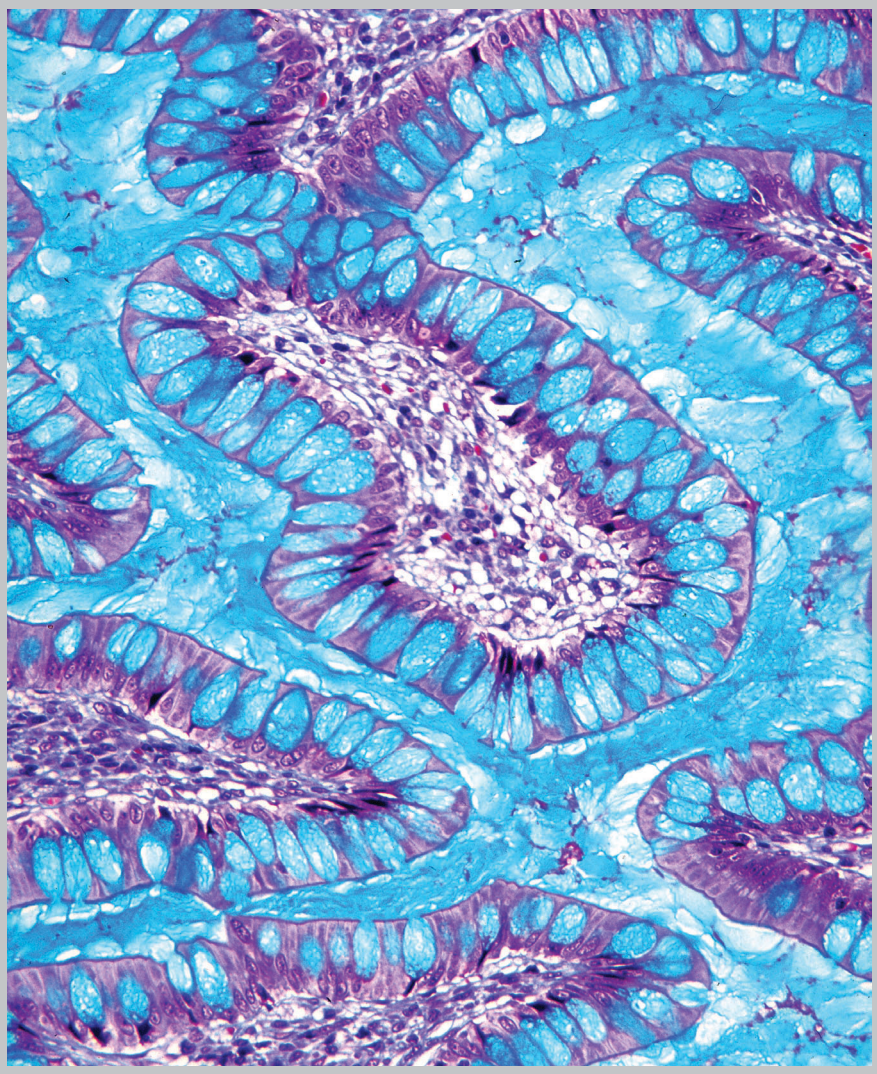
Microscope
Basics and Beyond

by Mortimer Abramowitz
Fellow, New York Microscopical Society

For Olympus America Inc.

Volume 1



MICROSCOPE

Basics and Beyond

REVISED EDITION 2003

By Mortimer Abramowitz

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For Olympus America Inc.
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Back Cover: Mouth Parts of the Blowfly

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PART ONE

An Introduction to the Compound Microscope

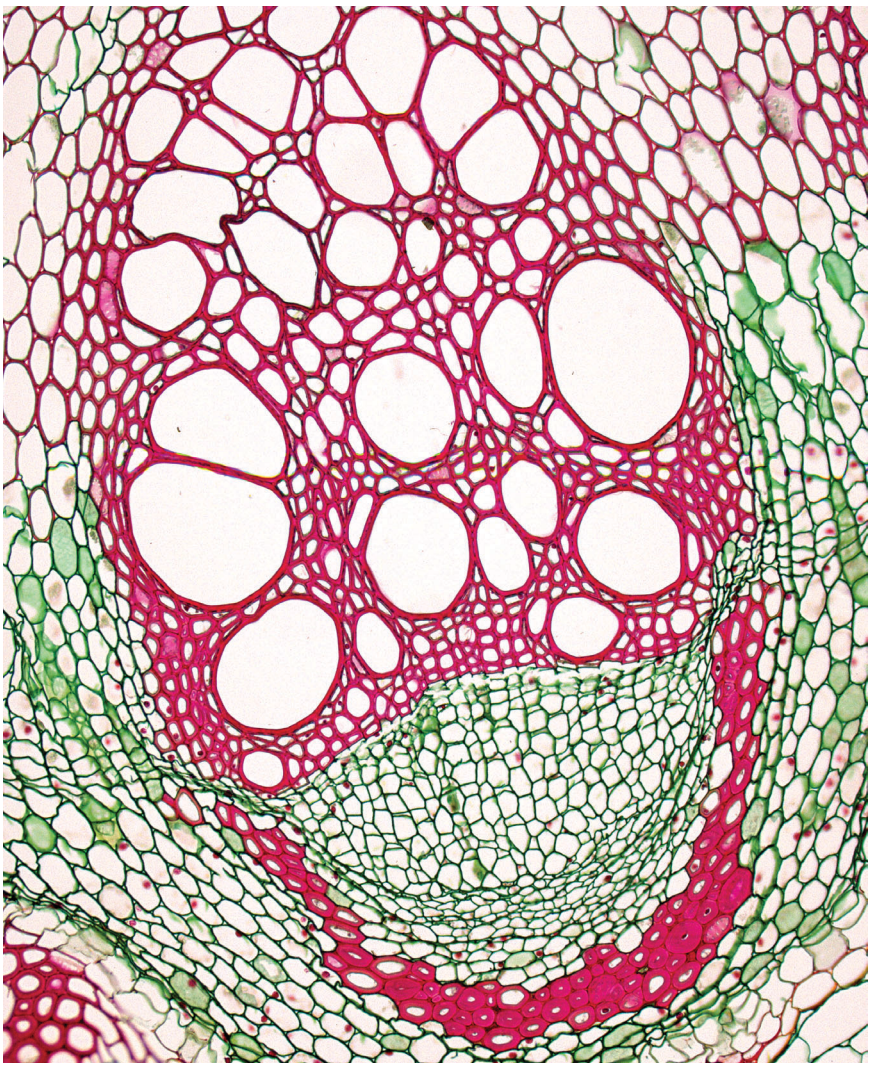


Photo: Clematis Stem Thin Section

What is a microscope? How does it work?

A microscope is an instrument designed to make fine details visible. The microscope must accomplish three tasks: produce a magnified image of the specimen (magnification), separate the details in the image (resolution), and render the details visible to the eye, camera, or other imaging device (contrast).

It is the purpose of this basics booklet to explain how the microscope achieves these tasks and to explain the components and their use in simple, non-technical language.

The Human Eye

Since so many microscope users rely upon direct observation, it is important to understand the relationship between the microscope and the eye. Our eyes are capable of distinguishing color in the visible portion of the spectrum: from violet to blue to green to yellow to orange to red; the eye cannot perceive ultra-violet or infra-red rays. The eye also is able to sense differences in brightness or intensity ranging from black to white and all the grays in-between. Thus for an image to be seen by the eye, the image must be presented to the eye in colors of the visible spectrum and/or varying degrees of light intensity.

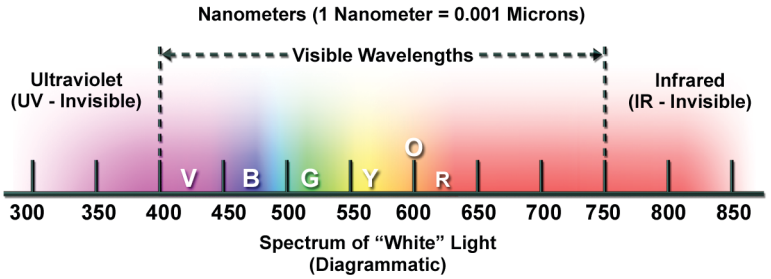


Fig 1. Spectrum of of "white" light. (Diagrammatic)

The eye receptors of the retina for sensing color are the cone cells; the cells for distinguishing levels of brightness, not in color, are the rod cells. These cells are located on the retina at the back of the inside of the eye. The front of the eye, including the iris, the curved cornea, and the lens are respectively the mechanisms for admitting light and focusing it on the retina. From there, the "message" is sent to the brain via the optic nerve.

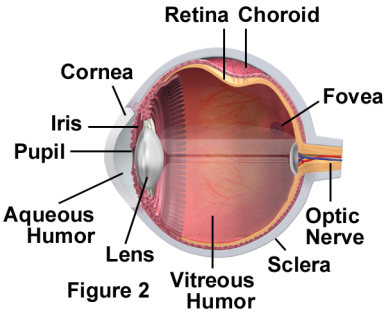


Figure 2

Fig 2. Anatomy of the Human Eye.

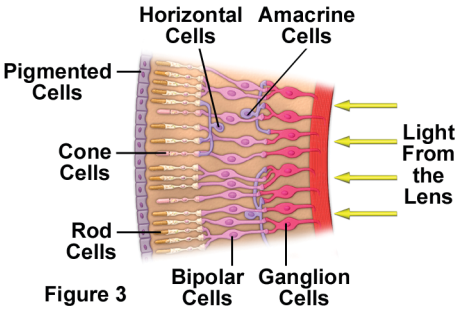


Figure 3

Fig 3. Microscopic Anatomy of the Retina.

For an image to be seen clearly, it must be spread on the retina at a sufficient visual angle. Unless the light falls on non-adjacent rows of retinal cells (a function of magnification and the spreading of the image), we are unable to distinguish closely-lying details as being separate (resolution). Further, there must be sufficient contrast between adjacent details and/or the background to render the magnified, resolved image visible.

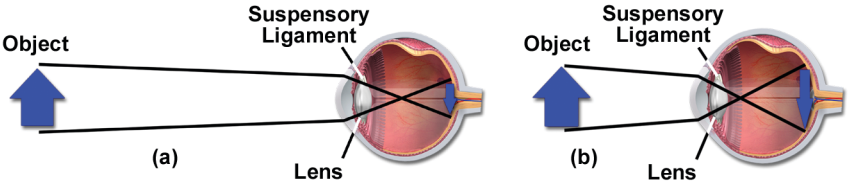


Fig 4. Accommodation of the human Eye. a. Object far away from the eye. b. Object very close to the eye.

"SIMPLE" MICROSCOPE

Because of the limited ability of the eye's lens to change its shape, objects brought very close to the eye cannot have their images brought to focus on the retina. The accepted minimal conventional viewing distance is 10 inches or 250 millimeters (25 centimeters).

More than five hundred years ago, simple glass magnifiers were developed. These were convex lenses (thicker in the center than the periphery). The specimen or object could be focused by use of the magnifier placed between the object and the eye. These "simple microscopes", along with the cornea and eye lens, could spread the image on the retina by magnification through increasing the visual angle on the retina.

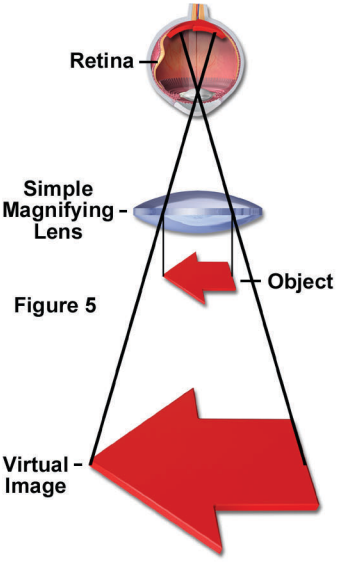


Fig 5. Simple magnifier. A simple magnifier uses a single lens system to enlarge the object in one step.

