

23 MICROSCOPE OPTICS

23.1 INTRODUCTION

23.1.1 Scope. The material in this section will be devoted primarily to a discussion of the compound microscope, its characteristics, components, and various special purpose adaptations. However, in any discussion relating to visual instruments, the designer must keep in mind that the eye of the observer is an integral part of the optical combination, and that the degree of optical perfection in the human eye is as influential on the final retinal image, as is the degree of image perfection formed by the instrument's optical elements. The reader is urged therefore, to refer to Section 4 for a discussion of visual optics.

23.1.2 Functional relationships of microscope components.

23.1.2.1 The primary function of the high-power, compound microscope is to obtain information regarding the structure and optical characteristics of small specimens. This information is obtained by visually interpreting the manner in which the light transmitted by, or reflected from, the specimen is affected.

23.1.2.2 Usually, the specimen must be illuminated by intense artificial light, and it is only in rare and special cases that the specimen can be self-illuminated. However, the action of the specimen on the illumination system used may consist of absorption, reflection, diffraction, scattering, birefringence, or localized changes in the phase of the illuminating light waves. The purpose of the microscope then, is to form an image, based on the action of the specimen on the illuminating light waves, which can be interpreted in terms of the particular information with respect to the specimen, that is desired.

23.1.2.3 Since the human eye is only sensitive to color and intensity contrasts, the information derived from the image by the observer must be interpreted from these two effects.

23.1.2.4 The primary source of light can be of any number of high intensity light sources, however the light used must be concentrated on the specimen by a condenser system. The specimen affects the light as stated in paragraph 23.1.2.2, and the objective system of the microscope must be capable of receiving the altered light so that the maximum effects of diffraction, absorption, scattering, etc., may be transmitted by the objective and appear in the image as interpretable spacial variations.

23.1.2.5 In order to interpret the spacial variations in illumination, the objective must be capable of accepting and transmitting a wide angular beam of light, since the effects of the specimen on the light, as previously mentioned, especially diffraction, fan out from the specimen over wide angles. In the important case of diffraction, the more of the spectral orders the objective can receive, the more exact is the correspondence between the specimen and the structural details of the image.

23.1.2.6 Another requirement of the microscope objective is that the points and lines in a specimen be imaged sharply, so that the details in the image have a point by point correspondence with those in the specimen. This requirement necessitates a high degree of correction for aberrations.

23.1.2.7 All the available information regarding the specimen, as a result of its action on transmitted or reflected light, is contained in the primary image formed by the objective. As long as this information is contained in the primary image it is useless, since it must be interpreted in the brain of the observer. The simplest method for increasing the interpretability of the image is by means of magnification. By means of the eyepiece, the smallest significant details of the image can be resolved by the human eye.

23.1.2.8 There are other intermediate means available for interpreting the information from a specimen. The primary image may be magnified by projection, and formed on a photographic plate. The image could also be viewed by a television tube and an enlarged image presented on a screen.

23.1.2.9 To summarize then, the compound microscope is an instrument which transforms the action of a small object specimen on light waves into interpretable visual impressions, and in a broad sense any apparatus which accomplishes this function may be designated as a microscope.

23.2 CHARACTERISTICS

23.2.1 General. The compound microscope is characterized by the following requirements: high magnification (without a sacrifice of definition over a restricted size of field), a comparatively small true angular field, an illumination system, and resolution limited only by the wavelength of light and the numerical aperture of the

objective. Similarly, it is desirable that the oblique aberrations be as well corrected as is consistent with the requirement for axial definition of the highest possible order. These characteristics determine the basic design of the compound microscope.

23.2.2 High magnification. Since the major function of the compound microscope is to view extremely small specimens, it must be capable of magnifying to such a degree that the smallest resolvable detail can also be resolved by the human eye. The highest useful magnification, expressed in diameters, is approximately a thousand times the numerical aperture of the objective used in the microscope. It should be noted however, that the compound microscope is not always used to view extremely small specimens and, in some instances, magnifications as low as 25 diameters are advantageous for viewing larger specimens.

