is called the field of view. As the focal length is reduced, and magnification is increased, the field of view is also reduced.

For these reasons, switching from a low power objective on the microscope to a higher power objective brings the objective closer to the object slide and also diminishes the field of the object which is visible through the microscope.

RESOLVING POWER

Resolving power in a microscope is the ability of the objective to distinguish fine detail in the specimen structure.

Resolving power is indicated by the Numerical Aperture, or N.A., of the objective. It may be defined as:

$$\text{N.A.} = n \sin a.$$

In this formula,

n is the refractive index of the transparent medium (air, water, or oil) between the objective lens and the object;
a is the angle between the extreme marginal ray and the central ray entering the objective.

Actually, then, N.A. is a mathematical rating based on the cone of light which an objective can utilize.

The higher the N.A. of the objective, the greater the resolving power and the finer the detail it can reveal. From our math we know that the largest value for the sine of an angle is 1. Since the refractive index of air is 1, the limit of N.A., when air is between the object and the objective lens, is 1. If oil or other suitable fluid with an index of more than 1 is introduced between the objective and the object, we can obtain a greater value of N.A. and, consequently, can obtain higher resolving power. The oil immersion objective can give greater resolution as well as higher magnification. To provide an illuminating cone with N.A. equal to that of the objective, oil must be substituted for air between the condenser and slide.

Resolution increases for a given N.A. with light of shorter wavelength. That is, with a given objective, blue light will provide greater resolution than red light.

The greater the cone of light which enters the objective lens, the more clearly visible is the detail. The greatest resolution possible for a particular objective is obtained when the cone of light completely fills the aperture of the objective. Ideally, it should be possible to match the N.A. of the objective with the N.A. of the condenser system.

Neither N.A. nor resolution creates magnification. While the prime function of the microscope may be to magnify, magnification alone is not sufficient. The objective lens of our microscope must be carefully designed to include a definite relationship between N.A. and magnification to make it useful.

ILLUMINATION

The purpose of illumination is to light the specimen so that it can be seen to maximum advantage for significant examination. Sky light provides sufficient illumination for elementary microscopy, but it varies unreliably and is restricted to daylight hours. The more advanced the microscopy, the more important the source of illumination, and the more stringent the requirements of constancy, intensity, flexibility, and control.

Objects are made visible to us because of the way they affect the light which reaches them from the sun, or, perhaps, from an incandescent lamp. We recognize an opaque object, for example, in form, color, and texture, because of the light reflected from it to our eye. On the other hand, we recognize some objects by the way they affect the light which passes through them. They are more or less transparent, or appear as outlines against a luminous background.

Objects too small, or structures too fine to be studied by the unaided eye are made visible under the microscope in the same manner. *Surface illumination* for opaque matter, to make details visible by the light reflected into the microscope, and *trans-illumination*—making the structure visible by

passing light through the matter—are the two main forms of illumination for the microscope. The latter form is known as *bright field illumination* and is the principal method used for the examination of biological materials.

There are two major methods of bright field illumination. Both are correct in principle but an unfortunate selection of names for the two methods has been a source of confusion among users of the microscope.

One method is referred to as *critical* illumination, and was described by Abbe and Nelson. In this system the bare light source, such as sky light through a window, or an open lamp flame (such as was employed at the time this method was described) is placed before the microscope mirror. The substage condenser then images the source—the lamp flame, filament, clouds in the sky, or the window pane—on the object plane. The disadvantages of the method are obvious. Any structure or irregularity of the source is seen directly in the field of view and limits, to some extent, the examination of the specimen.

The second method, prepared by Dr. August Koehler, eliminates the disadvantages of the first method and insures a full, evenly illuminated field of view. At the same time, it meets the requirements for illumination of the aperture. The Koehler form of illumination is more generally used today. The source, usually a tungsten filament lamp, is imaged by a lens at the opening of the substage condenser. It is magnified so as to fill completely the condenser aperture. The substage condenser, in turn, is adjusted to form an image of the lens at the light source, in the field of view. The microscope manufacturer designs the illuminator lens and the substage condenser so as to provide full field and aperture illumination for the microscope.

It is well to recall that both methods of illumination are critical in the full meaning of Nelson's and Abbe's definition: both meet the requirements for exact imagery. It would be better if one were to refer to Abbe-Nelson illumination on the

one hand and Koehler illumination on the other.

Most thin, fixed biological sections or smears must be stained to afford sufficient contrast for adequate study in bright field illumination. However, living materials can rarely be stained without damage to the subject. A recent development known as Phase Contrast Microscopy is a modification of bright field illumination which makes examination of living, or other thin, unstained preparations entirely practical and comparatively simple.

Phase Contrast

Phase contrast microscopy makes use of the relationship between two paths of light:

1. Light which enters the microscope objective *directly* through the object structure; and
2. Light which enters the objective after being *diffracted* by the object structure.

Special equipment is used to reveal, in terms of varying brightness, all points of divergence between these two paths of light. In this way a pattern is formed, revealing structure whose lack of contrast would make it invisible under other illumination conditions.