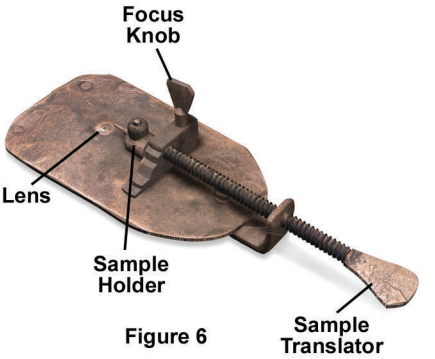


Fig 6. von Leeuwenhoek microscope. (circa late 1600s)

The “simple microscope” or magnifying glass reached its highest state of perfection, in the 1600’s, in the work of Anton von Leeuwenhoek who was able to see single-celled animals (“animalcules”) and even some larger bacteria. The image produced by such a magnifier, held close to the observer’s eye, appears as if it were on the same side of the lens as the object itself. Such an image, seen as if it were ten inches from the eye, is known as a virtual image and cannot be captured on film. These magnifiers had severe limitations in specimen positioning, illumination, lens aberrations, and construction.

COMPOUND MICROSCOPE

Around the beginning of the 1600’s, through work attributed to the Janssen brothers in the Netherlands and Galileo in Italy, the compound microscope was developed. In its basic form, it consisted of two convex lenses aligned in series: an object glass (objective) closer to the object or specimen, and an eyepiece (ocular) closer to the observer’s eye—with means of adjusting the position of the specimen and the microscope lenses. The compound microscope achieves a two-stage magnification. The objective projects a magnified image into the body tube of the microscope and the eyepiece further magnifies the image projected by the objective (more of how this is done later). For example, the total visual magnification using a 10X objective and a 15X eyepiece is 150X.

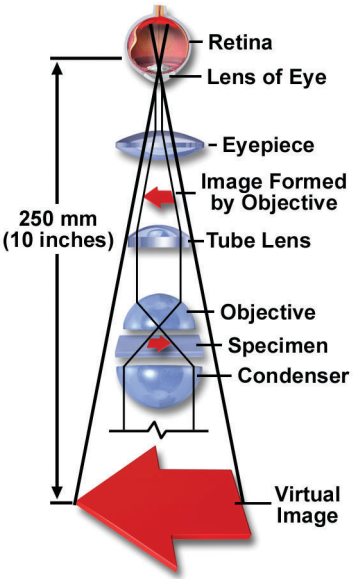


Fig 7. Compound magnifier. In the compound microscope, the intermediate image formed by the objective and tube lens is enlarged by the eyepiece. (diagrammatic)

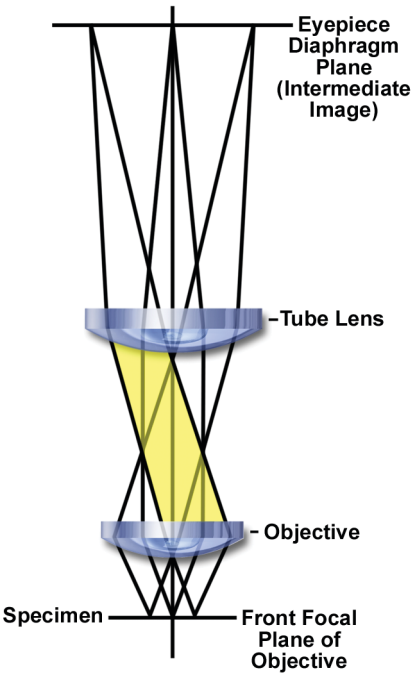
When you look into a microscope, you are not looking at the specimen, you are looking at an IMAGE of the specimen. The image is “floating” in space about 10 millimeters below the top of the observation tube (at the level of the fixed diaphragm of the eyepiece) where the eyepiece is inserted. The image you observe is not tangible; it cannot be grasped. It is a “map” or representation of the specimen in various colors and/or shades of gray from black to white. The expectation is that the image will be an accurate representation of the specimen, accurate as to detail, shape and color/intensity. The implications are that it may well be possible (and is) to produce (or even enhance) highly accurate images.

Conversely, it may be (and often is) all too easy to degrade an image through improper technique or poor equipment.

Essentially, this is how a microscope functions. Light from a lamp passes through a substage condenser and then through a transparent specimen placed over an opening in the stage. Light is then gathered by the objective. The objective, together with the built-in tube lens (more of this later), focuses the image of the specimen at the level of the fixed diaphragm of the eyepiece. The image is then seen by the observer as if it were at a distance of approximately 10 inches (250 millimeters) from the eye.

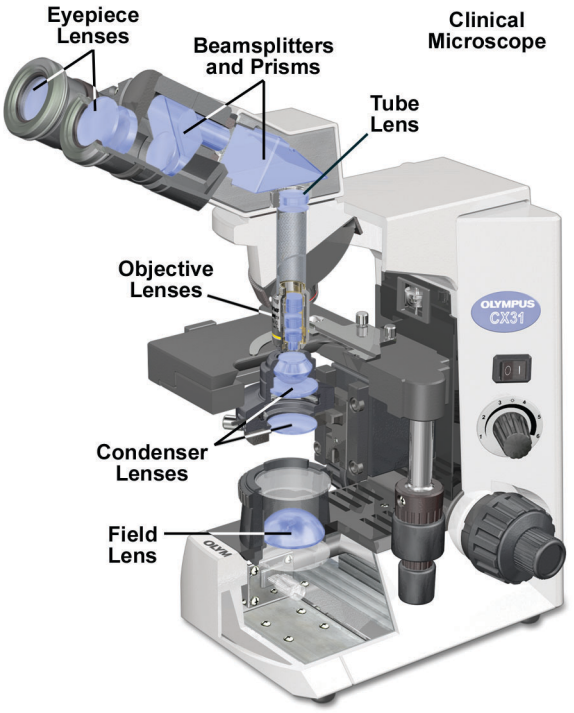
At the lowest part of the observation tube in infinity-corrected systems for Olympus equipment, there is a tube lens which gathers the parallel beams of light emerging from the objective and focuses the resulting image at the plane of the fixed diaphragm of the eyepiece. The eye lens of the eyepiece, together with the curved cornea and lens of the eye, focuses the image on the retina of the observer's eye.

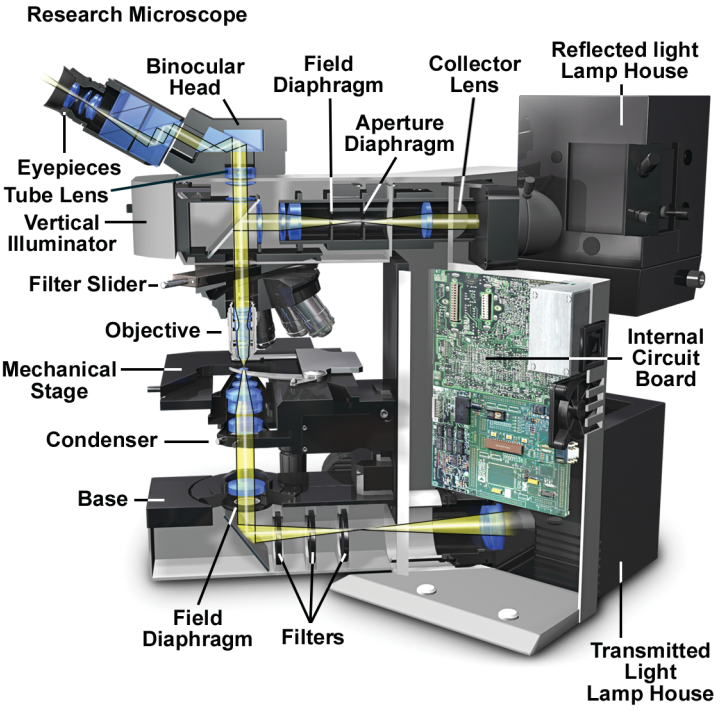
Fig 8. Infinity-corrected objective system. (diagrammatic)



Some microscopes, especially those used in tissue culture, are inverted rather than upright. Such microscopes have a fixed stage. Underneath the stage opening, there is a moveable nosepiece holding the objectives. The focusing knobs move the nosepiece closer or further from the specimen. Above the stage and specimen, there is a moveable condenser and a light source. The principles of functioning and the setting up of appropriate illumination are essentially the same as those for the upright microscope.

First an overview of the main parts of the microscope. Later, an elaboration of the details.


Eyepiece Lenses
Beamsplitters and Prisms
Clinical Microscope
Tube Lens
Objective Lenses
Condenser Lenses
Field Lens
OLYMPUS
CX31


Research Microscope
Binocular Head
Field Diaphragm
Collector Lens
Reflected light Lamp House
Aperture Diaphragm
Eyepieces
Tube Lens
Vertical Illuminator
Filter Slider
Objective
Internal Circuit Board
Mechanical Stage
Condenser
Base
Field Diaphragm
Filters
Transmitted Light Lamp House

Optical Components

The most important optical component of the microscope is the OBJECTIVE.

1. Its basic function is to gather the light passing through the specimen and then to project an accurate, real, inverted IMAGE of the specimen up into the body of the microscope.

2. Other related functions of the objective are to house special devices such as an iris for darkfield microscopy, a correction collar for counter-acting spherical aberration (more of this later), or a phase plate for phase contrast microscopy.

3. The objective must have the capacity to reconstitute the various points of the specimen into the various corresponding points in the image, sometimes called the "anti-points".

4. The objective must be constructed so that it will be focused close enough to the specimen so that it will project a magnified, real image up into the microscope.

5. The higher power objectives should have a retractable front lens housing to protect the front lens where the objective requires focusing very close to the specimen.

6. To the extent possible, corrections for lens errors (aberrations) should be made within the objective itself.

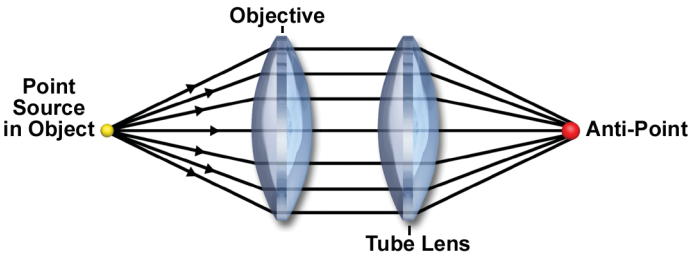


Fig 9. The image of a point, formed by a lens, is never a point, but a circular disk (Airy disk) of definite diameter, known as the anti-point.

A second important optical component is the EYEPIECE.

1. Its basic function is to "look at" the focused, magnified real image projected by the objective (and tube lens in infinity-corrected systems) and magnify that image a second time as a virtual image seen as if 10 inches from the eye.

2. In recording, a Photoeyepiece "picks up" the real image projected by the objective a second time as a real image able to be captured by a camera.

3. The eyepiece houses a fixed diaphragm. It is at the plane of that fixed diaphragm that the image projected by the objective will be "seen".

4. On the shelf of the fixed diaphragm, the eyepiece can be fitted with scales or markers or pointers or crosshairs that will be in simultaneous focus with the focused image.

The third important optical component is the SUBSTAGE CONDENSER.

1. Its basic function is to gather the light coming from the light source and to concentrate that light in a collection of parallel beams (from every azimuth) onto the specimen.

2. The light gathered by the condenser comes to a focus at the back focal plane of the objective (later, the explanation of this term).

3. In appropriately set up illumination, it is arranged that the image of the light source, comes to focus at the level of the built-in variable aperture diaphragm of the substage condenser (the front focal plane of the condenser).

4. Correction for lens errors are incorporated in the finest condensers, an important feature for research and photography.

5. Where desired, the condenser can be designed to house special accessories for phase contrast or differential interference or darkfield microscopy.

Other optical components:

1. The base of the microscope contains a COLLECTOR LENS. This lens is placed in front of the light source. Its function is to project an image of the light source onto the plane of the condenser's aperture diaphragm. In some instruments a diffusion or frosted filter is placed just after the collector lens (side closer to the specimen) in order to provide more even illumination.

2. Also in the base of the microscope, under the condenser, is a FIRST SURFACE MIRROR (silvered on its front surface only). Its function is to reflect the light coming from the lamp up into the substage condenser. Just before that mirror (closer to the lampside) is another variable diaphragm known as the field diaphragm.

3. At the lowest part of the observation tubes (binocular or trinocular) there is incorporated a TUBE LENS. Its function is to gather the parallel rays of light projected by the objective (in infinity-corrected systems) and bring those rays to focus at the plane of the fixed diaphragm of the eyepiece. In the instruments of some manufacturers, the tube lens is built into the body of the microscope itself.

