The THEORY of the MICROSCOPE

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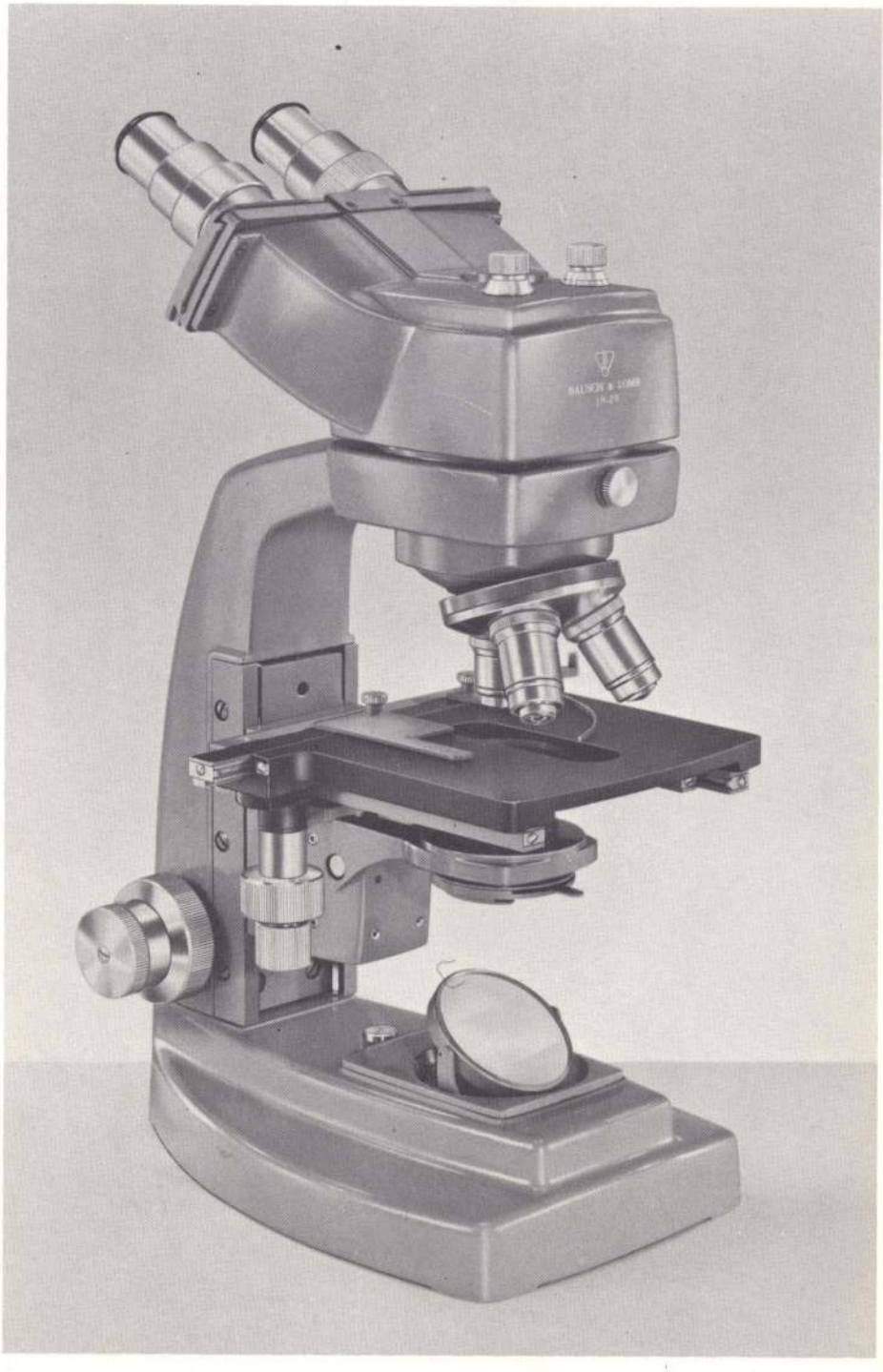

THE THEORY OF THE MICROSCOPE

BY

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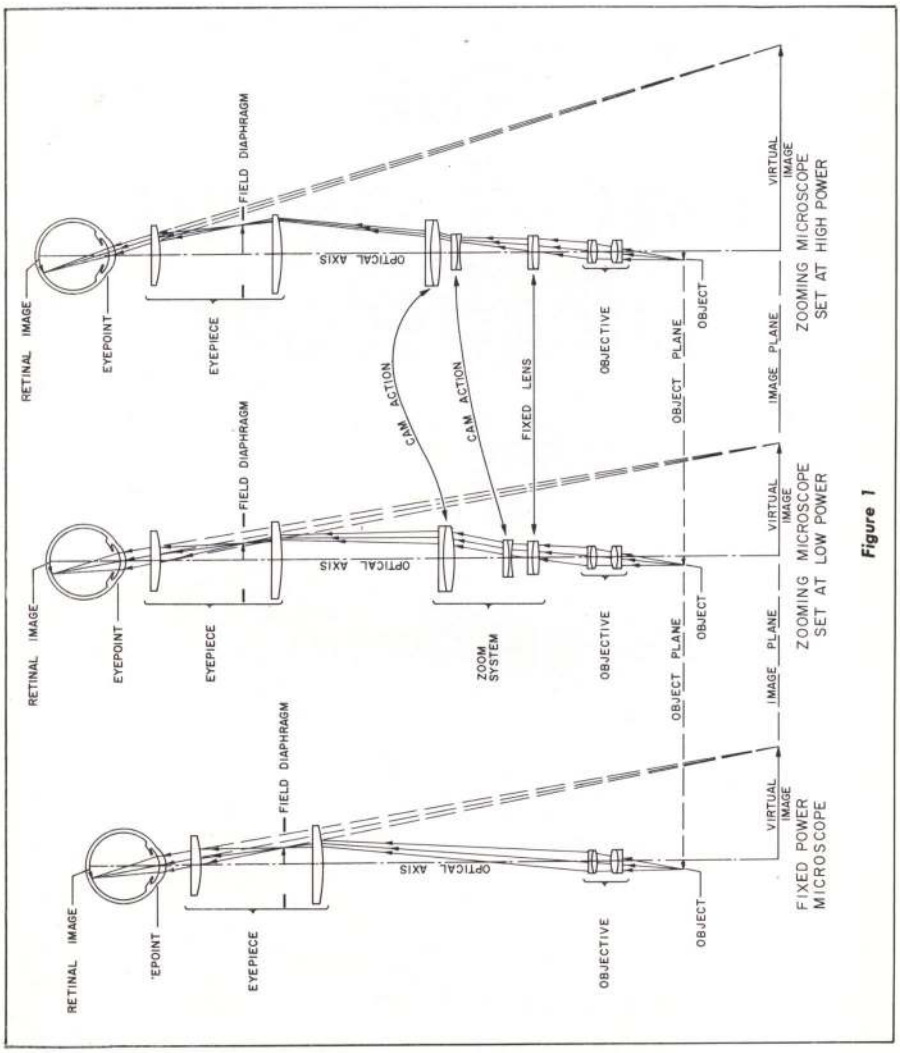

RETINAL IMAGE
EYEPOINT
EYEPIECE
FIELD DIAPHRAGM
OPTICAL AXIS
OBJECTIVE
OBJECT
OBJECT PLANE
IMAGE PLANE
VIRTUAL IMAGE
FIXED POWER MICROSCOPE
ZOOMING MICROSCOPE SET AT LOW POWER
ZOOMING MICROSCOPE SET AT HIGH POWER
ZOOM SYSTEM
CAM ACTION
CAM ACTION
FIXED LENS

Figure 1

THE THEORY OF THE MICROSCOPE

Introduction

The aim of this booklet is to provide the microscopist with a basic explanation of the theory of the microscope sufficient to enable him to understand the reasons behind accepted microscope techniques. It is felt that such an understanding will not only add to his interest in using the microscope, but will help him to work his way out of possible problems that may arise later on when detailed instructions, originally grasped, may have been forgotten. Where possible, mathematical formulae have been avoided, in favor of physical or pictorial explanations, as it is felt that such explanations are more easily grasped and better retained than explanations involving mathematics.

What's in a Microscope?

Figure 1 shows the way the lens elements of a miscroscope act to produce an enlarged image of a very tiny object. For the sake of clarity the 3 drawings are limited to strictly the lens elements of the microscope. Later on we will describe the complete system, including the illumination system, the substage condenser, and the mirrors and prisms in the binocular body.