Darkfield

This is a method of illumination by means of which the object structure is made to appear luminous against a dark field, hence the name. In this system a special form of substage condenser is employed. A central stop in the lens or, in some forms, a ring type of reflector, focuses a hollow cone of light on the object plane. The rays of light in the illuminating cone are at such an oblique angle that they do not enter the objective directly. As the rays strike the object structure, part of the light is reflected upward into the objective, because of the difference in refractive index be-

tween the structure and the mounting medium. Consequently the object detail is seen only by the light it reflects and therefore appears self-luminous.

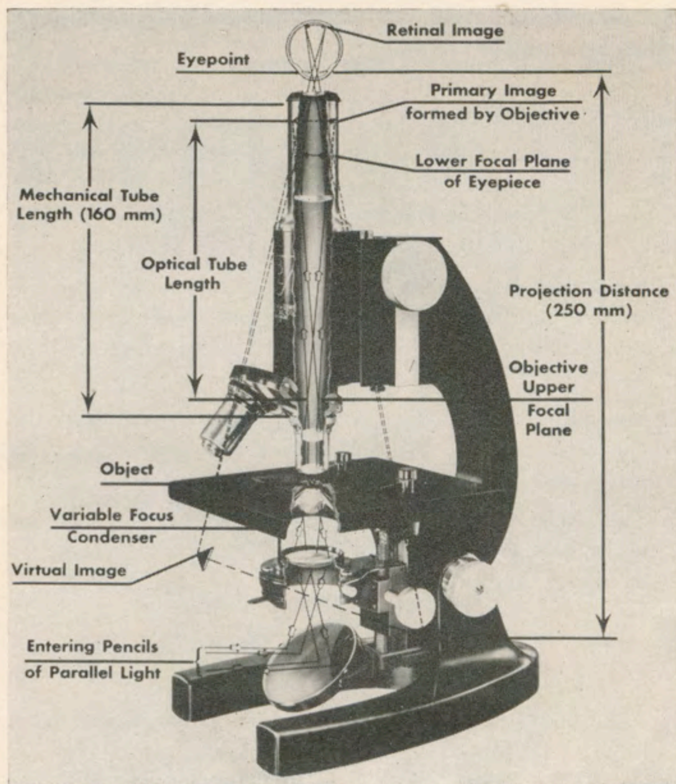
Polarized Light

Light rays vibrate at right angles to the direction of travel from the source. *Polarization* restricts this vibration to one desired plane or direction. Certain kinds of crystalline substances and minerals can be examined to great advantage when placed between two polarizing elements called a polarizer and an analyzer which are rotated at right angles to each other. The crystals appear bright against a dark background as they tend to change the direction of polarization. Application of this principle in the microscope provides a method for the identification, measurement, and study of certain characteristics of crystals and crystalline materials.

Identifying and studying crystals is only one of the many applications of the microscope to chemistry. The microscope is also used to identify compounds in gas and liquid states as well . . . in studying foams, emulsions and plastics. Use of the different types of illuminating systems . . . including systems utilizing wavelengths beyond the power of the eye to see, for photographic observation . . . has enabled the chemist and his co-workers in the other physical sciences to examine and identify compounds, matter or details of structure whose existence they had previously been able only to assume or postulate.

KNOW YOUR MICROSCOPE

The first requirement in learning to use your microscope with maximum effectiveness is to know all of its parts, their correct names, and the proper functioning of each part.



Study the diagram until you know all of the labeled parts. Test yourself by covering the labels and identifying the individual structures until you can do so accurately every time.

After doing this you should be able to compare the diagram with the actual parts corresponding to it on the particular microscope which you are using. In this way you can take the first important step toward becoming familiar with

the correct operation and structures of the microscope which is available to you.

Focusing Procedures

RIGHT from the start is the way to focus properly. The procedure becomes automatic in a very short time, so doing it properly from the beginning means taking a long step toward good microscopic technique.

1. Watching the microscope tube from the side of the stage, use the coarse adjustment to bring the body tube down until the low power objective is about one-quarter inch above the slide.
2. Using the coarse adjustment, and looking through the eyepiece, raise the objective until the specimen comes into approximate focus.
3. Still using the low power objective, search the field for various areas which might warrant more complete study at higher magnification.