23.2.3 True angular field. The true angular field of the microscope is, in most instances, small due to the following factors. The diameter of the primary image cannot be larger than that of the eyepiece, and present day eyepieces have become standardized in order to afford interchangeability with those of different manufacturers. The optical tube length, i.e., the image distance of the objective, in practice has become standardized, within limits, so that it is not difficult to interchange objectives made by different manufacturers, without significantly changing the magnification and correction of the objective. Since the true angular field, α , can be expressed as

$$\tan \frac{\alpha}{2} = \frac{y}{d} ,$$

where y is the half-diameter of the primary image, and d is the optical tube length, it may be seen that the true angular field has a maximum value in practice. The exceptions to this characteristic are the special microscopes which have extra large diameter eyepieces, and hence larger true angular fields. Actually, the size of the primary image is limited by the field diaphragm in the eyepiece, and this diaphragm must always be slightly smaller than the outside diameter of the eyepiece itself. In so-called negative type eyepieces, it is the virtual image of the diaphragm formed by the field lens which limits the primary image formed by the objective, but since the magnification of the field diaphragm by the field lens is not large, the statements above regarding field size are still applicable. The true angular field of the microscope may be considered to have a maximum value of less than 7° . For example, the half-diameter of the primary image field may be taken as not exceeding 10mm., and the optical tube length as 170mm. Substituting these values in the equation previously mentioned, it will be seen that the true angular field is $6^\circ 8'$.

23.2.4 Illumination. The intensity of illumination in a compound microscope is a major factor due to the usually small size of the specimen being viewed, and because of the high magnification required to resolve the details of the specimen. As a result of the intense illumination required, light from an artificial source must be condensed onto the specimen. It is noted that in some cases, sunlight or skylight are used for illumination. Most specimens are thin and transmit light. For such specimens, the illumination falls on the back of the specimen, and the light is transmitted through the specimen into the microscope. For opaque specimens, the illumination is condensed upon the upper surface and only the light which is reflected from the specimen enters the microscope. This method of illumination is designated as vertical illumination. For most specimens however, transmitted illumination is used.

23.2.5 High resolution. High resolution is a basic requirement of the compound microscope, for it is upon this characteristic that the ability of the microscope to distinguish the fine details thereof, is based.

23.2.5.1 Factors determining resolving power. In compound microscopes, the source of light is most often an incandescent lamp provided with a condenser (in photomicrography and micro-projection, arc lamps are often used). The lamp condenser concentrates the light into a second condensing system, which is a part of the microscope proper and is known as the substage condenser. When vertical illumination is required, the substage condenser is not used, and other various forms of condensers are employed to condense light onto the specimen from above. Therefore, the resolving power of the microscope depends on the following factors:

- (a) the size of the angle of the illuminating cone of rays passing through the specimen.
- (b) the ability of the optical system to accept that which has been transmitted by the specimen and to transmit a wide cone of rays.
- (c) the refractive index of the material between the specimen and the first surface of the optical system comprising the microscope's objective.

23.2.5.2 Limit of resolution. The concept of numerical aperture (N. A.) is essential in expressing the limit of resolution of the microscope. As illustrated in Figure 23.1, n is the refractive index of the medium in which the specimen, S , is immersed; θ is the half angle of the cone of incident rays; and the numerical aperture (N. A.) of the cone of rays is $n \sin \theta$. In a compound microscope, a glass cover slip, and in the case of immersion objectives a layer of immersion fluid, intervenes between the specimen and the entrant surface of the optical system. In this case, the numerical aperture becomes the product of the lesser index of refraction and the sine of the angle θ in that medium. The limit of resolution of the compound microscope, i.e., the least distance between two objects that can be seen as separate, is equal to the wavelength of light (λ) divided by the sum of the numerical apertures of the substage condenser and the microscope's objective lens.