At the left in Fig. 1 is shown the traditional microscope. The objective acts much like a small projection lens, but instead of projecting an image onto a screen, it projects an enlarged primary image of the object up near the top of the microscope tube. This primary image is formed in the air and is called an "aerial image." (The presence of this image could be shown by removing the eyepiece and putting a small translucent screen in the plane of this aerial image.) In actual use, however, we do not use a screen, we look at this image through the *eyepiece*. This eyepiece acts very much like a magnifier, the principal difference being that it is used to magnify an aerial image instead of an actual object.

The final image is formed on the retina of the eye, but appears to the eye to be in the plane of the *Virtual Image* down near the bottom of the diagram. This latter image is called a "virtual image" because the light rays do not actually come from this image, they merely *appear* to come from it. The dashed lines going to the ends of this virtual image indicate that these are not actual rays of light, merely extensions of the actual rays. The actual rays are shown in solid lines in between the eyepiece and the eye. It is instructive to lay a straight-edge along the dashed lines to get a clearer picture of the fact that these are extensions of actual rays.

The microscope attains its magnification in two stages. The first stage of magnification is produced by the objective, the second by the eyepiece. The final magnification is the product of these two stages. If the objective magnification is 10 and the eyepiece magnification is 10, the final magnification is the product of the two, or 100.

The central and right diagrams in Figure 1 show the formation of images in microscopes equipped with zoom lens systems. These systems vary the magnification in a continuous manner, rather than in discrete intervals such as is obtained by changing objectives or eyepieces. With such a system, one closes the gaps in the magnification range, making possible attainment of any magnification desired for optimum viewing of a specimen.

The actual range of magnification factor of the zoom system is 1X to 2X. Thus, with a 10X objective and 10X eyepiece, one can vary the magnification continuously from 100X up to 200X. Then by inserting a 20X objective, one can progress continuously from 200X to 400X, etc.

Note that the zoom lens system does not form an intermediate real image, but simply moves the image plane outward. The image is held in constant focus during zoom by accurately cut cams which control the lens motions. The cams are shown schematically between the central and right diagrams in Figure 1.

What Limits the Magnification?

Actually there is no upward limit to the magnification of a microscope, there is only a limit to the *useful* magnification. The basic limitation is not magnification but *resolving power*, the ability of the microscope to render visible the fine detail of the object. If the object has been magnified to the point that its image is becoming fuzzy or indistinct, due to the limited resolving power, further magnification does nothing but make the image larger, and less distinct without showing any more detail. Such useless increase in magnification is called "empty magnification," meaning that it has exceeded the actual useful limit in showing specimen detail.

Resolving Power

The resolving power of a microscope depends generally on the design of the objective. An objective capable of utilizing a large angular cone of light coming from the specimen will have better resolving power than an objective limited to a smaller cone of light. This is shown in the comparison pictures, Figure 2. The objective which picks up the larger cone of light gives considerably more detail in the image.

The image of a point object is not a point but, due to the phenomenon of diffraction, is a small circular spot of light surrounded by rings of light as shown in Figure 3. This phenomenon was first mathematically investigated by the astronomer Sir George Airy in 1834. He showed that the distribution of light in this pattern (which came to be known as the "Airy disc") is such that the radius of the first dark ring (h′ in

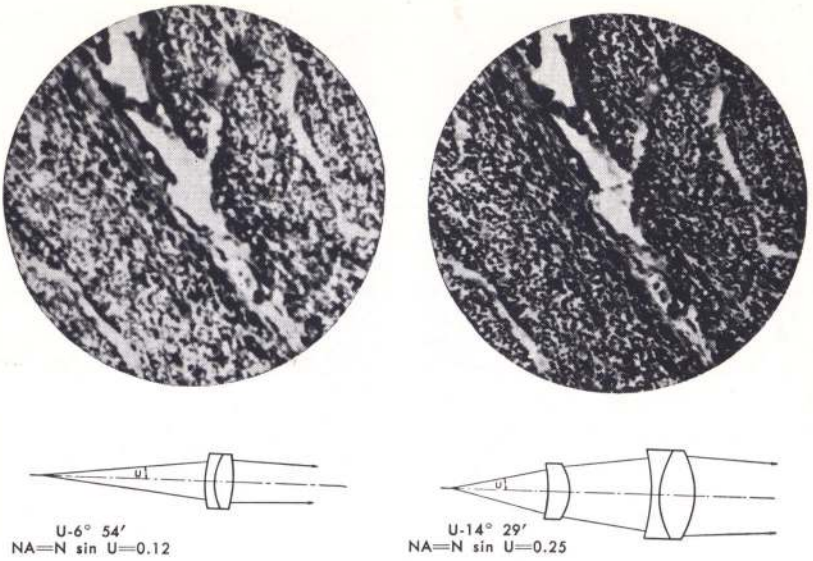


Figure 2
Photomicrographs showing the dependence of Resolving Power on numerical aperture (N.A.)

Figure 3) is a measure of the resolvable separation in the image. It can further be shown that this separation in the image can be referred back to the object as a separation h defined by the equation:

$$h = \frac{0.61\lambda}{N.A.}$$

where λ is the wavelength of light (about 0.0005mm or 0.00002") and N.A. is the "Numerical Aperture" of the objective.

Numerical Aperture

The quantity N sin U in Figure 2 is called the Numerical Aperture or N.A. of an objective. Thus:

N.A. = N sin U (by definition)
where N is the refractive index in the object space.

Manufacturers customarily engrave the N.A. on an objective, as it is an important characteristic of the lens. The higher the N.A. the more complex and expensive the lens system becomes, hence in buying a microscope it is wise to see that the N.A.'s are up to standard practice.

The equation $h = \frac{0.61\lambda}{N.A.}$ indicates that the fineness of detail h which can just be resolved is inversely proportional to the objective N.A.

As indicated by the formula above, there are three ways to increase resolving power, i.e. to decrease the resolvable separation h. The first method is to decrease the wavelength λ, the second is to increase the angle U in the object space, (see Figure 2), and the third is to increase the index N in the object space.

The wavelength λ can be decreased by going toward the violet,

or short wavelength, end of the spectrum by means of selective filters. By means of special optics and special techniques this effect can be extended into the ultraviolet for still further lowering of the resolvable separation.

The angle U can be increased toward the 90° theoretical maximum, (i.e. N.A. = 1.00) only to a certain practical limit. The 0.95 N.A. apochromat represents a design giving the highest value of U which is practicable. N.A.'s higher than 0.95 are achieved by the use of immersion fluids, as explained in the next paragraph.

The final method of decreasing the resolvable separation is to increase N, the index in the object space. This is accomplished by "immersion objectives," in which a fluid is used between the object slide and the front lens of the objective. The immersion fluid is normally oil (N = 1.52) although water (N = 1.33) and monobromonapthalene (N = 1.66) have also been used to some extent. By means of immersion fluids, objectives as high as 1.60 N.A. have been produced, but the practicable limit has been found to be about 1.40 N.A.

Depth of Focus

When one focuses a microscope on an object, there is a finite range above and below this object in which other objects appear in sharp focus. This range is called the depth of focus of the microscope. It varies markedly with objective N.A., in accordance with the relation.

$$d = \frac{\lambda\sqrt{N^2-(NA)^2}}{(NA)^2}$$

where d is the depth of focus for photomicrography. For visual use, one must add further depth, since the eye is capable of a certain amount of accommodation. In this case, the depth d' becomes

$$d' = d + \frac{250}{M^2}$$

where M is the magnification of the microscope. The assumption is made here that the eye can accommodate on an image 250mm away.

Putting actual numbers in the above expression, we find that for the three most common objectives, we have the following depths of focus:

Objective	Eyepiece	Depth of Focus	
		Photomicro	Visual
16mm,0.25 N.A.	10X	.008mm	.0335mm
4mm,0.65 N.A.	10X	.0010mm	.0023mm
1.8mm,1.30 N.A.	10X	.0002mm	.0005mm

The visual depth of focus of the 1.8mm, 1.30 N.A. oil-immersion objective is here shown to be only .0005mm, or about a wavelength of light. This very tiny value indicates how closely one must focus when using such a high power objective, and indicates why a microscope needs a very finely controlled focusing motion.