WITH PARFOCAL OBJECTIVES

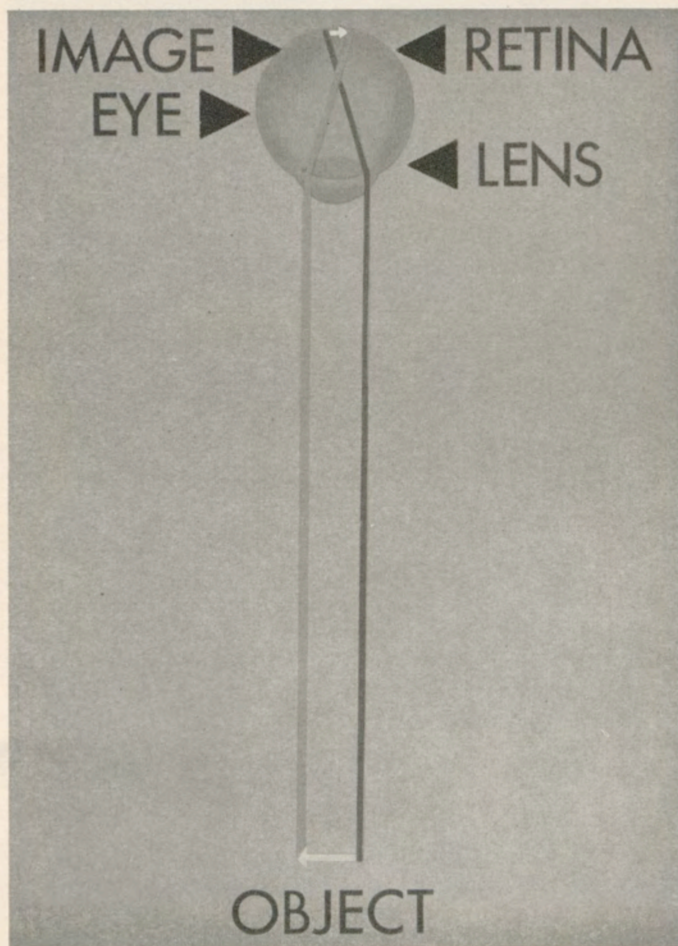
4. Turn the high power objective into place.
5. Slight adjustment of fine focus brings the specimen into sharp focus.

WITH NON-PARFOCAL OBJECTIVES

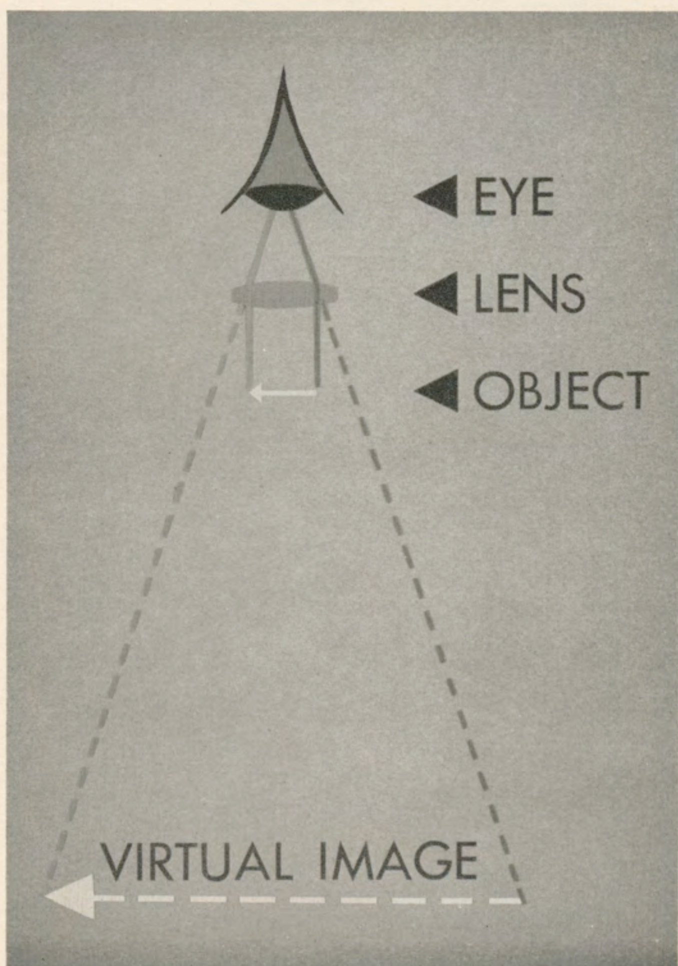
4. Raise the body tube, turn the high power objective into place.
5. Lower the objective carefully to the slide, watching it from the side of the stage.
6. Focus *up only* with fine adjustment, until specimen comes into sharp focus.

The Optical System

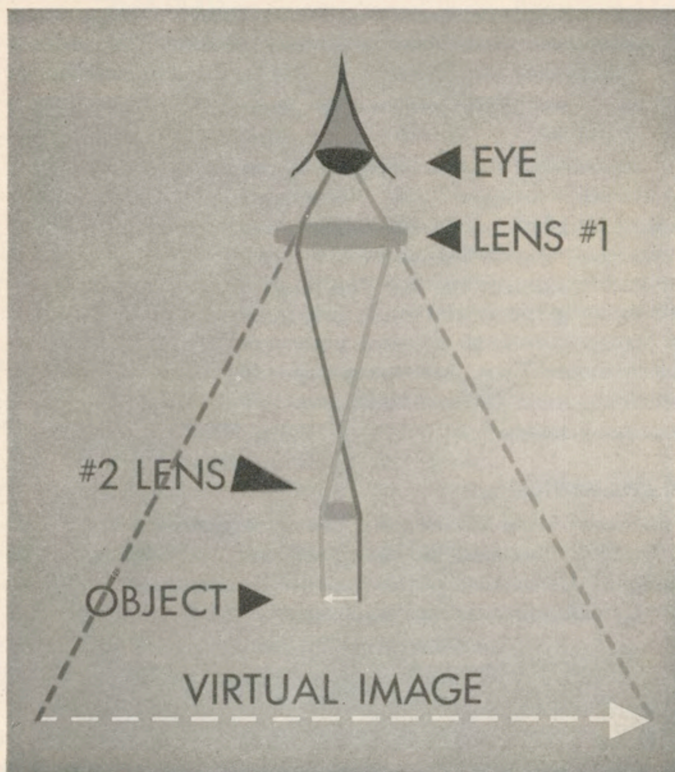
This diagram can represent normal vision of an object by the human eye.