Bear in mind for later elaboration the important diaphragms: the variable aperture iris diaphragm in the condenser, the variable field diaphragm in the base of the microscope and the fixed diaphragm in the eyepiece.

Mechanical/Electrical Components

The STAND of the microscope houses the mechanical/electrical parts of the microscope. It provides a sturdy, vibration-resistant base for the various attachments.

1. The BASE of the Olympus microscopes is Y-shaped for great stability. It houses the electrical components for operating and controlling the intensity of the lamp. The lamp may be placed, depending on the instrument, at the lower rear of the stand or directly under the condenser fitting. The base also houses the variable field diaphragm. The base may also have built in filters and a special circuit for illumination intensity for photomicrography.

2. Built into the stand is a fitting to receive the microscope STAGE. The stage has an opening for passing the light. The specimen is placed on top of the stage and held in place by a specimen holder. Attached to the stage are concentric X-Y control knobs which move the specimen forward /back or left/right.

3. On the lower right and left side of the stand are the concentric COARSE and FINE FOCUSING KNOBS. These raise or lower the stage in larger / smaller increments to bring the specimen into focus.

4. Under the stage there is a built-in ring or a U-shaped CONDENSER HOLDER. This holder receives any one of several types of condenser. The holder has a tightening screw to hold the condenser in place and may have 2 small knobs (at 7 o'clock and 5 o'clock positions) for centering the condenser to the optical axis of the microscope in Koehler Illumination (explained later). Adjacent to the condenser holder there are either one or two knobs for raising or lowering the condenser.

5. Above the stage, the stand has a NOSEPIECE (may be fixed or removable) for holding the objectives of various magnifications. The rotation of the nosepiece can bring any one of the attached objectives into the light path (optical axis). The nosepiece may also have a slot for special attachments.

6. Removable OBSERVATION TUBES, either binocular or trinocular, are attached to the stand above the nosepiece. The binocular is used for viewing and the trinocular is used for viewing and /or photography. The observation tubes are usually set at approximately a 30 degree angle for comfortable viewing and may be tiltable or telescoping push-pull for greater flexibility. The bottom of the observation tube holds a special lens called the TUBE LENS. The tube lens has the function of gathering the parallel beams projected by the objective and bringing the image to focus at the level of the eyepiece diaphragm (intermediate image plane). On the instruments of some manufacturers (not Olympus) the tube lens may complete optical corrections not made in their objectives.

To Sum up:

The microscope stand has the following functions:

1. To insure stability and rigidity of the microscope.
2. To provide the frame for holding the objectives and eyepieces at opposite ends of the stand.
3. To make it possible, by means of adjustment knobs, to bring the specimen into focus.
4. To hold the specimen on a flat surface stage and to be able to move the specimen on that surface.
5. To carry the moveable substage condenser which receives the light deflected by the built-in mirror and transmits that light up through the specimen.
6. To hold the lamp and the electrical controls to operate the lamp and control its brightness.

The key characteristics of modern microscopes are their MODULARITY and their ERGONOMICS.

Various components are readily interchangeable, e.g. condensers, objectives, eyepieces, stages, lamps, observation tubes.

The stage controls are concentric; the focusing knobs are concentric and placed close to the table top for ready access for the user without need to raise arms or hands. The front base is narrow to allow easy reach to accessories on the tabletop. The angle of viewing enables long periods of observation without undue tiring. Observation tubes may be tiltable for various angles of view and push-pull for greater comfort. The nosepiece is designed so that, when the desired objective is in the light path, the other objectives are not in the way of the user's fingers.

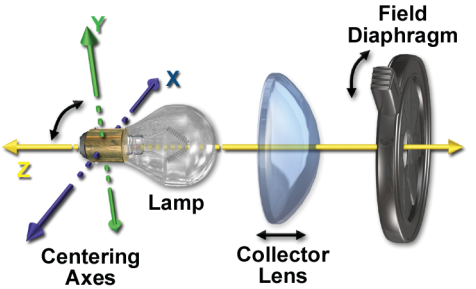


Fig 10. Microscope illuminator. The essential elements of the illuminator are the lamp, a collector lens, and the field diaphragm. The diaphragm is adjustable.

Illumination

Since specimens rarely generate their own light, illumination is usually furnished by means of a built-in lamp. The light beams pass through the substage condenser after deflection by a built-in mirror. The light transmitted by the condenser then passes through the specimen on the stage, into the objective, thus illuminating the specimen. If the lamp is of high intensity (tungsten-halogen), its brightness is controlled by a built-in or separate transformer.

Light

Light travels in straight lines (this interpretation is sometimes called geometric optics). Its path can be deflected or reflected by means of mirrors or right angle prisms. Light can be "bent" or refracted by means of glass lenses that are thicker or thinner at their center or their periphery.

Light travels at different speeds in air and in glass (faster in air which is usually taken as the standard of 1). Light is slowed and "bent" or refracted when it passes through air and enters a convex lens. Thus light is refracted when it enters a convex lens from air; refracted when it leaves the convex lens and reenters air; refracted when it passes from air through oil; or from oil through air. Oil has a refractive index of 1.515 as does common glass. The refractive index of a vacuum is 1 and air has a refractive index of 1.00+.

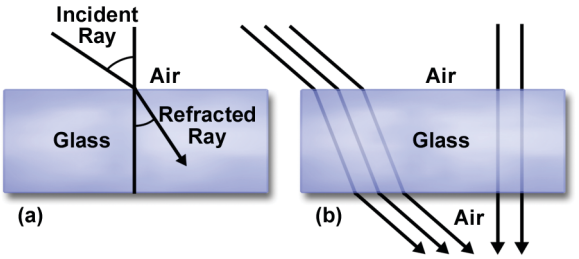


Fig 11. a. Snell's Law. Refraction of a light ray at a glass surface. b. A light ray is laterally "bent" by a sheet of glass unless it passes perpendicularly through it.

Refractive index is the ratio of the speed of light in a vacuum compared to the speed of light in a medium. Thus, except for light traveling in a vacuum, the refractive index is always greater than 1.

When light at an angle, other than 90°, passes from a less dense medium (e.g. air) into a more dense medium (e.g. glass), the rays are "bent" toward the perpendicular. When light at an angle, other than 90°, passes from a more dense medium (e.g. glass), into a less dense medium (e.g. air), the rays are "bent" away from the perpendicular. When light passes from glass into air, if the angle is too great, (critical angle), the rays do not emerge but are totally internally reflected. When light passes from glass (refractive index 1.515) into immersion oil (refractive index also 1.515), the rays are not refracted since the refractive indices are identical.

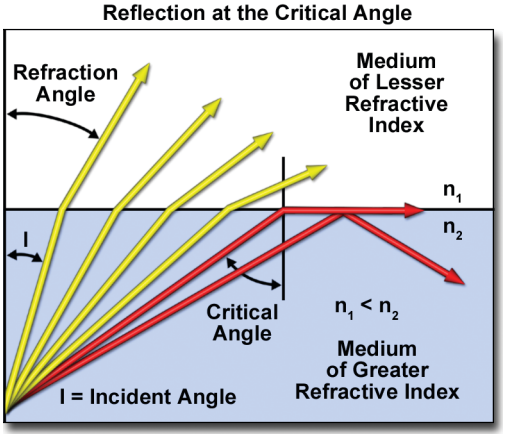


Fig 12. Reflection at the critical angle.

Another explanation of the nature of light is that it is made up of very small waves (wave optics) vibrating at right angles to the direction of the light's travel path. Light is composed of visible and invisible waves. The frequencies (number of vibrations per second) of visible light are represented by the familiar spectrum or rainbow, from violet to red. The violet end of the spectrum has higher frequencies and shorter wave lengths; the red end of the spectrum has lower frequencies and longer wavelengths.

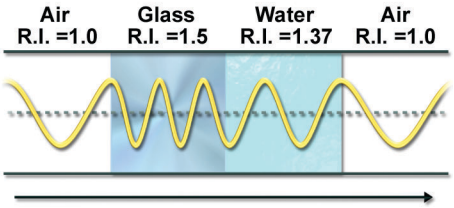


Fig 13. Variation of wavelength with light velocity.

Invisible to the eye, parts of the spectrum that may be of use in microscopy are the ultra-violet (shorter wavelengths than violet) and infra-red (longer wavelengths than red).

It will be seen later in this booklet how the microscope uses the fundamentals of illumination and light in making possible excellent image rendition of the observed specimen.

REMEMBER: It is the enlarged IMAGE of the specimen, NOT the specimen itself that is seen or recorded.

PART TWO

The Significant Details

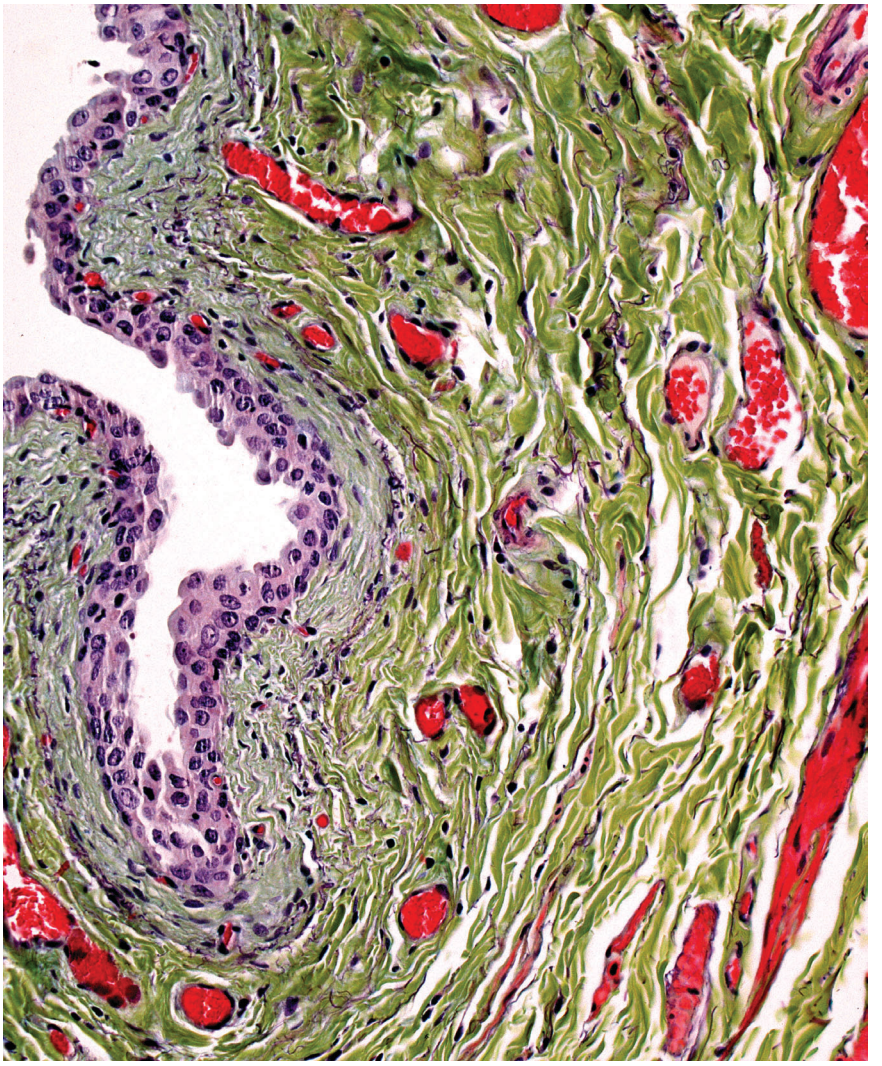


Photo: Epidermis Tissue Thin Section

The Microscope Stand

Just a few additional details on the microscope stand:

The sensitivity of the fine adjustment knob is calculated by the size of the interval of motion as the knob is turned clockwise or counter-clockwise. In basic school instruments, this interval is usually .002mm or 2 microns; on the better clinical and research instruments, the sensitivity of the fine adjustment is .001 mm or 1 micron or better.

The microscope stage may be rectangular or circular. It is more useful if the stage can be rotated either for viewing or image capture or for special contrast techniques.

The microscope observation tubes may be binocular, trinocular, or monocular. For comfortable viewing, binocular tubes produce less eyestrain. For photomicrography or video imaging, a trinocular (one of its three tubes is upright for transmission of light to an imaging device) offers the greatest convenience, since a simple movement of a lever redirects the light or a portion of the light to the camera. Thus, in a trinocular microscope, it is possible to simultaneously view the specimen and to record the image. In modern microscopes, the observation tubes are removable and interchangeable.