Image Aberrations

The perfect lens system has yet to be designed. Lens systems all have aberrations or defects to a greater or lesser extent, depending on the skill of the designer and the magnitude of the lens design problem. Lens systems are composed of lenses having spherical surfaces, and a spherical surface does not form a perfect image. The lens designer, by judicious combinations of lens shapes and glass choices, is

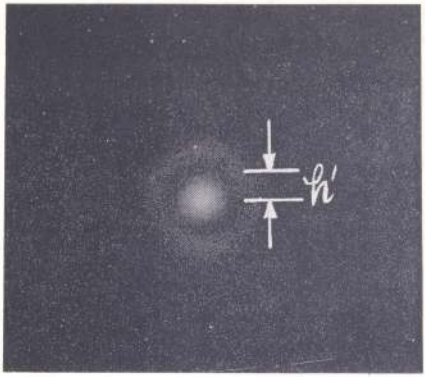


Figure 3 The Airy disc. Photomicrograph of a pinhole in an aluminum mirror taken with a 4mm, 0.65 N.A. objective. h', the radius of the first dark ring, is a measure of the resolving power.

Figure 4—The appearance of the Airy disc when spherical aberration is present. Photomicrograph taken with a 4mm, 0.65 N.A. objective, purposely spaced incorrectly to show the effect of lens spacing on spherical aberration. Compare with Figure 3.

generally able to counteract the defects of one surface by equal and opposite defects in other surfaces, so that the end result approaches perfection, even though it never fully reaches this goal.

The principal aberrations in the image formed by a spherical lens surface are:

1. **Spherical Aberration**, the (somewhat unfortunate) term used to express the fact that the outer portion of a spherical surface has more power than the inner portion. The lens designer overcomes this problem by judicious combinations of convergent and divergent lens elements, properly shaped to minimize the variation of focal power with aperture. Figure 4 shows the appearance of the Airy disc (image of a point object) in the presence of spherical aberration. Note, by comparison with Figure 3, that spherical aberration has caused some of the light which should be in the central spot to diffuse out into the ring structure. This undesirable diffusion causes a loss in contrast in the normal microscope preparation.
2. **Astigmatism** is the defect whereby a marginal point object is drawn out into two separate line images lying at different distances from the lens surface. Like curvature of field, it results in a general deterioration of the off-axis image, but unlike curvature of field, an astigmatic image can never be focused sharply except for detail parallel or perpendicular to a radius of the field. Figure 5 shows the bad deterioration of the image of a point object (pinhole in an aluminum mirror) due to astigmatism.
3. **Coma** is the name given to the defect in which different circular concentric zones of the lens surface give different magnifications to an offaxis image.

This defect results in a point object being imaged as a comet-shaped image, and, like the preceding two aberrations, causes the off-axis image to deteriorate. Figure 6 shows the bad deterioration of the image of a point object due to the presence of coma. Coma in the center of the field is an indication of damage to the objective.

4. **Distortion** is the aberration which renders a square object as an image with curved sides, as shown in Figure 7, an image of a rectilinear cross-ruling. Note how the rulings near the edge appear curved inward. This is called "cushion distortion." The opposite effect is sometimes encountered, where the rulings appear to be curved outward, and in this case the effect would be termed "barrel distortion." Distortion is caused by the lens surface having different magnifications at the marginal and central portion of the image.
5. **Curvature of Field,** as the name implies, is the aberration of a spherical lens surface which produces a curved image of a flat object due to the marginal portions of the image coming to a focus at a different distance than the central portions of the image. The end result is that when the central part of the image is focused sharply the marginal portions are out of focus, and vice versa. Figure 8 shows the appearance of the same cross-ruling as in Figure 7, but taken with a lens having curvature of field.
6. **Chromatic Aberration** is the property of a spherical lens surface which brings light of short wavelength to a focus closer than light of a longer wavelength. The defect is brought under control by proper combinations of glass types used in the convergent and divergent lens elements which make up the lens system.
7. **Lateral Color,** or chromatic difference of magnification, re-

Figure 5—The appearance of a point object due to the presence of astigmatism. Compare with Figure 3.

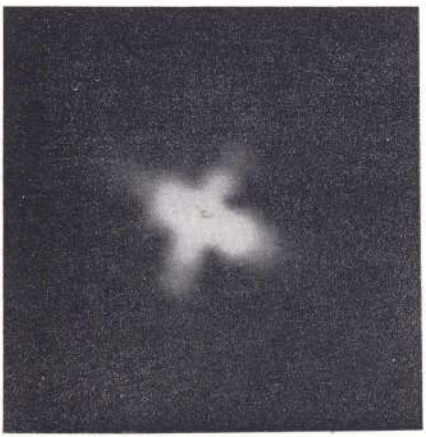


Figure 6—The appearance of a point object due to the presence of coma. Compare with Figure 3.

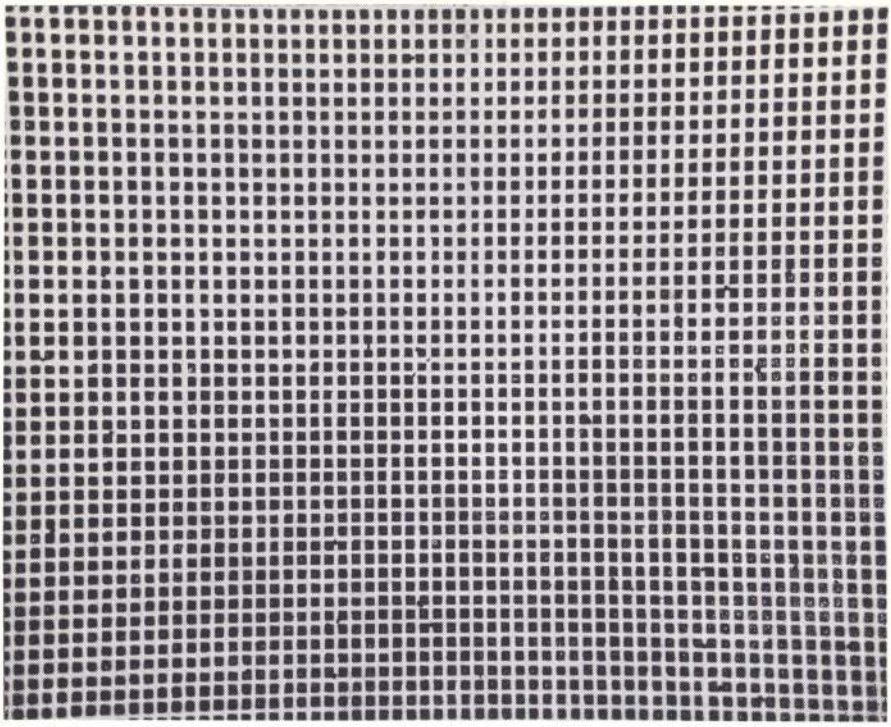


Figure 7—Distortion in a lens system causes straight lines to appear curved. Note curving lines at edges.

sults in light of one color being imaged at a greater magnification than light of another color. This aberration causes an off-axis image of a point object to be spread out into a tiny spectrum or spread of color.

Aberrations in the Microscope

The aberrations which are of particular interest in the microscope are spherical aberration, curvature of field, and lateral color. All the other aberrations are ordinarily under good control in a properly designed microscope.

Spherical aberration is sometimes apparent in microscope objectives, not due to design, but to the use of different thickness cover glasses on the specimen slide. The high-powered "dry" (i.e., not oil immersion) objectives are particularly sensitive to cover glass thickness. Objectives are normally designed to work with 0.18mm thick cover glasses, and any wide divergence from this thickness causes spherical aberration with the high-powered dry objectives, resulting in a "washed out" image of low contrast.

Curvature of field is extremely difficult to remove in the design of the high-powered objectives. It has long been known that the way to flatten the field in an optical system is to (roughly) equalize

the positive and negative power in the lens system[1], but the nature of the construction of high-powered micro-objectives, where the front element is a hemisphere, leads to an extreme preponderence of positive power which is very difficult to neutralize by negative power elsewhere in the system. As a consequence the field of high-powered systems is more strongly curved than in the lower-powered systems.