If a simple convex lens is placed between the object and the eye, we have a case of simple magnification.



If we now place a second lens in the same system, we achieve even greater magnification.



THE FIELD . . . becomes much smaller with each increase in magnification.

FOCAL LENGTH . . . (the approximate distance between the lens and the object when the object is in sharp focus) . . . becomes shorter with each increase in magnification.

MAGNIFICATION . . . depends directly upon the curvature of the lenses introduced into the system, and the total magnification is the product of the magnifying powers of the individual lenses.

RESOLUTION . . . increases as each objective of higher magnification and higher N.A. is brought into use. But for any one objective, increase in eyepiece magnification does not increase resolution.

Actually the modern compound microscope is, in effect, a system of two lenses, or magnifying components, which enable the eye to distinguish structures in the object indistinguishable with the naked eye.

Follow the paths of light through the microscope in the diagram (page 17) so that you can distinguish and understand the functions of the various optical components of the instrument. Your own microscope, regardless of make or specific minor differences, operates on precisely the same optical principles.

Illumination

The function of the mirror is to reflect light from an outside source up through the condenser onto the specimen, and into the lenses and optical system of the instrument. Problems in illumination are of several varieties.

SOURCE . . . The source must provide light of proper intensity for the needs of the particular work being done.

CONTROL . . .

(a) *Intensity*—Ideally, the intensity should be controllable within wide limits. Some system of filtering is essential so that light can be adjusted to a comfortable level for optimum observation.

(b) *Color*—Control of the color of the light is important to vary contrast and to select light toward the shorter end of the spectrum for better resolution, when required.

(c) *Size*—Control of the size of the light beam reaching the mirror or condenser system is desirable to eliminate stray light and increase effectiveness of the condenser system.

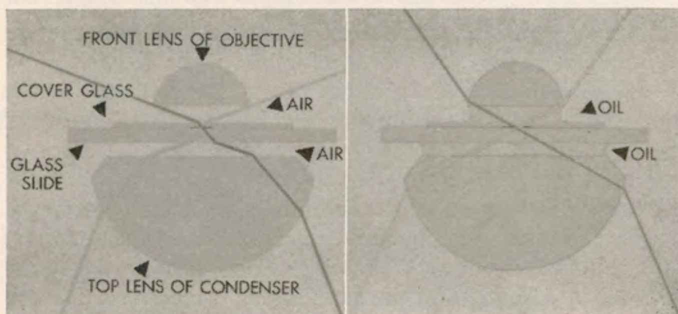
Condenser

Proper use of a condenser, either the variable focus type or one which will give similar flexibility and practical results, is an important part of precise microscopy.

The variable focus condenser is an important improvement in the Abbe substage condenser which provides greater convenience and flexibility in the adjustment of illumination for the different objectives . . . a valuable contribution to optimum imagery. Its effect upon the angle of light which enters the lens can be seen on your microscope by racking the condenser up and down and observing the change of angle in the light which reaches the specimen. This change in angle can actually be seen by removing the eyepiece and observing the objective lens.

It is the function of all microscope condensers, of whatever variety, to provide suitable specimen illumination to permit objectives to obtain their maximum resolution. Ideally, the

Left, condenser, slide, and front lens of objective without immersion oil. Right, note change in path of light when oil replaces air.



condenser should be capable of matching the N.A. of the particular objective in use.

By substituting oil on the top and bottom of the slide we can exclude the air. Since the oil used has optical properties similar to the glass of the objective, we can make it possible for the objective to include diffracted light rays which, without the oil, would pass outside the objective or be reflected away from it. By embracing rays diffracted at the extreme angle in this manner, and recombining them with the corresponding undiffracted rays, the oil immersion objective gives us the maximum resolution.

Use of the Oil Immersion Objective

1. Place a drop of oil on the top lens of the condenser, or on the bottom of the slide.
2. Determine the approximate position of the area to be observed, and survey the field carefully, using the 10 power objective.
3. Turn the 43 power objective into place and bring the image into focus in the center of this field by using the fine adjustment.
4. Without altering the fine adjustment, use the coarse adjustment to rack the body tube up from the slide.
5. Place a drop of oil on the top of the slide.
6. Using the coarse adjustment, and observing carefully from the side of the stage, lower the oil immersion objective to the surface of the slide. At the precise moment of contact, a flash of light will be visible.
7. Looking through the microscope, use the fine adjustment to bring the specimen into sharp focus.

Care of the Microscope

Dust and dirt not only interfere with microscope efficiency, but can actually be magnified to the point where they obscure or distort your view of the specimen. Make microscope cleanliness a habit from the start. Make it a point to inspect

and clean your microscope before and after you use it—until it becomes second nature.

Keep It Covered

During extensive periods of disuse the microscope should be kept in its case.

A microscope which is used regularly or intermittently should be protected by its plastic cover when not in actual use.