23.3 COMPONENTS OF A COMPOUND MICROSCOPE

23.3.1 General. In order to realize the requirements for microscopic observations, the simplest form of an optical system is shown schematically in Figure 23.2. Figure 23.3 illustrates how these optical components (except the light source A and the lamp condenser B of Figure 23.2) are incorporated into a modern compound microscope. In Figure 23.3, the mirror below the substage condenser and the reflecting prism in the body between the objective and eyepiece are for mechanical convenience and are of no optical significance. The initial optical element of many compound microscopes is a mirror, which reflects light from the source into the remainder of the optical system. The mirror usually presents no design problems. One side is flat and reflects the light into the substage condenser. However, when extremely low powered objectives are used, the substage condenser usually will not illuminate the entire field of view because of its increased size. In this instance, the substage condenser may be removed, and the second side of the mirror, which is concave, will condense the light onto the specimen.

23.3.2 Illumination systems.

23.3.2.1 Simple illuminator. The simplest form of illumination for a compound microscope is a broad diffusing source, such as a ground glass, placed in front of an incandescent bulb. This source is imaged directly onto the specimen by means of the substage condenser (Figures 23.2 and 23.3). However, any granules in the ground glass would be visible in the field of view unless the image of the ground glass was slightly defocused. Therefore, such a source is satisfactory only for low power microscopy, because of its lack of illumination for high

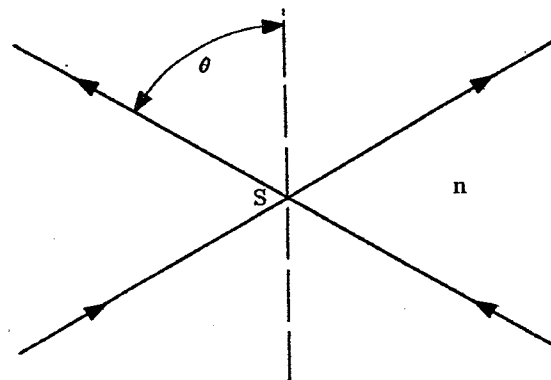


Figure 23.1- Determination of numerical aperture.

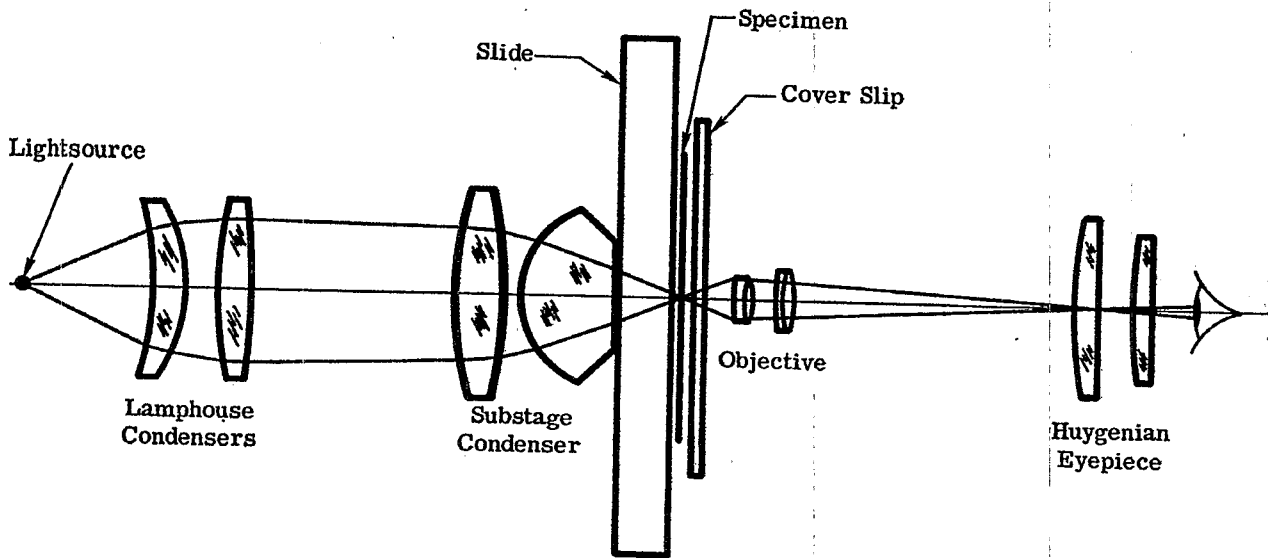


Figure 23.2- Optical schematic of a compound microscope.