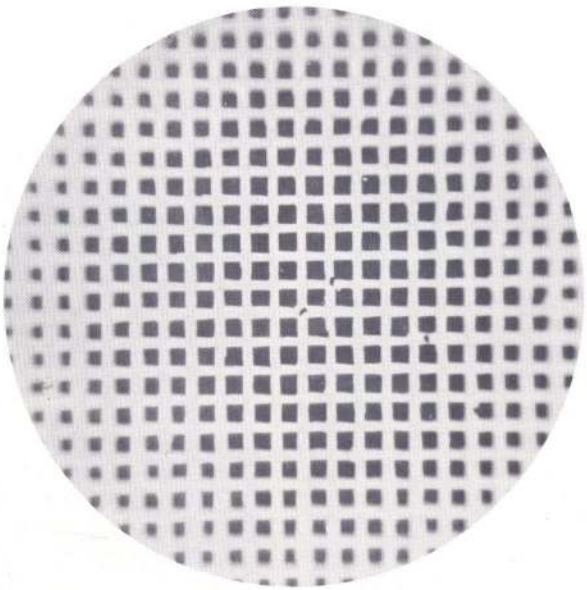
Lateral color is also a problem in high-powered objectives and, like curvature of field, results from the chromatically uncorrected strong hemisphere at the front of the system. The rear elements correct the chromatic aberration but cannot at the same time correct the lateral color coming from the front hemisphere. The defect is easily overcome, however, by the proper choice of eyepiece type. The Compensating eyepieces have overcorrected lateral color, and, when used with the high-powered objectives, give color-free imagery. For intermediate powers, where lateral color in the objective is less, the Hyperplane eyepieces, with less overcorrection for lateral color, give the best results. For the low power objectives, which are well corrected for lateral color, the Huygenian eyepieces give the best results. The Huygenian eyepieces have no overcorrection for lateral color.

Types of Objectives

There are three major types of

[1]*More precisely, the Petzval sum* $\frac{(N'-N)}{(NN'R)}$, *must equal zero in order for the possibility to exist of producing a flat-field objective which is at the same time free from astigmatism.*

Figure 8—Curvature of Field. The center is sharply focused, the periphery is out of focus.



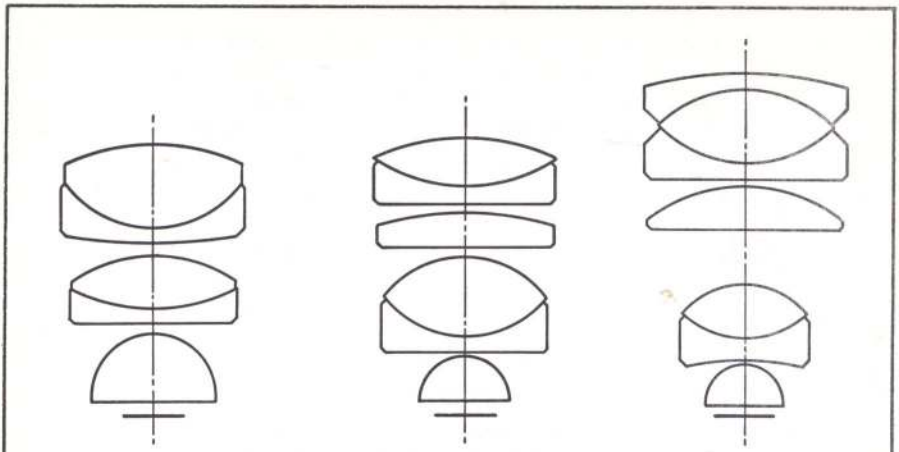


Figure 9—General construction of the 4mm Achromat, the 4mm Fluorite, and the 4mm Apochromat in that order.

microscope objectives. They are called "achromats," "fluorites," and "apochromats." The achromats are the simplest and least expensive. For most purposes achromats do an adequate job, and consequently they are popularly used on most medical and laboratory microscopes. The term achromat means that the lens is corrected for color aberration, but, as indicated previously, complete correction is generally the goal rather than the accomplished fact in lens design. The correction for both chromatic and spherical aberration in low power achromats is generally very good, but with increasing power these aberrations become more difficult to control.

A good many years ago it was found that an objective lens system which combined fluorite with glass lenses achieved an improvement over what was possible in the achromats which used glass elements alone. Such lenses became known as "fluorites" and "apochromats." The fluorites are sometimes called "semi-apochromats" as they represent a compromise between the achromat and the apochromat in their degree of correction. The apochromats represent the finest lens making known to the art. Their correction for spherical and chromatic aberration is markedly superior to that achieved by the less complicated lens designs in the achromatic series. Figure 9 shows the general construction of the 4mm achromat, the 4mm fluorite, and the 4mm apochromat.

Types of Eyepieces

As pointed out in the early sections of this booklet, the eyepiece is essentially a magnifier to enlarge the image formed by the objective. The most popularly used eyepiece is the Huygenian type, named after its inventor Christian Huygens, a famous Dutch scientist of the 17th century. The Huygenian eyepiece works well with the low power achromats, but as pointed out in

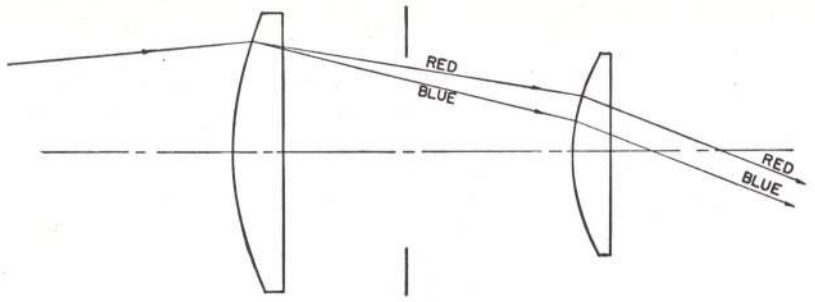


Figure 10—General construction of the Huygenian Eyepiece.

the preceding section, intermediate and higher power objectives tend to have under-corrected field curvature and lateral color, so that the eyepiece requirement for these objectives is one in which these aberrations are compensated. The degree of compensation required increases with objective power, so that a graded series of eyepiece is indicated. It has been found, however, that three types suffice to cover all cases. The Huygenian eyepieces cover the low powers; the Hyperplane eyepieces, the intermediate powers, and the Compensating eyepieces, the high powers.

The Huygenian eyepiece utilizes two simple lenses to achieve its correction for lateral color. Its construction is shown in the diagram, Figure 10.

The light on going through the first lens, called the "collective lens," is spread out into a spectrum with red at one limit and blue at the other. However, by proper spacing of the lenses, the red light is caused to strike the second lens, called the "eyelens," at a sufficiently greater height than the blue to compensate the angular spread between the rays and cause the red and blue rays to emerge parallel. To the eye, focused for infinity, the red and blue rays unite on the retina to

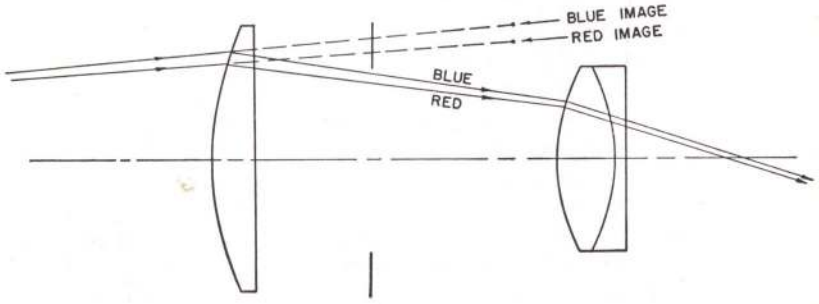


Figure 11—General construction of the low power Hyperplane and Compensating Eyepieces.

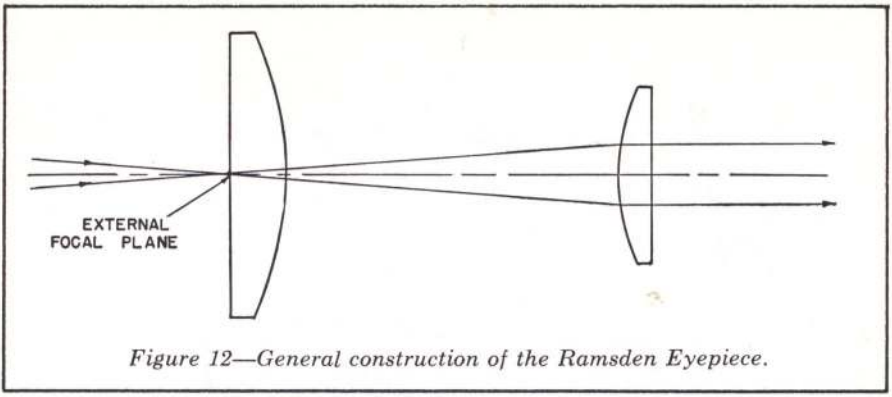


Figure 12—General construction of the Ramsden Eyepiece.

form a color free image.

The Hyperplane eyepiece utilizes a doublet eyelens to accomplish an intermediate degree of overcorrection for lateral color.