Present day binocular observation tubes are constructed at an angle (about 30°) for viewing ease. Some variations of these tubes may be tiltable (from 0° to 30°) and even ergonomically-telescoping push-pull closer or further from the viewer. The horizontal distance between the eyepiece sleeves is adjustable to fit the interpupillary distance of the user's eyes. There also may be a rotatable, knurled ring on one of the sleeves to allow adjustment for individual eye acuity. (See appendix for instructions on how to focus a microscope).

Modern microscopes have ergonomically low-positioned coarse and fine adjustment knobs; these are concentrically positioned. X-Y stage controls are available for either right-handed or left-handed users.

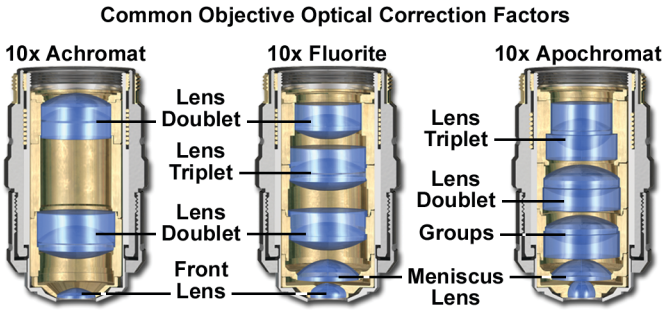
Objectives, Eyepieces, Condensers

Objectives

Objectives are the most important components of the microscope. Modern objectives, made up of many glass elements, have reached a high state of quality and performance. The extent of corrections for lens errors (aberrations) and flatness of the image field determines the usefulness and cost of the objective.

The least expensive objectives are the achromatic objectives. These are corrected chromatically to bring red and blue light to a common focus. Further, achromats are corrected for spherical aberration (see later explanation) for the color green. Thus, achromats yield their best results with light passed through a green filter and, when employed for black/white imaging. If an objective is not otherwise labeled, you can assume it is an achromat.

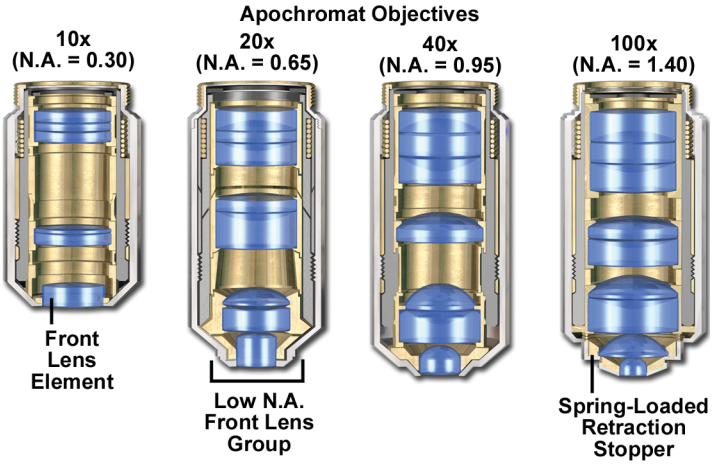
Fig 14. Lens complexity of common objectives as a function of optical correction factors: achomat, fluorite, and apochromat.



The next higher level of correction and cost is found in objectives called fluorites or semi-apochromats. These objectives are also corrected chromatically for red and blue light, usually also closer to the green focus. The fluorites are corrected spherically for two colors, blue and green. Hence fluorite objectives (their lens elements traditionally contain natural or synthetic fluorite) are better suited than achromats for color photomicrography or recording in white light.

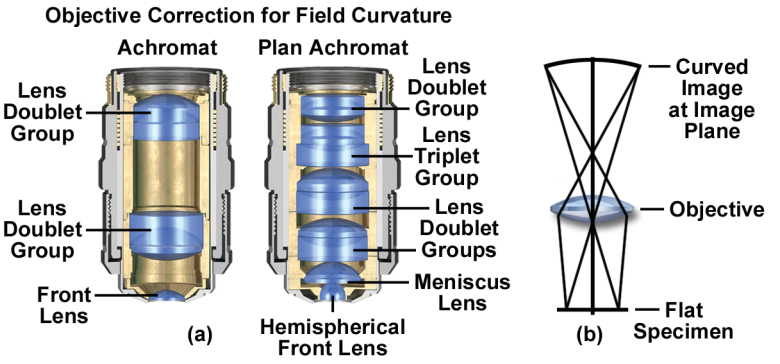
The highest level of corrections (and expense) is found in apochromatic objectives. These objectives are corrected chromatically for four colors, deep blue, blue, green, and red; they are spherically corrected for two or three colors: deep blue, blue and green. Apochromats are the best objectives for color recording and viewing. Because of their high level of correction, such objectives have, for a given magnification, higher numerical apertures (see later explanation of this term) than do achromats or fluorites.

Fig 15. Apochromat objective lens complexity at increasing magnification.



All three types of objectives project images that are curved rather than flat. To overcome this inherent condition, lens designers have produced objectives which yield flat images across the field of view. Such objectives are called plan-achromats, plan-fluorites, or plan-apochromats and are labeled plan on the objective itself. The plan correction is invaluable for image recording.

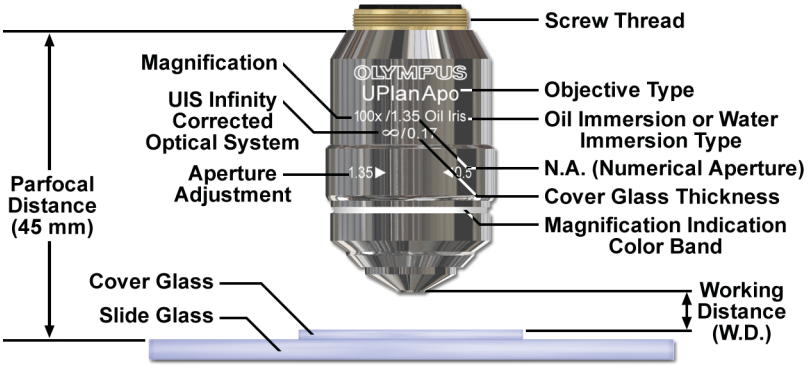
Fig 16. Correction of objectives for curvature of field. a. Lens arrangement in uncorrected and corrected objectives. b. Curvature of field ray-trace diagram.



Each objective has inscribed on it the magnification (e.g. 10X, 20X, etc.); the insignia "∞" for infinity-correction; the thickness of the cover glass, covering the specimen, which was assumed by the lens designer (usually 0.17 mm). Also the level of correction if it is a fluorite or an apochromat. If the objective is designed to operate in a drop of oil between its front lens and the specimen, it will be labeled OIL or HI (homogeneous immersion) or OEL. If these latter labels are not on the objective, it is a so-called "dry objective" meant to operate with air between its front lens and the specimen.

Objectives also always have the inscription for numerical aperture, N.A. (see later explanation of this term). The numerical aperture may vary from 0.04 for low power objectives to 1.4 for high power oil plan-apochromats.

Fig 17. Standard objective nomenclature.



Objectives are also inscribed with a color ring to enable the user to easily identify the magnification-red for 4X, yellow for 10X, green for 20X, blue for 40X or 60X, white for 100X.

Immersion color code [a]	Immersion type
Black	Oil
Orange	Glycerol
White	Water
Red	Special

Magnification color code [b]	Magnification
Black	1x, 1.25x
Brown	2x, 2.5x
Red	4x, 5x
Yellow	10x
Green	16x, 20x
Turquoise blue	25x, 32x
Light blue	40x, 50x
Cobalt (dark) blue	60x, 63x
White (cream)	100x

a Narrow colored ring located near the specimen of objective.

b Narrow band located closer to the mounting thread than the immersion code.

Some objectives, usually the higher power “dry” objectives of 40-60X magnification are fitted with a correction collar Since these objectives are particularly susceptible to incorrect thickness of the cover glass covering the specimen, the rotation of the correction collar can compensate for cover glasses thicker or thinner than 0.17mm.

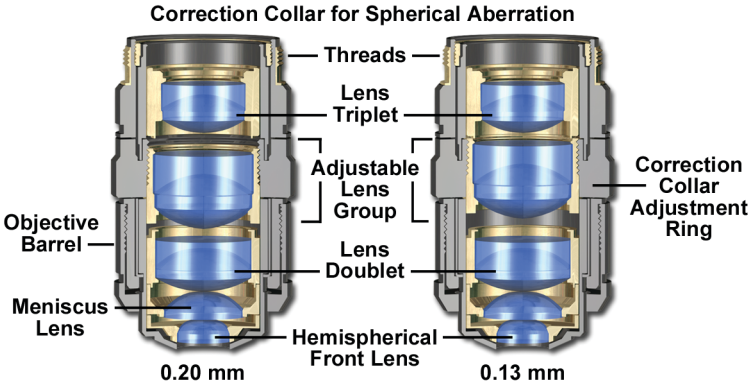


Fig 18. Objective correction collar to eliminate or reduce spherical aberration.

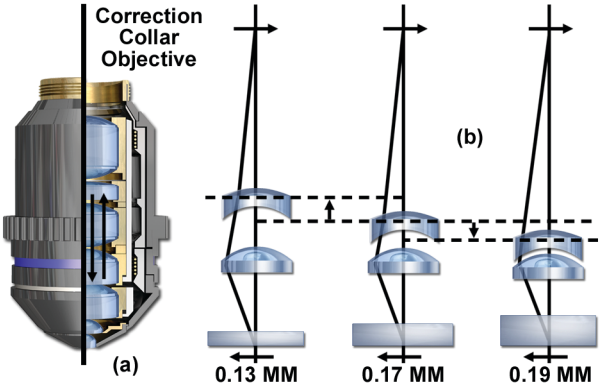
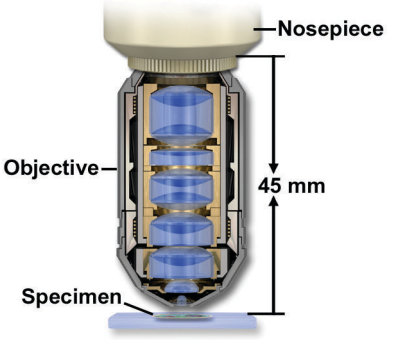


Fig 19. a. 4mm objective of 0.95 N.A. with correction collar marked in terms of coverslip thickness. b. Action of a correction collar (diagrammatic).

When a manufacturer's set of similar objectives, e.g. all achromatic objectives of various lengths and magnifications, are mounted on the nosepiece, they are usually designed, along with the tube lens, to project the image to approximately the same plane, the fixed diaphragm of the eyepiece. Thus changing objectives, by rotating the nosepiece, requires only minimal use of the fine adjustment knob for refocusing. Such a set of objectives is described as being parfocal-a useful convenience and safety feature. Sets of objectives are also designed to be parcentric, that is, a feature of the specimen which is centered in the field of view remains centered when the nosepiece is rotated to change objectives.

Fig 20. Parfocalizing distance.



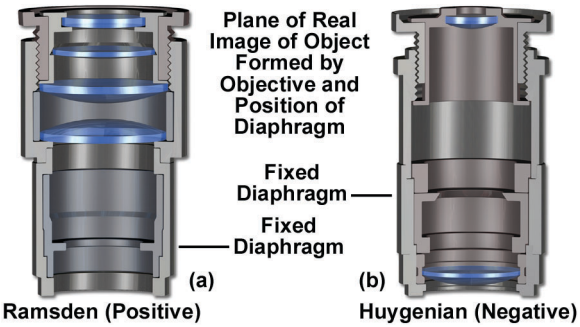
For Olympus infinity-corrected objectives, corrections for chromatic aberration and for spherical aberration are made in the objectives themselves.

Eyepieces

Best results require that objectives be used in combination with eyepieces that are appropriate to the correction and type of objective. There are two main kinds of eyepieces: Ramsden and Huygenian.