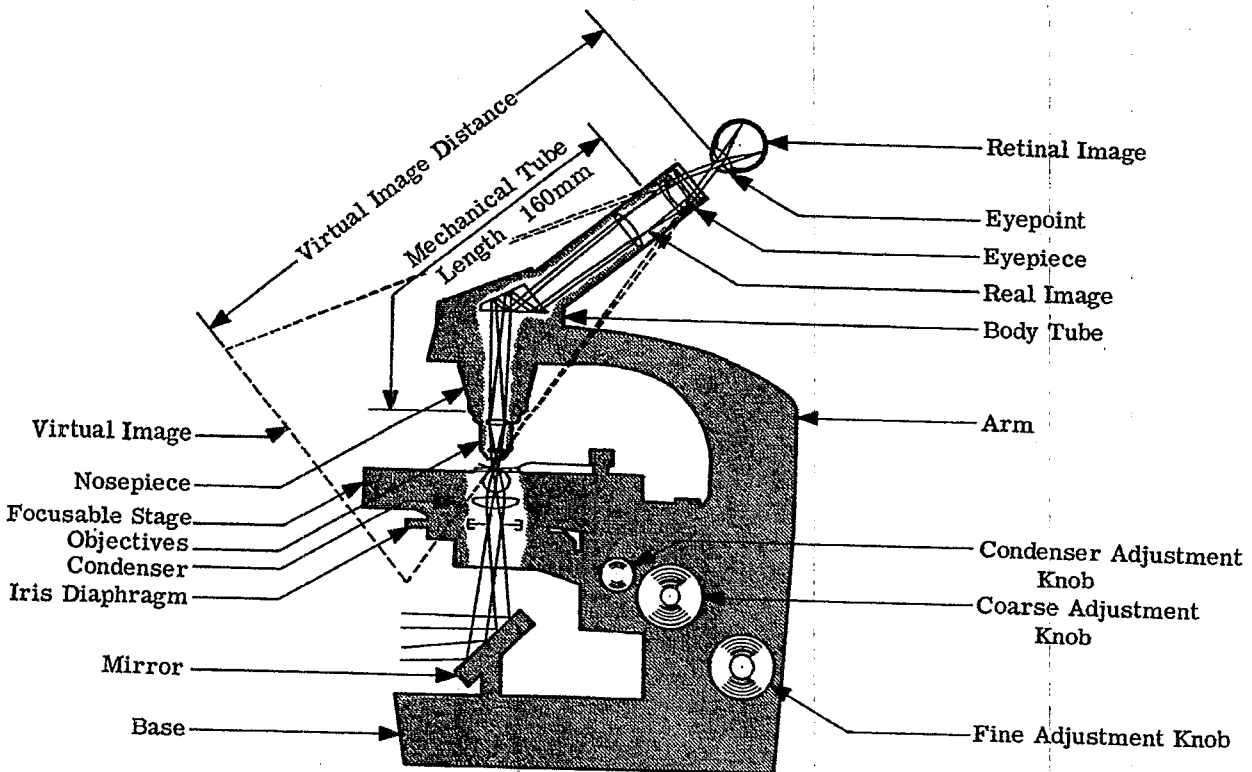


Figure 23.3- Optical and mechanical features of the microscope.

power work. It should be noted that when the source of light is focussed directly onto the specimen, the illumination is designated as critical illumination. A more efficient microscope illuminator than that discussed here previously is shown in Figure 23.4 and consists of a monoplane filament lamp behind which is a spherical reflector and in front of which is a condenser--generally a two lens system. The filament of the lamp is near the center of curvature of the spherical reflector, and the reflected images of the strands of the lamp are located between the strands themselves. This not only allows the reflected light to be utilized, but also forms a more nearly uniform primary source of light. The condenser in this system may be focussed so that the light source is imaged in the vicinity of a ground or opal glass, which in turn is focussed by the substage condenser onto the specimen (critical illumination).