The Compensating eyepiece is of the same basic construction but by the use of different glasses and lens shapes accomplishes a greater degree of over-correction for lateral color. The general construction of the (low power) eyepieces in these series is as shown in Figure 11. In this diagram the red and blue rays are shown aimed toward different heights in the primary image, due to the objective having under-corrected lateral color. The eyepiece compensates for this, and causes the red and blue rays to emerge parallel so that they unite in a single color free image on the retina of the eye. The higher power eyepiece in both the Hyperplane and Compensating series are of a more complex form than indicated in Figure 11.

Besides these three eyepiece types there are other types which find use in special applications on the microscope. Among these are the Ramsden eyepieces, the Wide-

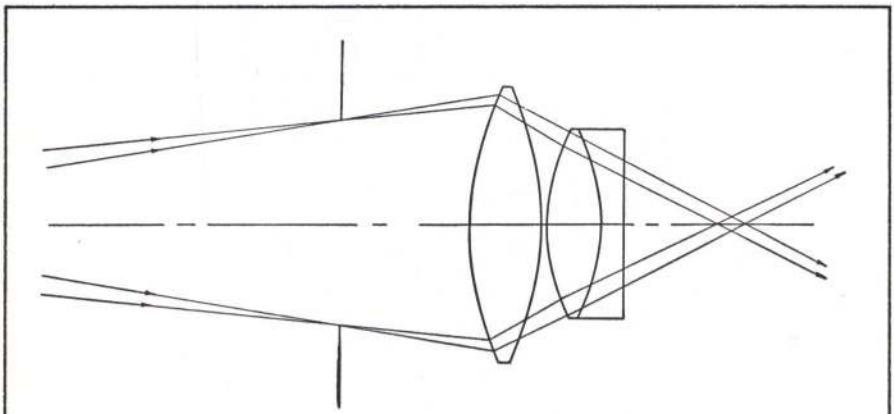


Figure 13—General construction of the Widefield Eyepiece.

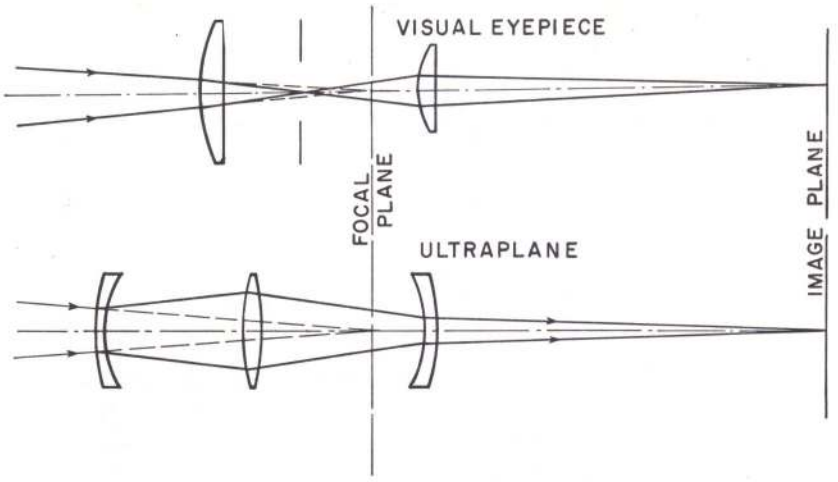


Figure 14—Comparison of Ultraplane with Visual Eyepiece.

field eyepieces, and the Ultraplanes.

The Ramsden eyepieces are similar to the Huygenian eyepieces but have their focal planes either on the outer surface of the collective lens or just outside this surface. The construction of the Ramsden eyepiece is shown in Figure 12. The Ramsden construction is used chiefly in measuring applications where the external focal plane is an advantage. It is possible to use measuring scales in a Huygenian eyepiece, but the image quality of the scale is not as good as in the Ramsden eyepiece, since in the Huygenian it is viewed by only one uncorrected lens.

The Widefield eyepieces are of the general construction shown in Figure 13. They are characterized by a very wide field of view and are particularly suited for low power microscopes, such as the Greenough Stereoscopic Microscope. Their use in high power microscopy is generally to be discouraged as their color correction is not suitable for this application.

The Ultraplanes are not true eyepieces, but are projection lens systems for photomicrography. Their general construction is indicated in Figure 14. In the Ultraplane, there is no intermediate formation of an image as in the preceding types. Instead the Ultraplanes intercept the image before it comes to a focus, and project it outward to the photographic film. The purpose of this type of lens system is to achieve greater field flattening than is possible by means of any of the visual eyepieces. This is due to the use of negative or diverging lens power which makes possible some compensation for the curvature of field arising from the objective. The

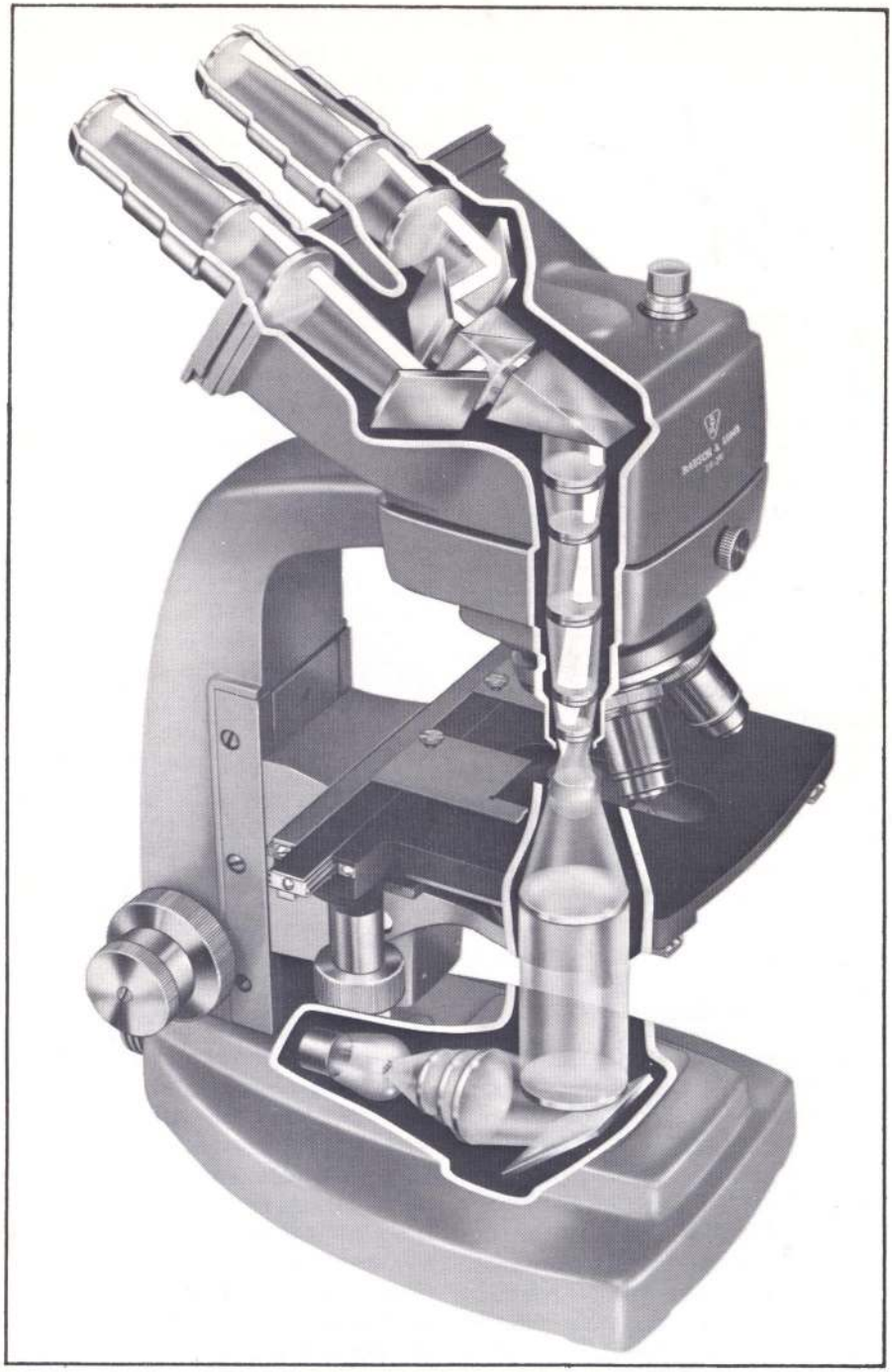


Figure 15

correction for lateral color in the Ultraplane varies, the High Ultraplane having a high degree of overcorrection making it suitable for use with high powered objectives, while the Medium and Low Ultraplanes are designed for use with the medium and low power objectives respectively.