Handle It, Don't Manhandle It

Carry the microscope with two hands, and when placing it on a desk or table, do so with care. Avoid jarring it. Treat it like the precision instrument it is.

Keep It Clean

Inspect and clean the eyepieces and objectives before using them. Dust should be removed with an air syringe or a small camel's hair brush. Wiping optical glass without first removing the dust may result in scratches and mars. Optical glass should be wiped with lens paper or a well-washed, soft, clean cloth. Handle the lenses carefully so as not to smudge them with oil from your fingers.

Immersion oil should be kept covered in its bottle, as dust may settle in it if it is left uncovered. Dirty immersion oil should never be used on a microscope.

After using the oil immersion objective, carefully wipe the lens, slide and condenser until they are clean and dry. Use solvent sparingly, and only when necessary.

Troubles and Trifles

When you first start using your microscope it will seem as though there are innumerable "little things" that keep you from getting maximum results. Here are a few of these troubles and trifles which you'll quickly learn to correct.

Image Shift

You may sometimes observe that the specimen you are examining appears to shift sidewise as you focus in and out. This is caused by the light entering the microscope system off the axis. It is easily corrected by adjusting your mirror and/or source of illumination so that the illuminating beam passes through the optical system centrally.

Cover Glass

Your microscope is probably designed for use with slides equipped with No. 2 cover glass (nominal thickness 0.18-mm). If the cover glass is too thick or too thin, you will not get the optimum image quality with the higher power dry objectives (21×, 43× and similar). You'll save trouble if you select cover glasses carefully when preparing slides. Cover glasses are an important part of the optical system when observations are to be made with the high power dry objectives.

Upside Down

Sometimes labels are placed on the wrong side of the slide by mistake. Make sure the slide is right side up (so that you look through the cover glass, not the slide), or you'll spend a long time seeing nothing.

And finally, remember: never use the condenser iris to cut down on illumination intensity. Moderation of light intensity should always be done at the microscope illuminator or in the light path between the microscope mirror and illuminator. Transparent filters of neutral density provide the most desirable means of adjusting intensity. If you have no neutral density filters, and your illuminator provides too intense a light, examine the lamp. If it is a high wattage lamp, change it to a lower wattage lamp.

GLOSSARY OF OPTICAL TERMS

ABERRATION

Failure of a lens to converge to a common focus all light rays coming from an object point; the deviation of such rays from a single focus.

ABERRATION, CHROMATIC

The inability of a lens to focus light of different colors at a single point. An *aberration* which causes different colors (wavelengths) of light to be focused at different distances from the lens is referred to as *axial* or *longitudinal* chromatic aberration. Difference in image size for different colors is called *chromatic difference of magnification*, or lateral color.

ABERRATION, SPHERICAL

The inability of a lens to bring light rays from all points on a lens surface to a common focal point.

ACHROMATIC

Referring to a lens designed to converge light rays of different colors to a single focus. Free from *chromatic aberration.* Corrected for two colors chromatically, and one color spherically.

ADJUSTMENT, COARSE

The mechanism—usually a rack and pinion—which rapidly sets the microscope in approximate focus on the specimen.

ADJUSTMENT, FINE

A mechanical arrangement, generally employing a micrometer screw and head, for effecting slow or minute changes in focus of the microscope.

ANGLE OF INCIDENCE

The angle between a ray of light striking a surface and a line (called the normal) perpendicular to the surface at this point.

Angle of Reflection

The angle between a ray of light reflected from a surface and the normal. (See *angle of incidence*).

Angle of Refraction

The angle between a refracted ray and the normal. (See *angle of incidence*).

Angstrom Unit

1/10 of a millimicron.

Aperture

The open area of a lens, or the opening in a diaphragm.

Apochromatic

Referring to a lens which has a higher degree of correction than the *achromatic* form. Specifically, in the apochromatic lens *chromatic aberration* is corrected for three colors and *spherical aberration* is corrected for two. This is accomplished with the aid of fluorite lens elements being incorporated in the optical system.

Arm

The rigid member of the microscope which supports and aligns the substage, stage, and body tube elements. Its form generally provides a convenient handle for carrying the instrument.

Astigmatism

Failure of a lens to unite at a single image point the rays from a single point in the outer regions of the field. This aberration causes two separate line images at different distances from the lens, displaced at right angles to each other.

Balcoted Lens

A lens which has been given the Bausch & Lomb anti-reflection treatment. The thin, tough coating applied to the lens surface helps produce a sharper, clearer image by reducing external and internal reflections.

Base

The foot, generally horseshoe-shaped, that supports the microscope.

Circle of Least Confusion

The circular cross section of a convergent, image-forming pencil of light, at the point of least diameter. The point of best focus.

Coddington

A form of corrected magnifier lens, named after its inventor.

Coma

A defect causing points not in the center of the *field* to undergo different magnifications in various zones of the lens. This *aberration* is named for the comet-shaped image such a lens forms of a single point.

Concave

Hollowed, or curved inward, like the interior of a sphere.

Condenser

A simple or compound lens used, as in a microscope, to "gather" light rays and focus them on the object to be illuminated.

Condenser, Abbe

A condenser used under the stage of a microscope to illuminate the specimen. It usually has two simple lenses in a fixed mounting, with the upper lens removable to increase focal length for low magnifications.