In the Ramsden type (sometimes referred to as positive eyepieces) the lenses, often several elements cemented together, are situated above the fixed diaphragm or opening of the eyepiece. It is usually easy to unscrew the lower portion of the eyepiece for dropping a reticle (glass discs with various rulings or markings) or pointer on the shelf of the fixed diaphragm. These devices will be in focus simultaneously with the focused image. The eyepieces are currently well-corrected to function optimally with the infinity-corrected objectives.

Fig 21. a. Ramsden Eyepiece, b. Huygenian Eyepiece. Both illustrated in longi- section.



In Huygenian (called negative eyepieces), there are two lenses, the upper or eye lens and the lower or field lens. In their simplest form both lenses are plano-convex, with convex side facing the specimen. Approximately midway between these lenses is a fixed circular opening or diaphragm which, by its size, defines the circular field of view that is observed when looking into the microscope. These will be found on some older microscopes and often completed corrections which were not made in some of the former series of objectives.

Inscribed on the eyepiece are its magnification and its field number, which is the diameter in millimeters of the diaphragm opening of the eyepiece. Depending on the eyepiece, the diaphragm opening may vary from as low as 18 mm to 26.5 mm.

Condensers

The substage condenser is fitted below the stage of the microscope, between the illumination lamp and the specimen. Condensers are manufactured according to different levels of correction needed.

The simplest and least well-corrected condenser is the Abbe condenser, numerical aperture up to 1.25. While the Abbe condenser is capable of passing bright light, it is not well-corrected chromatically or spherically. As a result, the Abbe is most suitable for routine observation with objectives of modest numerical aperture and correction.

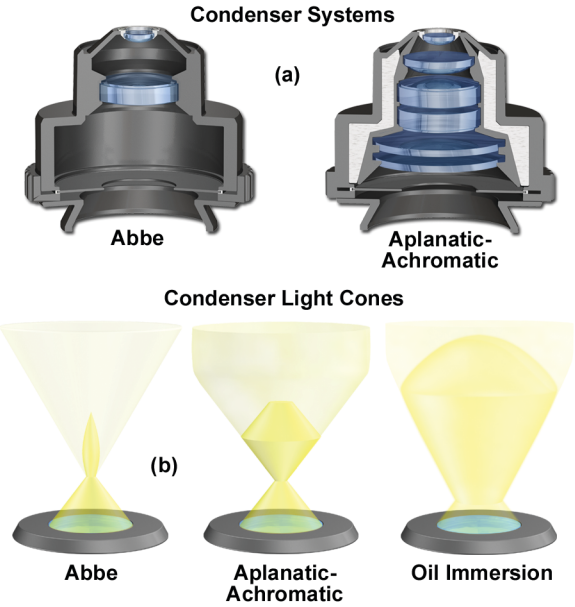


Fig 22. a. Abbe and aplanatic-achromatic condenser systems. b. Cones of light transmitted.

The best level of correction in condensers is found in the aplanatic-achromatic condenser. Such a condenser is well-corrected for chromatic aberration and spherical aberration. It is the condenser of choice for use in color observation and recording in white light.

The engraving on the condenser includes its numerical aperture and its correction, if aplanat-achromat. Condensers with a numerical aperture above 1.0 perform best when a drop of oil is applied to their upper lens and is brought into contact with the underside of the slide.

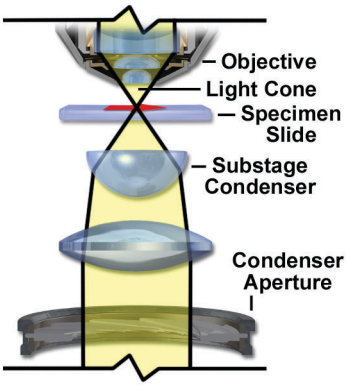


Fig 23. Cone of illumination. The substage condenser must be focused and the diaphragm adjusted so that the cone of illumination completely fills the aperture of the microscope objective.

The condenser aperture and the proper focusing of the condenser are of critical importance in realizing the full potential of the objective in use. Likewise, the appropriate use of the adjustable aperture iris diaphragm (incorporated in the condenser or just below it) is most important in securing excellent illumination and contrast. The opening and closing of the aperture iris diaphragm controls the angle of the illuminating rays which pass through the condenser, through the specimen and into the objective.

For low power objectives (4X or below), it may be necessary to unscrew the top lens of the condenser or to use a condenser with a flip-top upper lens. Special low power condensers are also available. Specialty condensers are available for darkfield microscopy, for phase contrast, polarized light, and for interference microscopy.

The height of the condenser is regulated by one or a pair of condenser knobs which raise or lower the condenser. This adjustment is described later in the section entitled Koehler illumination.

Light

Knowledge of the behavior of light and the effects resulting when light passes from air through a glass convex lens and out into air again is fundamental to the understanding of image formation. When light passes from air into a convex lens, the speed of light is slowed. The various colors, differing in wave length, are slowed at different rates (dispersion). This bending (refraction) effect differs for different colors. Those rays which strike the central area of the lens at a perpendicular emerge unrefracted. Light passing through the other parts of the convex lens are refracted or “bent”. The “blue rays” are bent more than the “green rays”, more than the “red rays.”

When white light passes through convex lenses of objectives, eyepieces, or condensers, two main kinds of aberrations may occur; chromatic aberration and/or spherical aberration. These aberrations can be corrected in the design of the lenses.

a) **CHROMATIC ABERRATION**

The various color frequencies (wave lengths) of white light pass through an uncorrected convex lens and, instead of being brought to a common focus, come to different foci. The lens designer strives, by combining various kinds of glass and lens elements of different shapes, to bring the main colors of red, green, and blue to a common focus.

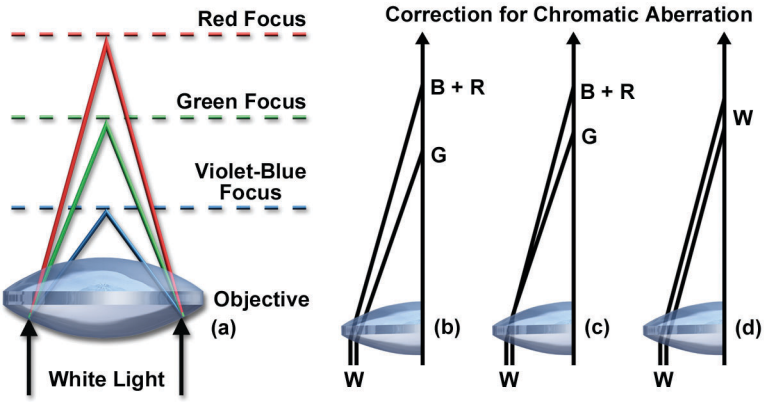


Fig 24. a. Chromatic aberration of white light. Failure of a simple lens to bring light of different wavelengths to a common focus. b. Achromatic lens. Green is brought to the shortest focus. The color error is much reduced. c. Fluorite lens. The color error is similar to (b) but still further reduced. d. Apochromatic lens. For all practical purposes chromatic aberration may be considered eliminated.

b) **SPHERICAL ABERRATION**

Light passing through an uncorrected convex lens will be brought to different foci depending upon whether the light passes through nearer the center of the lens or closer to the periphery. Lens designers correct this kind of zonal aberration by using lens elements of different shapes to bring the more central and more peripheral rays to common focus.

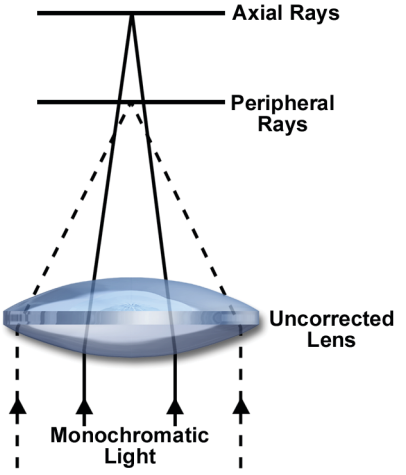


Fig 25. Spherical aberration. Failure of the lens system to image central and peripheral rays at the same focal point arises with spherical lenses. Optical correction is possible, but care must be taken not to introduce additional spherical aberration when setting up the microscope.

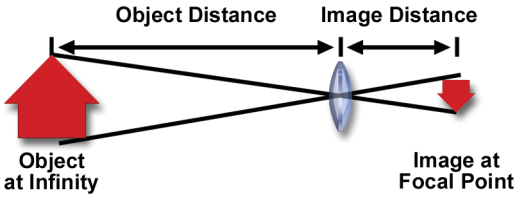
In image formation, light from all the illuminated points of the specimen passes through the objective which then, with the aid of the tube lens, reconstitutes the rays into an image. The finer and more accurate this reconstitution, the clearer the image will be.

To understand how the microscope's lenses function, you should recall some of the basic principles of lens action in image formation:

CASE 1

Light from an object that is very far away from the front of a convex lens (we'll assume our "object" is a self-lighted arrow) will be brought to a focus at a fixed point behind the lens. This is known as the FOCAL POINT of the lens. We are all familiar with this idea of a "burning glass" which can focus the essentially parallel rays from the sun to burn a hole in a piece of paper. The vertical plane in which the focal point lies is the FOCAL PLANE. The distance from the center of the idealized simple convex lens to the focal plane is known as the FOCAL DISTANCE. (For an idealized thin convex lens, this distance is the same in front of or behind the lens.) The IMAGE of our arrow now appears at the focal plane. The IMAGE is smaller than the object (arrow); it is inverted; it is a real image capable of being captured on film or another imaging device, e.g. a CCD camera. This is the case for the camera used for ordinary scenic photography.

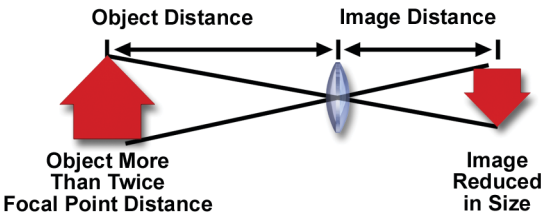
Fig 26. Object at infinity. Image reduced in size.



CASE 2

The object is now moved closer to the front of the lens but is still more than two focal lengths in front of the lens. Now, the image is found further behind the lens. It is larger than in case 1 but is still smaller than the object. The image is inverted, and is a real image. This is the case for ordinary portrait photography.

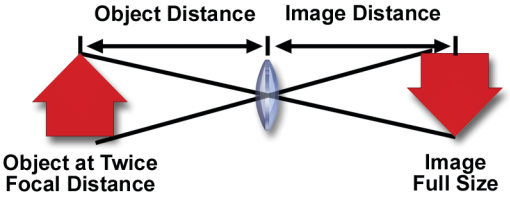
Fig 27. Object at a distance more than twice the focal length. Image reduced in size.



CASE 3

The object is brought to twice the focal distance in front of the lens. The image is now two focal lengths behind the lens. It is the same size as the object; it is real and inverted. This is the case for so-called 1 to 1 photography.

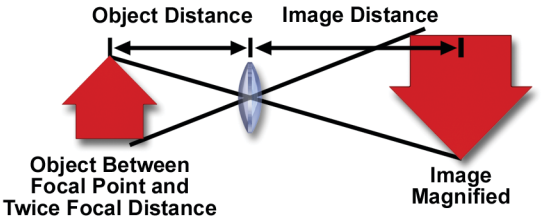
Fig 28. Object at twice the focal length. Image at full size.



CASE 4

The object is situated between one and two focal lengths in front of the lens. Now the image is still further away from the back of the lens. This time, the image is magnified and is larger than the object; it is still inverted and it is real. Case 4 describes the functioning of all finite tube length objectives used in microscopy. Such finite tube length (see appendix) objectives project a real, inverted, magnified image into the body tube of the microscope, designed to come to focus at the plane of the fixed diaphragm in the eyepiece.

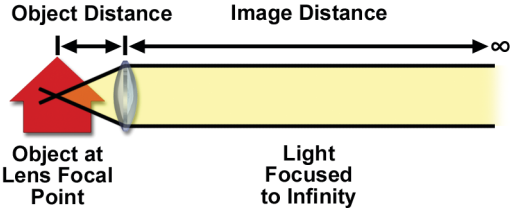
Fig 29. Object closer than twice the focal length. Magnified image.