Binocular Observation

Thus far we have considered only the monocular microscope. For prolonged use, the inclined binocular form is preferred since it gives a more natural and restful condition of observation. Figure 15 shows a cut-away view of the optical system of a binocular microscope. Binocular vision is attained by the use of a beam-dividing prism and three mirrors. This system divides the light equally, sending half to the left eye and half to the right. The coating on the mirrors is enhanced aluminum, having a multiple-film transparent coating on top of the aluminum to increase the reflectivity and protect the aluminum. Cover glass seals are used to keep dust from entering the binocular body.

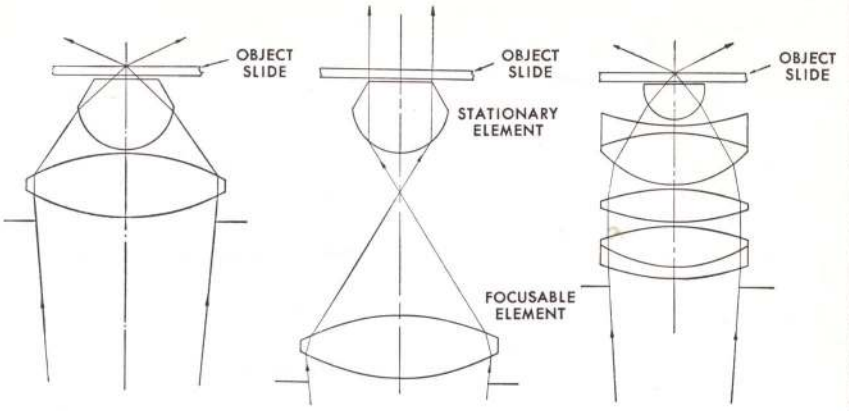
The Illumination System

The illumination system is a very important part of the microscope. It is the part with which the operator can do most in controlling the microscope performance, and is at the same time probably the least understood part of the microscope. It has been pointed out in the preceding sections that the resolving power depends on the N.A. of the objective. To get the most out of the objective, the condenser system must be capable of delivering as large an angular cone of light as the objective is capable of utilizing.

This statement should not be interpreted as meaning that the full N.A. of the objective should always be illuminated. Generally speaking, full N.A. illumination is *not* used, because contrast drops as the illuminated N.A. approaches the full objective N.A. Each specimen is a law unto itself in regard to the proper illuminating N.A. With high contrast specimens having very fine detail one would want to use very close to full N.A. illumination, but with low contrast objects, the illuminated N.A. would have to be reduced to prevent complete "washing out" of the image due to low contrast.

The illuminated N.A. is controlled by means of the substage iris. It is instructive to remove the eyepiece and look at the back lens of the objective while opening and closing the substage iris. An image of the iris will be seen in focus close to the back lens of the objective. Full-aperture illumination occurs when the iris is opened up just enough to include the full aperture of the objective lens.

Closing down the illuminated N.A. excessively is a common fault with beginners in microscopy. The contrast gets very good by so doing, but at a loss in resolution. The loss in resolution is not so easy to notice as the gain in contrast. Excessive closing down of the illuminated N.A. also gives rise to image artifacts due to diffraction. The experienced microscopist learns to make the optimum setting for illuminated N.A. as a best compromise between resolving power and contrast.



Abbe Condenser *Variable Focus Condenser* *Achromatic Condenser*

Figure 16

Where the microscope illuminator has an iris diaphragm, it is intended to be used to control the size of the illuminated field of view on the specimen. The substage condenser should be adjusted to form a sharp image of the lamp iris on the specimen plane. The size of the iris should then be adjusted to lie just outside the field of view of the microscope.

Condenser Types

The function of the substage condenser is to direct a light beam of the desired N.A. and field size onto the specimen. There are several types of condensers, three of which are shown in Figure 16.

The Abbe Condenser is a 1.30 N.A. condenser utilizing only two lenses. Because of its simplicity and good light-gathering ability, it has become extensively used for general microscopy. It is, of course, not corrected for spherical or chromatic aberration, but for general visual observation it serves very well.

The Variable Focus Condenser is a two-lens condenser, 1.30 N.A. maximum in which the upper lens element is fixed and the lower one focusable. By this means it is possible to fill the field of low power objectives without the necessity of removing the top element when the lower lens is raised to its top position. This condenser is basically similar to the 1.30 N.A. Abbe. When the focusable lens is lowered, the focus of the light is brought in between the elements, and when this focus is at the point indicated in the diagram, the light emerges as a large diameter parallel bundle.

The Achromatic Condenser is a 1.40 N.A. condenser which is corrected for both chromatic and spherical aberrations. Because of its high degree of correction it is recommended for research micro-

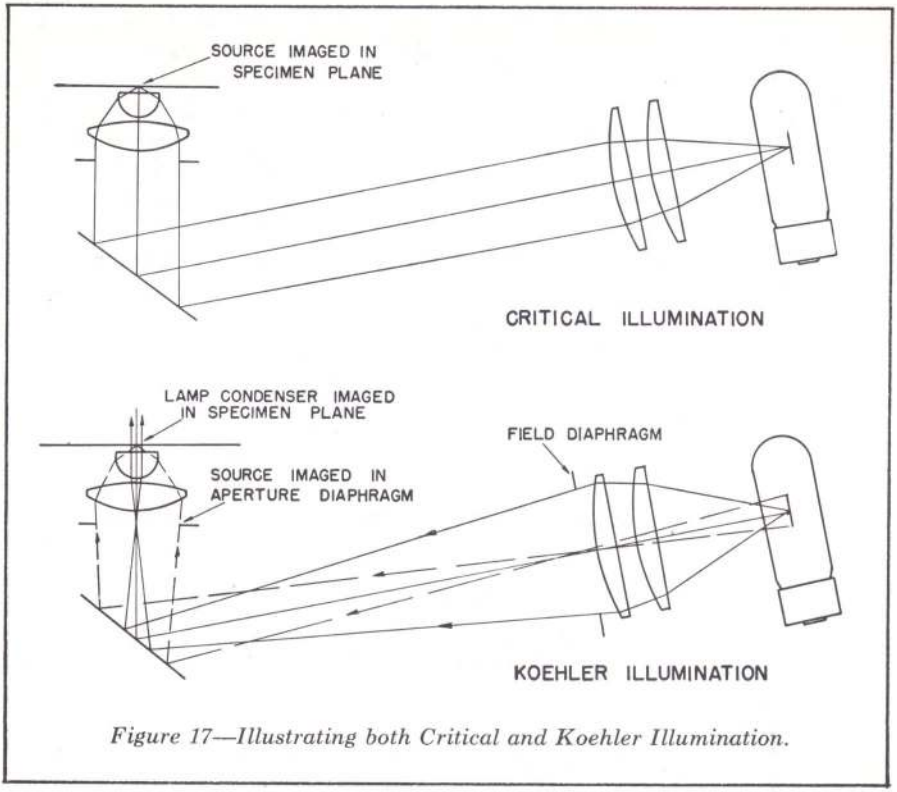


Figure 17—Illustrating both Critical and Koehler Illumination.

scopy and color photomicrography where the highest degree of perfection in the image is desired.

It will be recalled, from the previous discussion on immersion objectives, that to obtain N.A.'s over about 0.95 it is necessary to oil-contact the lens system to the specimen slide. A drop of immersion oil, placed on the lower surface of the object slide or the upper surface of the condenser hemisphere, effects this result.

Generally it is not necessary to immerse the condenser, but special tasks occasionally do require immersion for optimum results.

Critical Illumination

Critical illumination (sometimes called "Nelson Illumination") is that form of illumination in which the light source is imaged directly on the specimen. It is used in high power microscopy, micro-projection, and photomicrography, where an intense and controlled beam of light is necessary. Critical illumination was held in highest regard for many years, as early theoretical consideration indicated that it should permit higher resolving power than other forms of illumination. The reasoning back of this was founded on the theoretical

[2]*M. Berek, Optik, 1, 1946; 3,4, 1948; 4,6, 1948-49; 5, ½, 1949; 5,3, 1949; 5,6, 1949; 6,1, 1950; 6,4, 1950.*

basis that two adjoining points in a specimen could be better resolved if their illuminated background had no point-to-point phase relationship. Such a background is provided by critical illumination where a source is imaged directly on the specimen, since the various points of a light source have the completely random phase distribution characteristic of thermal emission. With the passage of years, critical illumination has been gradually replaced by another intense form of illumination known as Koehler Illumination. The two systems are shown in Figure 17.