Condenser, Dark Field

A substage condenser used to illuminate the specimen by oblique rays, causing a nearly transparent object to appear self-luminous in a dark background by virtue of light reflected by the object.

Condenser, Paraboloid

A type of *dark field condenser* which converges the beam of light from a parabolic reflecting surface.

Condenser, Variable Focus

The Bausch & Lomb patented condenser which achieves a desired simultaneous change of illuminated field and *numerical aperture* rating by a simple focusing motion of part of the condenser lens system.

Convex

Bulging or curving outward, like the exterior of a sphere.

Corrected

Referring to a lens or lens system in which aberrations have been overcome to a degree sufficient to produce a clear, sharp image.

Counting Chamber

A ruled slide, usually with a depression of known volume and a cover plate, used to evaluate small particles in respect to individual size or frequency per unit volume.

Cover Glass

A thin plate, usually of glass, rectangular or circular in form, which is placed over a specimen slide preparation and cemented in place by the mounting medium.

Critical Angle

The *angle of incidence* formed by a light ray in passing from a dense to a less dense medium, at which the refracted ray emerges just grazing the refracting surface. (See *angle of refraction*). Any increase in this angle causes internal reflection.

Crown Glass

Optical alkali-lime glass having a low *dispersion* and usually a low index of *refraction.*

Curvature

In a lens surface, the radius of curvature or "sharpness" of curve.

Curvature of Field

The failure of the image of a plane object to conform to a plane in the image space.

Depth of Field

The distance along the *optical axis* throughout which the object can be located and yet be imaged with satisfactory clarity in a given plane.

Depth of Focus

The distance along the *optical axis* throughout which the image formed by a lens is focused with satisfactory clarity.

Deviation

The angular difference between the original direction of a light ray and its new direction after passing through one or more optical boundaries.

Diaphragm, Iris

An adjustable opening or stop such as employed under the stage or substage condenser, or on an illuminator, for regulating the size of the light beam entering the microscope.

Diffraction

The spread of light waves occurring at the edge of an obstacle, or on passing through a narrow aperture, resulting from *interference* phenomena.

Dispersion

The separation of light into its component colors, on passing through a *refracting* medium, due to variation in the refractive index of the wavelengths of these components.

Distortion

The appearance of straight lines, in the object, as curves in the image.

Doublet

A pair of simple lens elements cemented together to form a single lens.

Double Lens Magnifier

A magnifier composed of two single lenses.

Empty Magnification

Magnification beyond that required to show to maximum advantage the detail resolved by the objective.

Entrance Pupil

A limiting stop in an optical system determining the effective or useful diameter of the entering light beam. It may be a real mechanical stop, but in most instruments it is the image of an internal aperture projected outside the optical system as if light were sent through it in a reverse direction.

Equivalent Focal Length

The *focal length* of a compound lens consisting of several simple lenses with a common *optical axis*, expressed in terms of an imaginary single lens having equal magnification.

Exit Pupil

A limiting stop outside the exit lens of an optical system. Although a real mechanical stop may be employed, it is more frequently the aerial image of an internal aperture formed by the exit lenses through which all light rays passing through the system emerge.

Eye-Lens

The lens of an eyepiece nearest the eye during observation.

Eyepiece (*sometimes called ocular*)

A lens or system of lenses serving primarily as a magnifier which provides a virtual enlargement of the *real image* formed by the objective lens.

Eyepieces may be designed to correct certain residual aberrations in the primary image; to project an external real image to a photographic material; or to project an enlarged image to a screen.

Eyepiece, Ampliplan

Used to project the microscope image to a photographic plate or to a screen. Corrected for minimum *curvature of field* in the ultimate image. Cannot be used for visual observation.

Eyepiece, Compensating

Corrects and compensates for the effects of *chromatic aberration* in the front lens in medium and high power objectives.

Eyepiece, Huygenian

Comprised of a plano-convex *field lens* and a similar, but higher power, *eye-lens*, with a diaphragm stop mounted between the two near the *focal point* of the eye-lens. This is the eyepiece most widely used with the microscope. Named after its inventor.

Eyepiece, Hyperplane

Similar to Huygenian eyepiece, but employing *achromatic* lenses. Particularly useful with the intermediate and high power dry objectives of the achromatic and semi-apochromatic (*fluorite*) types.

Eyepiece, Wide Field

Affords large *field of view* and high *eyepoint;* usually used with low power objectives to examine a large area of the specimen. Employs achromatic lenses.

Eyepoint

The point above the eyepiece at which the eye must be placed to see the full *field of view* afforded by the eyepiece. The eyepoint is defined by the *exit pupil* of the microscope optical system.

Field Lens

A lens of a system whose function is to provide the optimum *field of view*. For example, the anterior lens of certain forms of microscope eyepieces.

Field of View

The open or visible space commanded by a lens. In a compound microscope, the area that is visible through the eyepiece when the instrument is in focus.

Flatness of Field

Appearance of the image to be flat; conformity of the image to a flat plane, as opposed to *curvature of field.*

Flint Glass

A heavy, brilliant glass containing lead and having a high dispersion and usually a high index of refraction.