CASE 5

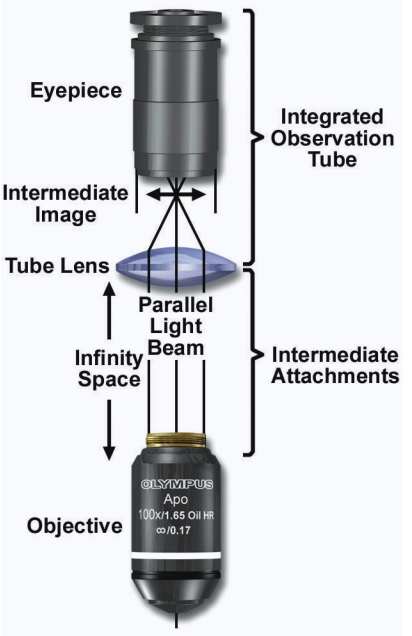
The object is situated at the front focal plane of the convex lens. In this case, the rays of light emerge from the lens in parallel. When brought to focus by the eye, the image, on the SAME side of the lens as the object, appears upright. It is a virtual image and appears as if it were 10 inches from the eye, similar to the functioning of a simple magnifying glass; the magnification depends on the curvature of the lens. This case describes the functioning of the observation eyepiece of the microscope. The “object” looked at by the eyepiece is the magnified, inverted, real image projected by the objective. When the human eye is placed above the eyepiece, the lens and cornea of the eye “look” at this secondarily magnified virtual image and see this virtual image as if it were 10 inches from the eye, near the base of the microscope.

Fig 30. Object distance equal to the focal length. Light focused to infinity.



This case also describes the functioning of the now widely-used infinity-corrected objectives. For such objectives, the object or specimen is positioned at exactly the front focal plane of the objective. Light from such a lens emerges in parallel rays in every direction. In order to bring such rays to focus, the microscope body or the binocular observation head (as in the new Olympus UIS optics) must incorporate a TUBE LENS in the light path, between the objective and the eyepiece, designed to bring the image formed by the objective to focus at the plane of the fixed diaphragm of the eyepiece. The magnification of an infinity-corrected objective equals the focal length of the tube lens (for Olympus equipment this is 180mm; other manufacturers use other focal lengths) divided by the focal length of the objective lens in use. For example, a 10X infinity-corrected objective, in the Olympus series, would have a focal length of 18mm (180/10).

Fig 31. Infinity space.



Basic Principles

Light in parallel beams entering a convex lens will emerge focused at the back focal plane of the lens.

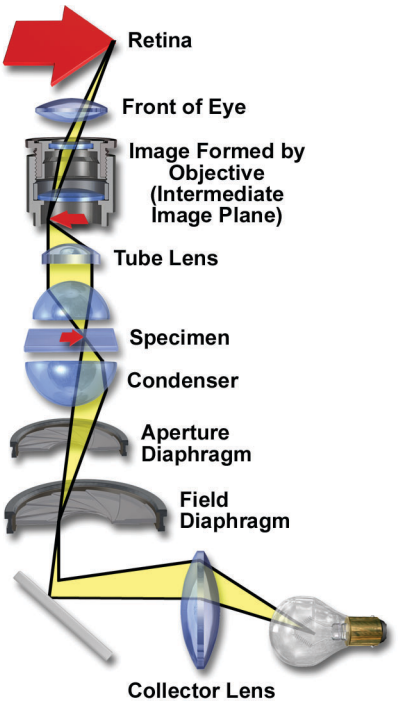
Light focused at the front focal plane of a convex lens will emerge from the lens in parallel beams.

ILLUMINATION

All too frequently, sophisticated and well-equipped microscopes fail to yield excellent images because of incorrect use of the light source and the substage equipment. Illumination of the specimen should be bright, glare-free and evenly dispersed in the field of view. Since modern microscopes achieve such excellence by use of Koehler illumination (named after its discoverer, August Koehler), this description will deal with the principles and steps in achieving Koehler illumination.

There are several physical-mechanical requirements. The substage condenser must be capable of being focused up and down, preferably by knobs operating on a rack and pinion. The substage condenser must be equipped with a variable aperture iris diaphragm that can be opened or closed by a lever or knurled ring. The microscope base must be fitted with a collector lens and must contain a variable field diaphragm positioned closer to the in-base first surface mirror. The lamp itself should either be prefocused or centerable.

Fig 32. Image-forming ray paths are traced from two ends of the lamp filament. Conjugate foci are the field diaphragm, specimen plane, intermediate image plane (entrance pupil of the eyepiece), the human eye or with camera in place, the film plane.



To repeat, there are two important variable diaphragms: the aperture iris diaphragm of the condenser and the variable field diaphragm in the base. The aperture iris of the condenser controls the angular aperture of the cone of light traversing the condenser toward the specimen. The field diaphragm controls the area of the circle of light illuminating the specimen.

KOEHLER ILLUMINATION

With the exception of fluorescence microscopy, the specimen being observed does not give off its own light; it must be illuminated. In the early days of microscopy, the source of illumination was usually the daylight sky, an extended, structureless source of illumination. More practically, microscopists sought artificial sources of illumination, e.g. gas lamps, oil lamps, electric lamps of various kinds. These sources were usually focused onto the already-focused specimen by means of the substage condenser. This kind of illumination, originally called critical illumination, frequently suffered from unevenness because of the limited size of the light source or because the image of the light source was obtrusively superimposed on the image of the specimen.

In the early 1900's, August Koehler and others developed a procedure for providing bright, even illumination superbly suited for both microscopy and photomicrography. Koehler illumination is the method of choice in all modern microscopy and photomicrography and imaging for transmitted as well as reflected light techniques. The rationale for Koehler illumination is elegant but simple. A collector lens is placed in front of the light source and is designed to project an enlarged image of the light source coming to focus at the level of the variable aperture diaphragm of the substage condenser.

Since the light source is focused at the front focal plane of the condenser (approximate position of the variable condenser aperture diaphragm), the light emerging from the condenser travels through the specimen in parallel rays (see Case 5 above) from every azimuth. Since the light source is not focused at the plane of the specimen, the light at the specimen plane is essentially grainless and extended. The opening and closing of the aperture diaphragm controls THE ANGLE OF THE LIGHT CONE reaching the specimen. The parallel rays are brought to focus at the back focal plane of the objective (see Case 1); here the image of the variable aperture diaphragm and the image of the light source will be seen in focus.

A second variable diaphragm, called the field diaphragm, is placed in front of the collector lens, most often in the base or so-called light port of the microscope. Its function is to control the DIAMETER (not angle) of the light bundle passing through the specimen.

A third diaphragm is the fixed diaphragm within the eyepiece. The plane of this diaphragm is known as the intermediate image plane. It is at this plane that the image projected by the objective and tube lens comes to focus.

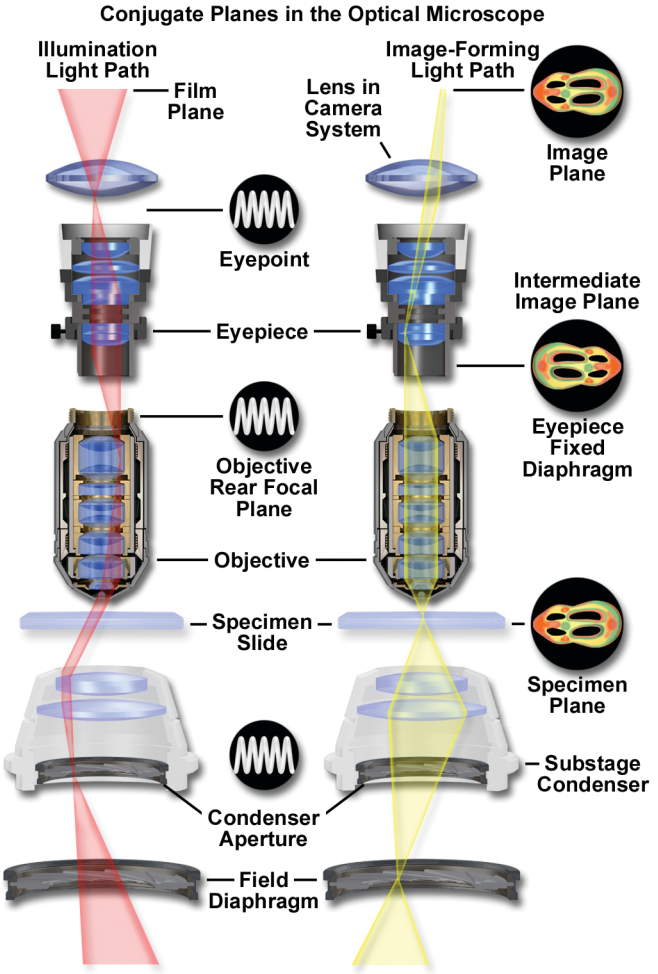
The light source itself should be precentered or centerable to the optical axis of the microscope. In practice, a frosted or diffusing filter is usually placed in front of the collector lens, but prior to the field diaphragm, to further ensure the evenness of the light.

It is the proper setting and manipulation of the two variable diaphragms, aperture and field, which are the keys to Koehler illumination—bright, even illumination yielding the best compromise between resolution and contrast.

Conjugate Planes in Koehler Illumination

It is advantageous to be able to distinguish between the successive planes in the illuminating path and in the image path in Koehler illumination. Such related planes are known as conjugate planes. By definition, in a given set of conjugate planes, what is in focus for one of the conjugate planes will also be in focus at the other conjugate planes of that set.

Fig 33. Conjugate planes and light ray paths in the compound microscope.



Conjugate planes in the ILLUMINATION path:

1. The lamp filament, burner arc, or the fiber bundle of a laser.

2. The variable condenser aperture diaphragm usually located at the front focal plane of the substage condenser.

3. The back focal plane of the objective.

4. The eyepoint (exit pupil of the microscope or so-called Ramsden disk) of the eyepiece, usually located less than a half-inch above the top lens of the eyepiece. This is where you place the front of your eye.

Conjugate planes in the IMAGE path:

1. The variable field diaphragm (usually in the base of the microscope).
2. The focused specimen on the microscope stage.
3. The intermediate image plane—located at the level of the fixed diaphragm of the eyepiece.
4. The retina of your eye or the film plane of an attached camera or the sensor of an imaging device, e.g. a CCD.

Setting Up Koehler illumination:

1. After switching on the lamp of the microscope, open up fully both the field diaphragm (in the light port of the microscope) and the aperture diaphragm (usually built into the substage condenser).
2. Rotate the nosepiece to bring the 10X objective into the light path. Place the specimen on the microscope stage and focus the specimen using the coarse and fine focusing knobs.
3. Close down the field diaphragm most of the way. Now raise the substage condenser (using the condenser focusing knob) and focus the image of the field diaphragm sharply onto the already-focused specimen. This image of the field diaphragm should appear as a focused polygon.
4. If the image of the field diaphragm is not centered in the field of view, use the condenser centering screws (or knobs) to center the image of the field diaphragm. Then open up the field diaphragm until it just disappears from view.
5. Next, take out one of the eyepieces and look down the tube of the microscope. As you look down the tube, open and close the aperture diaphragm of the substage condenser. You will see its image at the back focal plane of the objective. As a rule of thumb, adjust this diaphragm so that it is 2/3 to 3/4 open. This setting usually represents the best compromise between resolution and contrast. If there is a frosted or diffusion filter built into the light path in the base of the microscope, you will see an evenly lighted circle of light. If there is no such filter in the light path you will see the image of the filament of the light bulb. (A centering or phase telescope, inserted in place of the removed eyepiece, will make this adjustment easier to see.) Now replace the eyepiece.

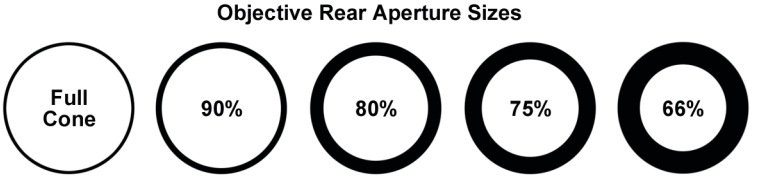


Fig 34. Appearance of light at the back lens of the objective.

6. You have now set up Koehler illumination with the 10X objective. If you wish to switch to a higher power objective, you must again adjust BOTH the field and the aperture diaphragms. For example, if you switch to the 40X objective, you will have to close the field diaphragm somewhat and may have to recenter it (looking at a smaller area of the specimen). You will also have to open up the condenser aperture diaphragm somewhat (the 40X objective has a higher numerical aperture - light-grasping ability - than does the 10X objective). Thus, every time you change objectives, you must adjust both diaphragms in accordance with the steps given above.