Koehler Illumination presents certain advantages over critical illumination and has gradually replaced the latter. With the Koehler system inclusion of field diaphragm control is made feasible. Another advantage of the Koehler system is that uneven distribution of energy in the source does not result in uneven brightness in the field of view, since the source is imaged in the aperture of the system. These practical advantages have led to the gradual replacement of critical illumination by Koehler Illumination, and when M. Berek showed[2] that the two systems were theoretically equivalent in resolving power, the final argument favoring critical illumination was removed.

Selective Filters

Contrast in the image of a colored microscope preparation can be controlled by the use of colored or selective filters. If, for example, the microscope preparation consists of red and blue areas, use of a red filter, which absorbs the blue but not the red, will darken the blue areas and cause the red areas to stand out bright by contrast with the blue. This type of contrast control is particularly useful in photomicrography and a set of selective filters is, accordingly, generally supplied with photomicrographic equipments. Colored glass filters and colored gelatine filters have been used extensively for this purpose in the past, but another type, operating on the principle of interference, has advantages over these, and will be described in the following section:

Interference Filters utilize the principle of optical interference to accomplish selective or colored transmission. The principle is illustrated in Figure 18. In the upper part of this figure the effect of adding two waves which are in phase is shown to result in a wave which is additive. The converse situation, where the waves are out of phase, is shown to result in destructive interference, or darkness. The former case is generally more easily understood than the latter. The "destruction" of energy by other energy is, however, understandable if labelled as a "redistribution" rather than a "destruction" of energy. The energy actually re-appears elsewhere. For example, if destructive interference at a surface causes the transmitted energy to lessen, it will at the same time cause the reflected energy to increase. The interference filter utilizes this principle to accomplish constructive interference for a narrow band in the visual spectrum and destructive interference for the rest of the spectrum, so that only a narrow band of colored light is passed by the filter. The

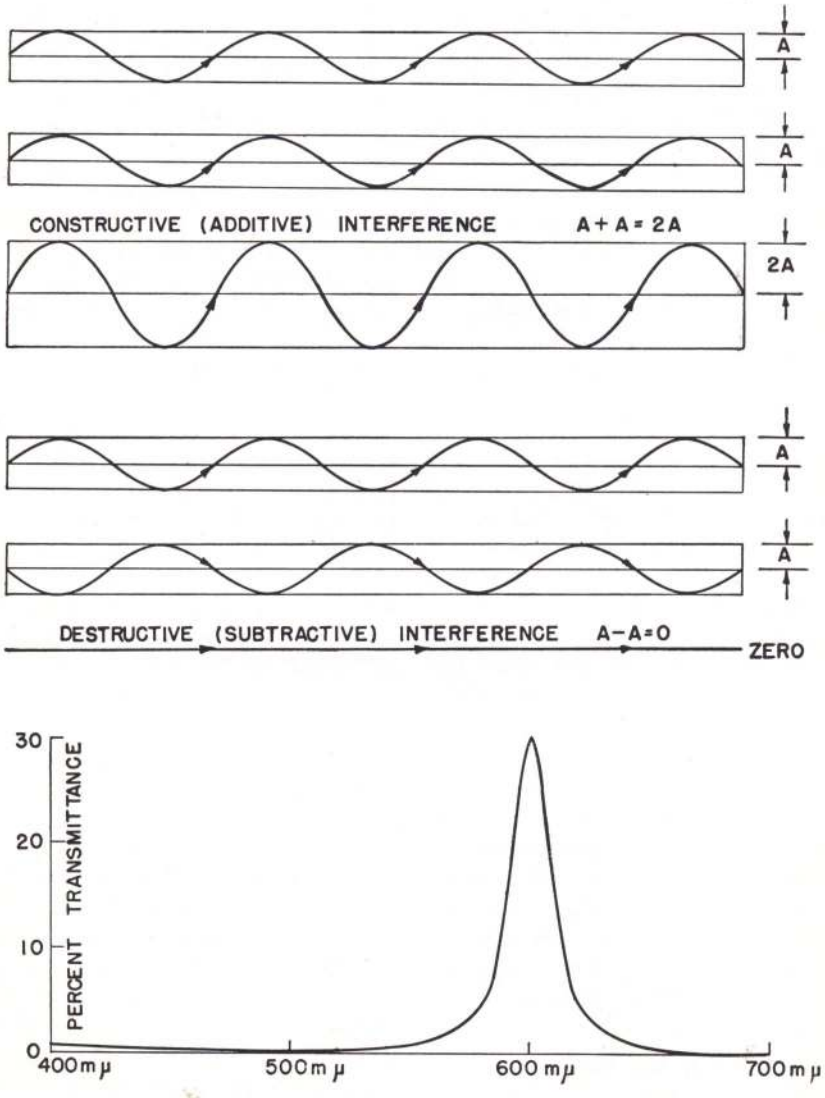


Figure 18—Interference Filters utilize the principle of optical interference to accomplish selective or colored transmission.

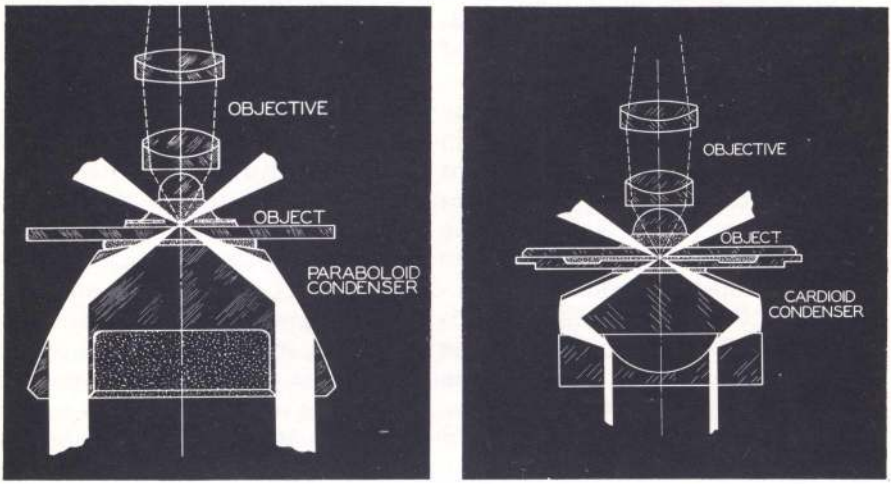
filter is composed of two semi-transparent silver layers separated by a thin layer of transparent material (magnesium fluoride generally). The thickness of the transparent layer is so controlled that multiple reflections between the silver layers are in a state of constructive interference in the transmitted beam for some chosen wavelength. The thickness chosen is slightly too great for constructive interference of shorter wavelengths, and slightly too small for longer wavelengths. As a consequence only wavelengths close to the desired wavelength get through the filter. The spectral transmission curve of a typical interference filter is shown in Figure 18.

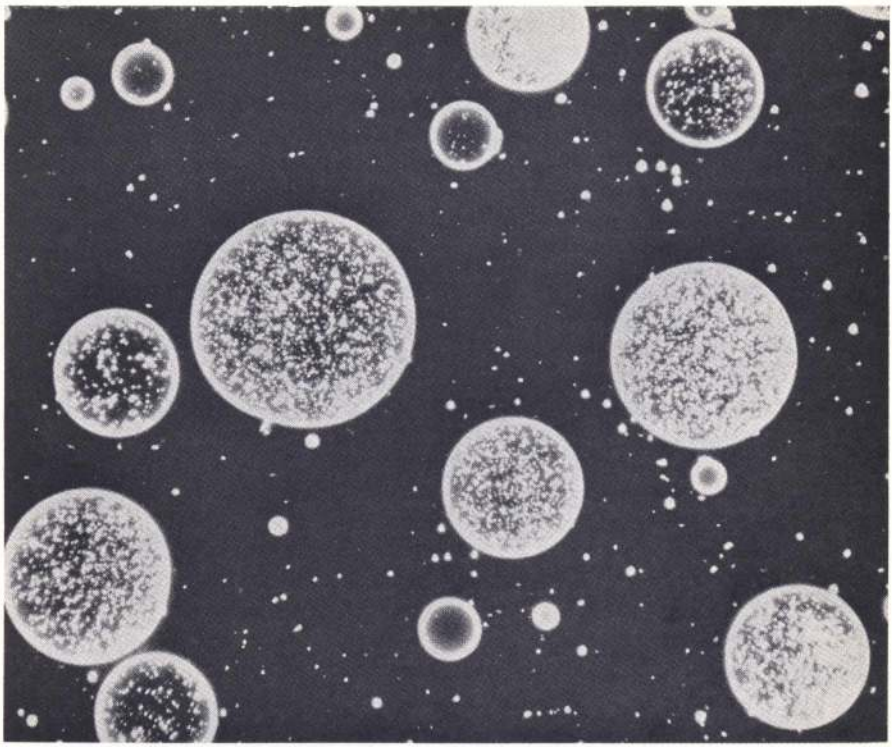
Darkfield Illumination

Two types of darkfield condensers are shown in Figure 19. These produce an intense hollow cone of light with apex (or focal point) in the plane of the specimen. If the specimen is completely transparent and homogeneous, the light continues directly on through and does not enter the objective, since the N.A. of the illuminating cone exceeds that of the objective. The field of view will thus look dark. If, however, the specimen has fine transparent detail which differs in refractive index from the embedding medium, it will scatter light due to refraction and reflection, and will appear bright, since some of this scattered light will enter the objective. This type of illumination, known as "darkfield," is useful principally on transparent unstained material where bright-field illumination fails to make the object visible due to the low contrast. Figure 20 is a photomicrograph of such a specimen taken in darkfield illumination.