Focal Length

For all practical purposes, for a single thin lens: the distance from the lens to the image of a distant object formed by the lens. For a thick lens, or system of lenses: the distance from the image of a distant object to the plane where an imaginary single lens might be substituted to perform the function of the thick lens or system of lenses. (See *equivalent focal length.*)

Focal Plane

The plane at right angles to the *optical axis* at a *focal point.*

Focal Point

The point at which light rays from a distant object, after passing through a lens, converge to a focus and form an image. If the light rays start from such a point they become parallel to each other after passing through the lens.

Focus

(Noun): The point at which light rays converge to form an image.

(Verb): To adjust a lens or system of lenses so as to produce an image.

Funnel Stop

A tubular form of diaphragm stop inserted in the back

of an oil *immersion objective* to reduce its N.A. to less than 1.0 for purposes of dark field illumination.

HASTINGS TRIPLET

A magnifier composed of three lens elements cemented together to form a single lens. It is corrected for spherical and *chromatic aberration* and covers a relatively large *field*. Named after its inventor.

HEMACYTOMETER

A form of *counting chamber* slide with special rulings and accessory pipettes, used to determine quickly the number of red or white blood cells per unit volume of whole blood.

ILLUMINATION, CRITICAL

Specifically, that condition of illumination in which all rays of light emanating from the source arrive at the specimen in phase (Nelson's definition). In practice, this means the source of light itself is imaged exactly in the object plane.

ILLUMINATION, KOEHLER

A form of illumination in which a lens images the light source in the lower focal plane of the substage condenser. An image of this lens aperture is formed in the object plane by the substage condenser. In this system the lens at the source becomes the effective source under the conditions of *critical illumination.*

ILLUMINATOR, MICROSCOPE

A light source to provide illumination for the microscope.

ILLUMINATOR, VERTICAL

An accessory device for illuminating the surface of an opaque specimen. In the compound microscope, the device fits between the body tube and the objective; the objective then serves as both illuminating lens system and objective for the viewing system.

Image

The likeness or picture formed by a lens; the optical counterpart of an object.

Image, Real

An image, formed by converging light rays, which can be seen by inserting a screen, such as a ground glass, into the optical system, or which can be recorded on a photographic plate.

Image, Virtual

An image in which light, originating from a point on the object, and having traversed an optical system, appears to be diverging. Such an image may be apparent to the eye, but since it is not formed outside the optical system it cannot be demonstrated on a diffusing screen or photographic plate as in the case of a *real image*.

Index, Refractive

A numerical value which expresses the ratio of the speed of light through a substance to the speed of light in a vacuum. For all practical purposes the speed of light in air is assumed to be the same as it is in a vacuum.

Infra-Red

The range of invisible radiation of long wavelength in the electro-magnetic *spectrum*, extending from the red end of the visible spectrum to the range of Hertzian or radio frequency radiation.

Interference

Any or all of the several effects produced by the interaction of two or more trains of light waves arriving simultaneously at the same point.

Lens

A transparent substance, commonly glass, formed with two regular opposite surfaces, both curved, or one plane and one curved, designed to change the direction

of rays of light in a definite manner. The term *lens* is used variously to refer to a single element, as defined, or to a combination of elements cemented or mounted separately as a complete unit for a particular purpose.

LENS SYSTEM
: Two or more lenses arranged to work in conjunction with one another.

LIGHT
: An electromagnetic radiation capable of inducing visual sensation in the eye. It travels in a vacuum at a constant speed of 186,000 miles per second. This speed is less when the light travels through a substance such as glass or water.

LIGHT, MONOCHROMATIC
: Light of one color (wavelength).

LIGHT, POLARIZED
: Light whose vibration is restricted within definite limits at right angles to its direction of travel, as compared with non-polarized light, which vibrates in all directions at right angles to its direction of travel.

MAGNIFICATION
: The apparent increase in size of an object by an optical element or instrument. It is the ratio of the height of the image to the actual height of the object under observation.

LENS, MENISCUS
: A lens of crescent-shaped cross section; one which is concave on one surface and convex on the other. It may be either converging or diverging.

MICROMETER DISC
: A circular glass plate with appropriate rulings for measuring purposes, used in the focal plane of an eyepiece.

Micrometer Eyepiece

An eyepiece incorporating a ruled disc or plate, specially designed for measuring. The scale may be fixed or adjustable.

Micron

1/1000 of a millimeter

Microscope

A single lens or system of lenses used to observe objects under magnification.

Bright Field Microscope

The instrument most ordinarily used in laboratory work. Preparations are observed in bright field illumination. (See Page 13.)

Dark Field Microscope

A microscope set up specifically for observation of matter by dark field illumination. (See Page 15.)

Phase Contrast Microscope

A microscope equipped with accessories for the observation of matter by the phase contrast system of illumination. (See Page 15.)

Polarizing Microscope

A microscope fitted with polarizing elements. (See Page 16.)

Stereoscopic Wide Field Microscope

A binocular form of microscope with double objectives, designed to give a three dimensional view of the specimen. Its magnifying power is limited to about 150 diameters.

Microscope, Binocular

A microscope with two eyepiece tubes, permitting observation with both eyes simultaneously.