The condenser aperture iris diaphragm may have a calibrated scale which tells the utilized numerical aperture of the condenser. The use of this scale makes it easy to repeat a desired setting related to the numerical aperture of the objective being employed. For example, if you want to have a setting of 80% of the objective being filled with light and the numerical aperture of the objective is 0.25, you would set the iris aperture diaphragm of the condenser at 0.20. 0.20 is 80% of 0.25.

It will now be found that the specimen is well-illuminated with even, glare-free light, giving good image contrast.

The intensity of the lamp is best adjusted by using neutral density filters (reduce brightness without affecting color temperature of the light source) or, if you are not doing image recording, adjusting the voltage by means of the built-in transformer lever or knob.
Once you have set up Koehler illumination, NEVER adjust brightness by lowering the condenser position or by closing the iris aperture diaphragm.

NUMERICAL APERTURE AND RESOLUTION

If geometric optics described earlier were the sole consideration in image formation, it would be possible to secure clear magnification of many thousands of times larger than the specimen itself. However, it was discovered by optics experts of the 19th century-Abbe, Rayleigh, Airy, et alia-that other factors operate to limit useful magnification. Additional magnification that does not yield clearer detail is called “empty magnification.”

These experts recognized that, when light from the various points of a specimen passes through the objective and is reconstituted as the image, the various points of the specimen appear in the image as small disks (not points) known as Airy disks. This phenomenon is caused by diffraction or scattering of light as it passes through the minute parts and spaces in the specimen and the circular back of the objective The Airy disks, at the plane of the image, consist of small concentric light and dark circles. The smaller the Airy disks projected by the objective in forming the image, the finer the detail of the specimen discernible because the disks are less likely to overlap one another. Objectives of better correction and higher numerical aperture (more of this follows later) produce smaller Airy disks than objectives of lesser correction and lower numerical aperture. The ability to distinguish (separate) clearly minute details lying close together in the specimen is known as resolving power.

Fig 35. Airy disk diffraction image of a point.

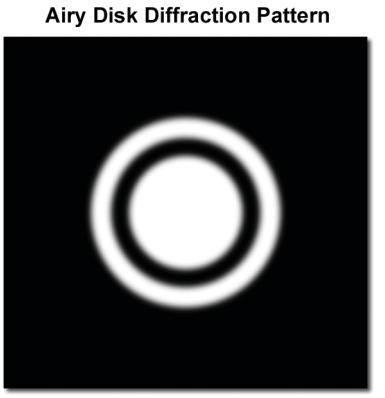


Fig 36. Decrease in size of the anti-point with increase in the numerical aperture of the lens. (diagrammatic)

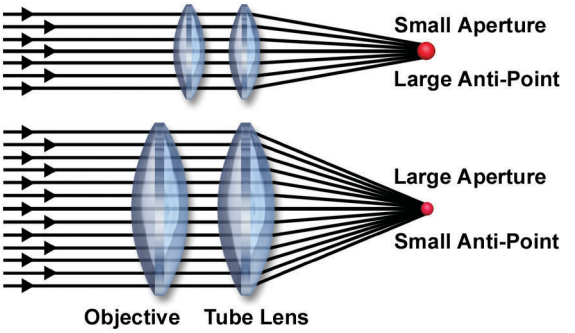
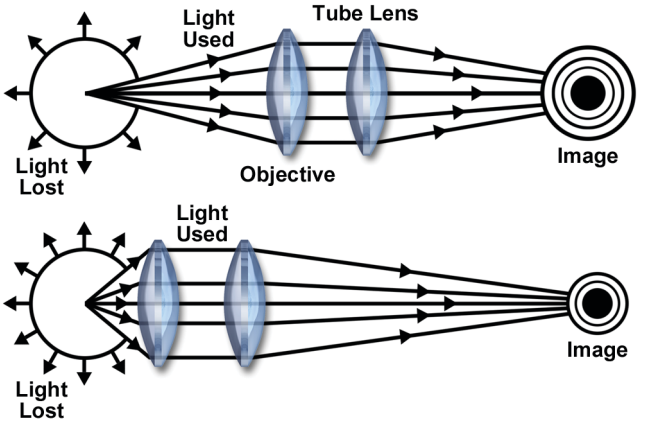


Fig 37. Decrease in size of Airy disks accompanying an increase of numerical aperture. (diagrammatic)



The phenomenon of diffraction and the limiting effect of the size of light waves dictate the “rule of thumb”, that the useful magnification of an objective is 500-1000X the numerical aperture of the objective. (e.g., upper limit of 250X for an objective of 0.25 numerical aperture; upper limit of 1400X for an objective with a numerical aperture of 1.4).

In achieving a desired magnification, it is generally good practice to use objectives of higher magnification accompanied by eyepieces of lower magnification (e.g., for a magnification of 200X, use an objective of 20X and an eyepiece of 10X, rather than an objective of 10X and an eyepiece of 20X.)

Now for the explanation of numerical aperture, referred to as N.A. The ability of an objective to include or “grasp” the various rays of light coming from each illuminated part of the specimen is directly related to the angular aperture of the objective. Objectives with lower angular aperture can include only a narrower cone of light as compared to objectives with higher angular aperture.

The equation for numerical aperture (N.A.) is: N.A.=n sine μ

In this equation N.A. is the numerical aperture; n is the index of refraction of the material in the object space, that is the space between the specimen and the front (lowest) lens of the objective. Sine μ is the sine of 1/2 the angular aperture of the objective. (refractive index of air is 1.00+; the refractive index of immersion oil is 1.515)

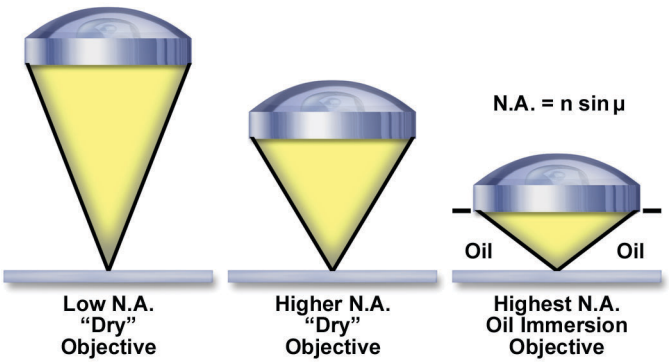


Fig 38. Numerical aperture and light gathering ability. N.A. = Numerical Aperture. n = Refractive index of medium between front lens and specimen.μ = 1/2 the angle of cone of light "captured" by the objecive.

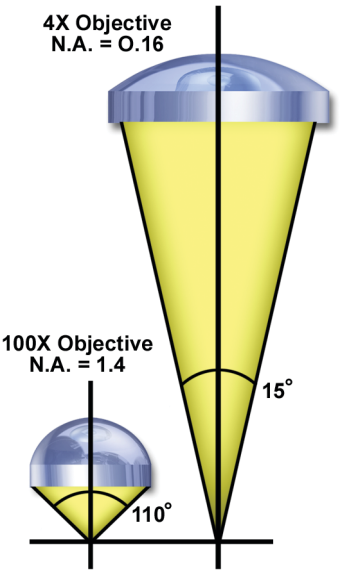


Fig 39. Angular apertures of objectives compared. The 15° narrow angle of a low power objective compared with the 110° wide angle of the high power oil immersion lens.

Study of the above equation will yield the following inferences:

- For a given angular aperture, oil immersion objectives can have higher numerical aperture since n=1.515 for oil.
- Since μ cannot exceed 90°, the sine of μ must be 1 or less. Since a "dry" objective is used with air in the object space (n for air is 1.00+), the maximum theoretical N.A. of a "dry" objective is 1; in practice, not more than 0.95.
- Increasing the angular aperture of an objective increases μ and thus increases sine μ and thus increases numerical aperture.
- Since immersion oil has a refractive index of 1.515, it is theoretically possible to utilize oil immersion objectives which can yield a numerical aperture of 1.5; in practice, not more than 1.4.

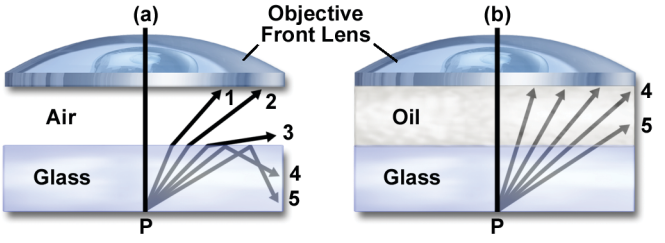
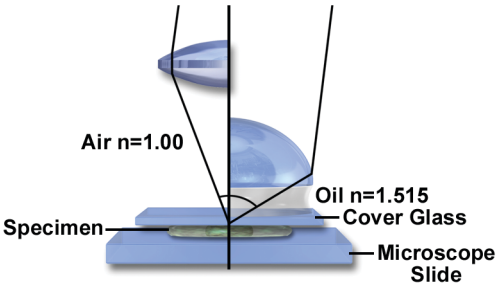


Fig 40. The principle of oil immersion. In a, five rays are shown passing from the point P in the object through the coverslip into the air space between the latter and the lens. Only rays 1 and 2 can enter the objectives. Rays 4 and 5 are totally reflected. In b, the air space is replaced by oil of the same refractive index as glass. The rays now pass straight through without deviation so that rays 1, 2, 3, and 4 can enter the objective. The N.A. is thus increased by the factor n, the refractive index of oil.

Fig 41. Comparison of a dry with an oil immersion objective.



And now, the important relationship between numerical aperture and resolving power. Resolving power has been defined as the ability of an objective to separate clearly two points or details lying close together in the specimen. Resolution has been defined as the actual (rather than theoretical) separation distance of two details lying close together still seen as separate. The equation for resolution for non-luminous objects (according to Abbe) is:

$$r = \frac{\lambda}{2N.A.} \quad \textit{for self-luminous objects (according to Rayleigh)} \quad r = \frac{0.61\,\lambda}{N.A.} \text{ or } \frac{1.22\,\lambda}{2N.A.}$$

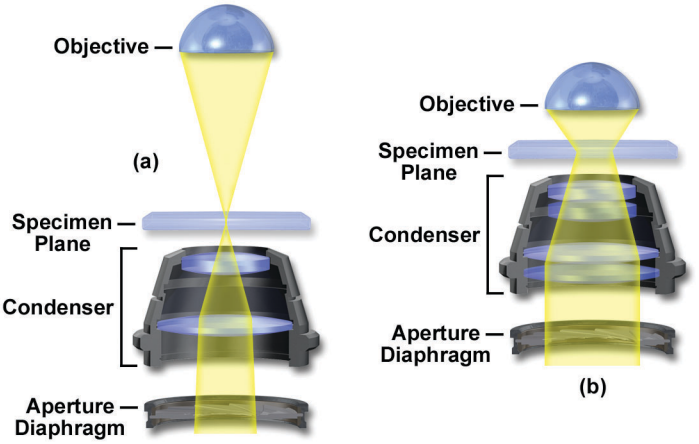
In these equations r = the size of the distance between two minute points lying close together in the specimen but still showing the points as separate; λ is the wavelength of light being used; N.A. is the numerical aperture of the objective.

Analysis of these equations will lead to the following inferences:

As N.A. increases, r becomes smaller; the size of the distance between adjacent points becomes smaller; hence resolution is better. If shorter wavelengths of light are used (e.g. violet-blue end of the spectrum) the resolvable distance becomes smaller; resolution is better. Longer wavelengths (e.g. red) yield poorer resolution. However, bear in mind that the human eye is most sensitive in the green wavelength. Resolution varies inversely with numerical aperture. Higher N.A. objectives are capable of yielding the best resolution; hence better for separating very minute details.

The numerical aperture of the entire MICROSCOPE SYSTEM depends on the N.A. of the substage condenser and the objective working together.

Fig 42. Comparison of low (a) and high (b) numerical aperture microscope systems.



$$\text{N.A. of the system} = \frac{\text{N.A of the objective + N.A. of the condenser}}{2}$$

The equation demonstrates that, for full realization of the aperture of the objective, it should be matched (but not exceeded) by the aperture of the condenser. In practice, the partial closing of the aperture iris diaphragm of the condenser reduces the working aperture of the system; the effect is to somewhat reduce resolution but to increase contrast for greater visibility.