Darkfield condensers depend for their effectiveness on the use of a

Figure 19—Two types of Darkfield Condensers. Each delivers a hollow intense cone of light, of greater N.A. than the objective N.A., hence objects are seen only by virtue of the light which they scatter.





Photomicrograph by G. G. Schneider

Figure 20—Emulsion of Sulphonated Oil in darkfield.

high N.A. hollow cone of light, and must be oil-contacted to the lower face of the object slide in order to obtain the required N.A. in the illuminated cone. It is also, of course, necessary to use an objective of N.A. somewhat under the illuminated N.A. of the darkfield condenser, to avoid direct light getting into the image. Objectives higher than 1.0 in N.A. must be provided with the appropriate "funnel stops" to reduce their N.A.'s to 1.0. The "funnel stop" is a small baffle, which fits into the back of an objective, reducing the aperture at the rear lens surface.

The effectiveness of a darkfield system is also dependent on the use of an intense, non-diffused, light beam from the lamp condenser. A homogeneous brilliant source, such as a ribbon filament or carbon arc, is required.

Polarized Light

Light energy is transmitted by waves, known as "transverse waves." This means simply that the waves vibrate at right angles to the direction of transmission of the light. Generally speaking, this vibration will be in *any* direction at right angles to the direction of transmission, but it is possible by means of devices known as "polarizers" to restrict the vibration to a single direction. If two such

polarizers are inserted in the optical beam in such a manner that the second one transmits in a direction at right angles to the first, the light will be extinguished. Such an arrangement is called "crossed polarizers." If, however, in between these crossed polarizers we insert an object which is crystalline in nature, it will in general appear bright against the dark background caused by the crossed polarizers. Furthermore it will brighten and darken upon every 90° of rotation about the optical axis of the microscope. The reason for this is that crystalline materials generally have different properties in different directions, and as a consequence they alter the state of polarization of the light and thereby effectively "uncross" the polarizers. This form of illumination is particularly valuable in the study of crystalline chemical compounds and minerals. Very beautiful and striking color effects can be obtained by this method.

Phase Contrast Microscopy

The normal microscopic object is seen because it has regions of varying density. In normal "brightfield" illumination a completely transparent specimen is very difficult to see in any detail, as all parts are equally dense. Darkfield illumination shows up border effects in such completely transparent specimens due to edge scattering and diffraction. Polarized light is also useful when transparent specimens have directional or crystalline properties. Another form of illumination, known as phase contrast, is of value in the study of transparent media, and has found extensive use in the study of transparent living media where staining for normal illumination methods cannot be used.

Phase contrast is basically a method of illumination in which a portion of the light is treated differently from the rest, and subsequently caused to interfere with the rest, in such a manner as to produce a visible image of an otherwise invisible transparent specimen. The arrangement necessary for phase contrast is shown in Figure 21. A clear annulus in the focal plane of the condenser is imaged at infinity by the condenser and then reimaged by the objective in its rear focal plane. The undiffracted energy all passes through this image and is both reduced in intensity and given a quarter-wave phase shift with reference to the diffracted energy, by means of an annular phase pattern in the rear focal plane of the objective. The end effect of these two changes in the undiffracted portion of the beam is to simulate the phase and intensity distribution which would be present in the objective focal plane if the specimen had density variations rather than refractive index variations, and as a consequence the image formed by this beam interfering with the diffracted beam simulates that of a specimen having density variations.

Concluding Remarks

This booklet has aimed toward helping the microscopist understand some of the basic theory of the microscope. It is admittedly an abbreviated text on a subject

which might well be extended to several times the length of this text. The aim has been to present the material in an interesting non-mathematical style, and it is recognized that in some cases this has over-simplified some of the explanations. The reader is encouraged to go on to more extensive treatments in the literature, and it is hoped that this booklet may have stimulated interest to do so.

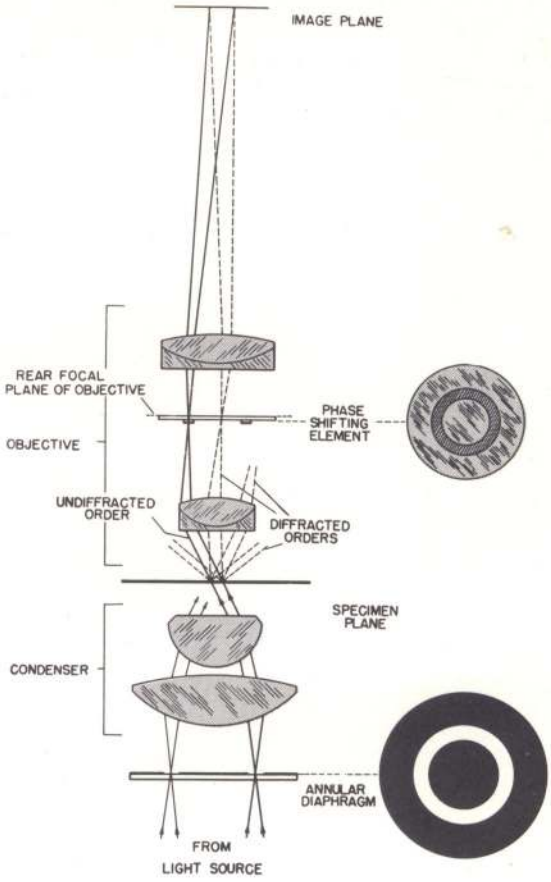


Figure 21—Image formation by Phase Contrast. An annular aperture in the diaphragm, placed in the focal plane of the substage condenser, controls the illumination on the object. The aperture is imaged by the condenser and objective at the rear focal plane, or exit pupil, of the objective. In this plane a phase shifting element, or phase plate, is placed.

Light, shown by the solid lines and undeviated by the object structure, in passing through the phase altering pattern, acquires a one-quarter wave length of green light advance over that diffracted by the object structure (broken lines) and passing through that region of the phase plate not covered by the altering pattern. The resultant interference effects of the two portions of light form the final image. Altered phase relations in the illuminating rays, induced by otherwise invisible elements in the specimen, are translated into brightness differences by the phase altering plate.

(The eyepiece is not shown in this diagram.)

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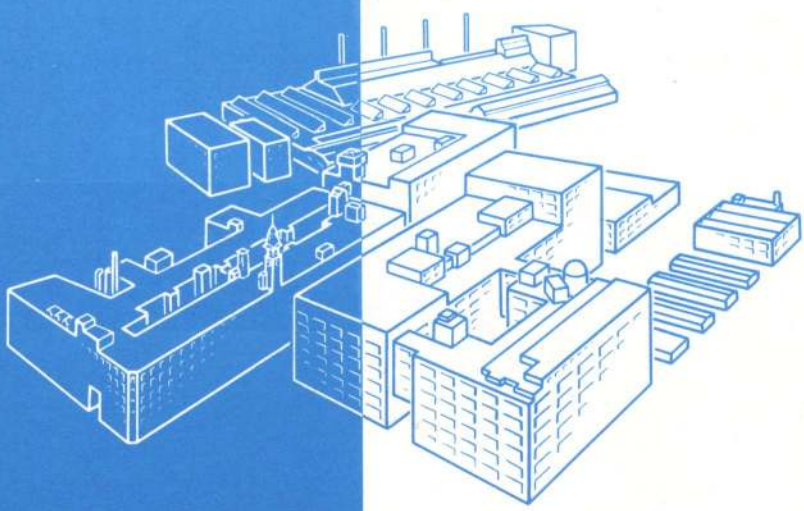

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