Microscope, Compound

A microscope including an objective and an eyepiece.

Microscope, Inclined Binocular
A microscope with eyepiece tubes inclined toward the observer.

Microscope, Monocular
A microscope with a single eyepiece tube.

Microtome
An instrument for cutting, with precision, thin sections of tissue or material for microscopical examination.

Millimicron
1/1000 of a micron.

Mirror
The plane or concave reflector used beneath the stage or substage condenser to direct light up through the microscope.

Nosepiece, Revolving
A device at the lower end of the microscope body tube for holding two or more readily interchangeable objectives.

Number, Magnification
The number engraved on an objective or eyepiece to indicate its magnification. Total magnification is objective number times eyepiece number.

Numerical Aperture (N. A.)
A numerical rating based on the cone of light that an objective can utilize. N.A. is an important factor in determining the resolving power of a lens.

Object
The matter examined by means of a magnifier or microscope; the part, particle or surface being viewed; variously referred to as subject, specimen, preparation, etc.

Objective
The lens or arrangement of lenses in a microscope or other optical system which receives light from the *field*

of view and forms the first image. So named because it is nearest the object. Sometimes referred to as the object lens or object glass.

OBJECTIVE, APOCHROMATIC

An objective having a lens system with *apochromatic* correction.

OBJECTIVE, DRY

An objective that does not require *immersion liquid* for full numerical aperture performance.

OBJECTIVE, FLUORITE

An objective that includes lens elements of fluorite and has slightly more correction for *chromatic aberration* than the conventional *achromatic* objective. Frequently termed "semi-apochromat."

OBJECTIVE, IMMERSION

An objective designed to include oil or other liquid instead of air between its front lens and the *cover glass.*

OIL, IMMERSION

Oil selected for use with oil *immersion objectives.*

OPTICAL AXIS

The straight line about which a lens or lens system is symmetrical. A ray following this line through the optical system would not be refracted from it.

OPTICAL CENTER

A point on the axis of a lens through which pass those rays of light whose paths on emerging from the lens will be parallel to their paths on entering it.

OPTICAL GLASS

Glass carefully manufactured to obtain controlled *index of refraction* and *dispersion,* purity, transparency, homogeneity and workability. The two most common types of optical glass are the crowns and the flints.

PARFOCAL

Referring to objectives or lenses that focus at the same position.

Plano

Pertaining to a plane; flat, without curvature.

Plano-Concave

A lens with one surface flat, the other curved inward. (See *concave*).

Plano-Convex

A lens with one surface flat, the other curved outward. (See *convex*).

Principal Focus

The focal point for a beam of light rays parallel to the *optical axis* of a lens or spherical mirror.

Prism

A transparent body (made of optical glass or crystalline material) with at least two polished plane faces inclined toward each other from which light is reflected or through which light is *refracted*.

Refraction

The change in direction occurring as a light ray passes from a medium of one optical density to a medium of another optical density.

Relative Aperture

The ratio of the *focal length* of the objective lens to the diameter of the *entrance pupil*, determining the "speed" or light-gathering power of the objective.

Resolving Power

The ability of a lens to distinguish fine detail in the structure of the specimen; the ability of a lens to image closely spaced objects so that they are recognized as separate in the ultimate image. This is in direct proportion to the N.A. of the lens.

Semi-Corrected

Referring to a magnifier or lens in which only part of the *aberration* is eliminated.

Slide

The glass support for the specimen or preparation to be studied.

Spectrograph

A spectroscope specifically constructed for photographic recording of a *spectrum.*

Spectrometer

A *spectroscope* used to determine the composition of a beam of light by comparing its *wavelengths* with *spectrum* lines.

Spectroscope

Any one of several forms of optical instruments used for dispersion of light and visual observation of the resulting *spectrum.*

Spectrum

An arrangement of dispersed radiation energy, such as light, in progressive order with respect to one of its characteristic properties, such as *wavelength.*

The visible spectrum consists of electromagnetic waves with wavelengths between 400 and 700 millimicrons. These wavelengths affect the sense of sight as colors, ranging from violet to red. White light is a mixture of all colors and wavelengths of the visible spectrum.

Stage, Mechanical

A device attachable to, or built into, the stage of a microscope to permit moving the specimen slide as desired while holding it in the plane of focus.

Stage Micrometer

A slide with appropriate interval rulings, used on a microscope stage for measuring, or for calibrating a *micrometer disc.*

Stage, Microscope

The platform on which the slide is placed for viewing with the microscope.

Tube, Body

The tube or barrel between the objective and the eyepiece of a compound microscope.

Tube Length, Mechanical

Distance from the top to the bottom of the body tube.

Tube Length, Optical

Distance from the plane of the primary image to the upper focal plane of the objective.

Ultra-Violet

A range of invisible radiation extending from the visible violet portion of the *spectrum* out to the low-frequency x-ray region of the electromagnetic spectrum.

Wavelength

The distance between two peaks in a wave train.
(Wave Train: a series of waves sent along the same axis by a vibrating body).
The numerical designation of specific bands of the electromagnetic spectrum.

Working Distance

The distance between the *cover glass* or object and the tip of the objective.

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D-133, 15, Apr '53 EB

Printed in U.S.A.

B&L