The N.A. of the system equation also shows that, in order to realize the full aperture of the system, any condenser with a numerical aperture of more than 1.0 should have oil placed on its top lens and brought into contact with the underside of the slide. Highly corrected, high numerical aperture objectives of N.A. greater than 1 should be used with oiled condensers of N.A. greater than 1.

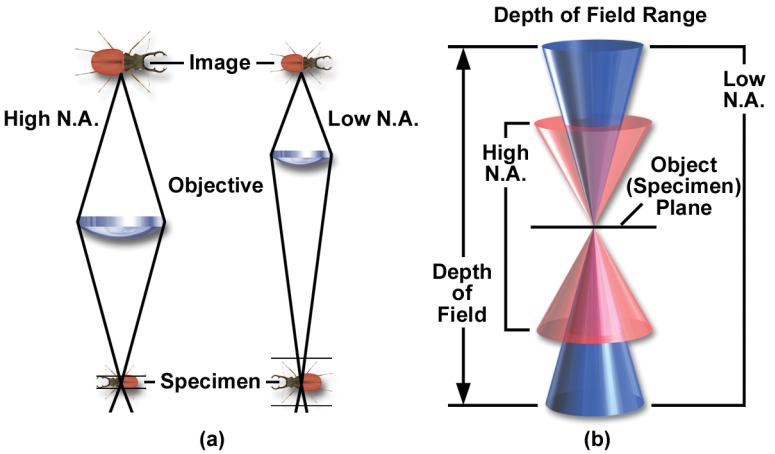


Fig 43. Comparison of low and high N.A. depth of field ranges. a. The diminution of field depth by increased N.A. in the system. b. Depth of field range in high and low N.A. objectives.

In many uses of the microscope, it may not be necessary to use objectives of high N.A. because the details of the specimen can be readily resolved with lower N.A. objectives. This may be important because high magnification, high N.A. objectives are accompanied by very shallow depth of field and short working distances from the specimen. (depth of field is the vertical distance above and below the actual plane of the focused specimen that still is in satisfactory focus). Thus in specimens where resolution is less critical and magnification can be lower, it may be better to use objectives of more modest N.A. to gain more depth of field and deeper distances between the front of the objective and the specimen.

Appendix 1

Procedure for Focusing the Microscope

1. Turn on the lamp and set the intensity for comfortable viewing.

2. While looking through the eyepieces of the binocular observation tubes, grasp the binocular tubes with both hands and bring the tubes closer together (or further apart) to fuse the two circles of light into one circle. This sets the interpupillary distance for YOUR eyes. If the viewing tubes have a scale for this setting, memorize the position so that you can readily return to it the next time.

3. Place a specimen slide on the stage. Using your RIGHT eye and your right eye Only, with the 10X objective on the nosepiece in the light path, slowly raise (or lower) the stage by use of the coarse adjustment knob (the larger of the two concentric focusing knobs). Bring the image into focus and then use the fine adjustment knob (the smaller of the two knobs) to perfect the focus.

4. Now, using your LEFT eye and your left eye Only, WITHOUT touching the focusing knobs, rotate the knurled ring on the left eyepiece tube to bring the image into focus for your left eye. This procedure adjusts for differences in acuity between your left and right eyes.

5. If you wish to move to a higher power objective, it should take very little movement of the fine adjustment knob to bring the image into focus. This is a built-in design feature which is known as Parfocality. Similarly, a particle in the image which is centered in the field of view should remain in the center as objectives are changed; a feature known as Parcentricity.

Appendix 2

Procedure For Using An Oil Immersion Objective

1. Focus the specimen, using Koehler Illumination, with the 10X objective. Then switch to the 40X “dry” objective and center a desired feature in the field of view.

2. Lower the stage and gently place a drop of immersion oil on top of the cover slip.

3. Rotate the oil immersion objective (usually the 100X) into the light path.

4. While looking at the microscope from the front or side (not through the observation eyepieces), slowly raise the stage until the front of the oil immersion objective makes contact with the oil drop. You will see a sudden flash of light.

5. Now, using the fine adjustment only, continue to raise the stage until the specimen comes into focus.

Some caveats;

For best results with oil immersion objectives, it is preferable to use a drop of oil on the top of the condenser (assuming the condenser has a numerical aperture above 1.0) and to raise the condenser slowly until the top of the condenser makes contact with the underside of the slide.

When finished with observations with the oil immersion objective, lower the stage and rotate that objective out of the light path so that you do not inadvertently dip the “high dry” 40X objective into oil.

It is important to clean the oil objective at the end of the day’s use. Take a piece of lens tissue (NOT facial tissue or abrasive eyeglass tissue) and gently blot the oil off the front of the objective. Moisten, NOT bathe, another piece of lens tissue in a lens cleaning solution and gently drag the tissue across the front lens of the objective. Take care not to rub the front lens vigorously in order to avoid effacing the lens coating. Dry the front lens by using a simple air blower, such as a child’s bulb ear syringe. Do NOT use compressed air can blowers.

Do not use an oil objective without oil immersion; the image will be poor.

Appendix 3

For Users of Finite Tube Length Microscopes

This appendix is addressed to users of finite tube length microscopes. For Olympus users, this would include the CH-2 series, the CH30-40, the CK-2 and CK 30-40, The BH-2 series, the IMT-2, The Vanox AHB2 and AHB3, AHBS and AHBT.

You can recognize a finite tube length microscope by looking at the objectives on the nosepiece. These objectives will be inscribed with 160/0.17 or 160/- or 160/0. The objectives do NOT have an infinity marking inscribed. The 160 refers to the mechanical tube length of such microscopes-the distance in millimeters between the shoulder of the mounted objective and the top of the binocular tube where the eyepiece is inserted.

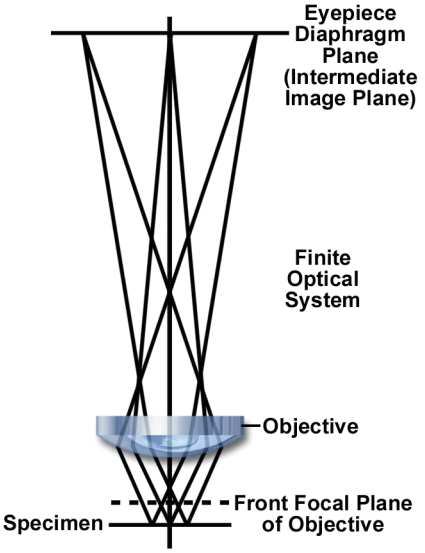


Fig 44. Finite optical system.

Finite tube length microscopes do NOT have a tube lens in the light path. The image projected by the finite tube length objective comes to focus at the plane of the fixed eyepiece diaphragm WITHOUT the intervention of a tube lens in the system. Otherwise, the same general principles described in this booklet will also apply to these microscopes.

The virtue of the infinity-corrected system comes from its better and less expensive adaptability, if you use intermediate pieces above the objective, e.g. vertical illuminators, polarizing devices, magnification changers, fluorescence illuminators, etc. You should NOT use finite objectives on an infinity-designed system and you should NOT use infinity-corrected objectives on a finite tube length microscope.

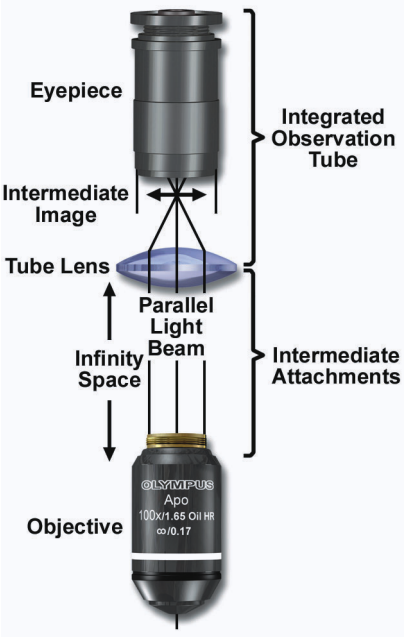


Fig 45. Infinity corrected microscope optical system.

Also, in previous series microscopes, the accompanying eyepieces were often labeled C or K (meaning compensating). Such eyepieces made the final optical correction for chromatic difference of magnification (lateral chromatic aberration), since this correction was not made in the objective itself. In current Olympus and Nikon microscopes, compensating eyepieces are not used because this correction is made in the objectives themselves. For Zeiss and Leica current microscopes, the correction for chromatic difference of magnification is accomplished by their tube lens.

Appendix 4

Some Useful Formulae

N.A. of a dry objective = sine μ This is the formula for numerical aperture, **μ** is ½ the angular aperture of an objective.

A.A. is angular aperture: the angle of cone of light capturable by the objective.

N.A. of an immersion objective = n sine μ; where n is the refractive index of the medium between the front of the objective and the top of the coverslip.

Refractive index is the ratio of the speed of light in a vacuum divided by the speed of light in a medium. n is the symbol for refractive index. See other page of appendices for common refractive indices.

n = c/*v* **c** is the speed of light in a vacuum; ***v*** is the speed of light in the medium.

$$\mathbf{r = \frac{\lambda}{2\,N.A.}} \quad \text{or} \quad \mathbf{\frac{0.61\lambda}{N.A.}}$$

This is the formula for resolution of two points lying close to each other and being shown as separate, **λ** is the wavelength of light (in nanometers) being used. **r** is the space between the two adjacent points of the specimen.

$$\mathbf{I = \frac{(N.A.)^2}{(Total\ M)^2}}$$

I equals the intensity of the image. **M** is the magnification (for transmitted light).

$$\mathbf{D_{fov} = \frac{F.N.}{M_{objective}}}$$

D is the diameter of the field of view in millimeters. **F.N.** is the field number inscribed on the eyepiece $\mathbf{M_{objective}}$ is the magnification of the objective being used.

$$\mathbf{M = F_{tube\ lens} / F_{objectives}}$$

M is the magnification of the objective. $\mathbf{F_{tube\ lens}}$ is the focal length of the tube lens (180mm for Olympus) and $\mathbf{F_{objective}}$ is the focal length of the objective (for infinity-corrected systems).

Appendix 5

Some Useful Numbers:

1 centimeter is 1/100 of a meter 1 centimeter equals 10^{-2} meters
1 millimeter is 1/1000 of a meter 1 millimeter equals 10^{-3} meters
1 micron is 1/1000 of a millimeter 1 micron equals 10^{-6} meters or 10^{-3} millimeters
1 nanometer is 1/1000 of a micron 1 nanometer equals 10^{-9}meters or 10^{-3} microns
1 Angstrom is 1/10 of a nanometer 1 Angstrom equals 10^{-10} meters or 10^{-4} microns

The highest N.A. of a dry objective, in practice, is 0.95
The highest N.A. of a water objective, in practice, is 1.2
The highest N.A. of an oil objective, in practice, is 1.4

The average wavelength of white light is 550 nanometers
The wavelength of violet light is about 400 nanometers
The wavelength of blue light is about 450 nanometers
The wavelength of apple-green light is about 550 nanometers
The wavelength of red light is about 650 nanometers
The wavelengths of the near ultra-violet are 330-390 nanometers
The wavelengths of the near infra-red are 800-1600 nanometers

The theoretical limit of resolution (not visibility) of the light microscope in white light is about 0.20-0.25 microns.

The standard coverslip thickness is 0.17mm. In a box of #1½ type coverslips, any individual coverslip may be 0.16mm to 0.19 mm thick.

Common refractive index numbers:

Vacuum	1.0000
Air	1.00028
Water	1.33
Immersion oil	1.51+
Glass	1.51+

Useful Total magnification equals 500-1000X the N.A.

Appendix 6

A “Bare Bones” List for further reading/study

Abramowitz, Mort. “Optics, A Primer” published by Olympus America 1994

Bradbury, Savile “An Introduction to the Optical Microscope” A Royal Microscopical Society Publication 1984

Eastman Kodak Bulletin P2, “Photography Through the Microscope” 1980, written by John Delly

Douglas B. Murphy “Fundamentals of Light Microscopy and Electronic Imaging”, 2001, publisher Wiley-Liss

Websites :

Molecular Expressions http://www.microscopy.fsu.edu

Olympus Microscopy Resource Center http://olympusmicro.com

Price $12.00