23.3.2.2 From the preceding paragraph it can be seen that critical illumination has the defect of not providing completely uniform illumination over the area of the specimen, and especially for photomicrography this is disadvantageous. A system known as Kohler illumination is used to overcome this difficulty. In this system, the lamp house condenser is used to focus the primary light source onto the substage iris diaphragm, placed at the front focal surface of the substage condenser, shown in Figure 23.4. Hence the light emerging from the substage condenser consists of parallel rays, which are re-imaged by the microscope's objective at its rear focal plane. The substage condenser now focuses the lamp house condenser, Figure 23.2, onto the specimen. Since the lamp house condenser is nearly uniformly illuminated by the light source, the field of the specimen is very uniformly illuminated. For cases in which the field must be uniformly illuminated, e.g., photomicrography, a form of Kohler illumination must be used unless the light source is very uniform as with a ribbon filament lamp.

23.3.2.3 Optical requirements for an illuminator.

23.3.2.3.1 The spherical mirror offers no design problem other than that of its aperture being large enough so that the reflected light will pass through the optical elements that follow.

23.3.2.3.2 The lamp house condenser should be large enough so that its image (formed by the substage condenser on the specimen--Kohler illumination) will fill the field of view. In addition, the focal length of the lamp house condenser should be correctly determined, in order that the particular primary light source will be large enough to fill the substage iris diaphragm.

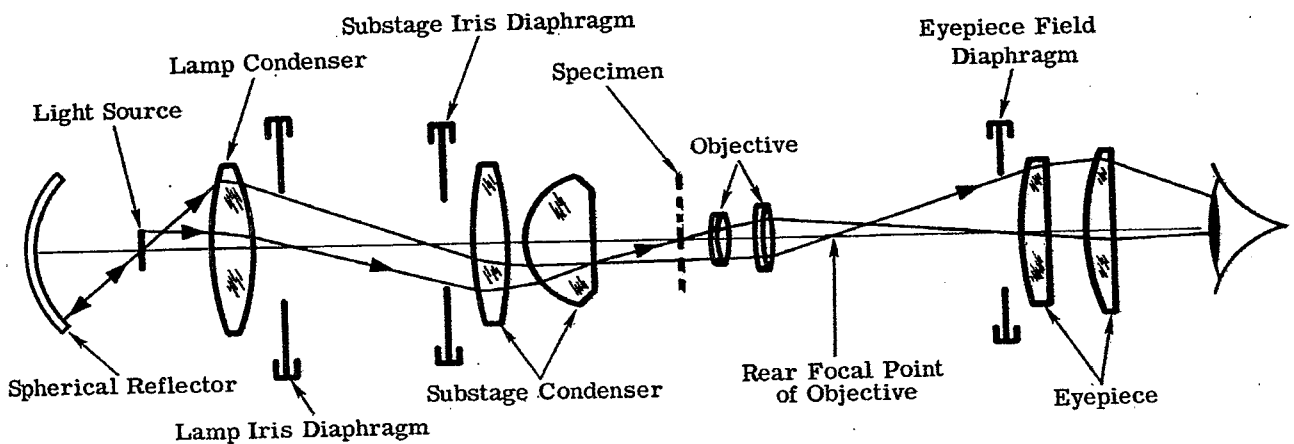


Figure 23.4- Kohler Illumination, schematic diagram.

23.3.2.3.3 An iris diaphragm is often located very close to the lamp condenser. With this design, the condenser (in Kohler illumination) and the iris diaphragm are imaged on the specimen, and if the iris diaphragm is adjustable in diameter, its image can be made to precisely fill the field. An adjustable iris diaphragm will prevent illumination of a greater area of the specimen than is necessary, and will also prevent scattered light, with its resultant loss of contrast, from entering the microscope.

23.3.2.3.4 The lamp condenser is usually a two lens, air-spaced system. It should be as well corrected for spherical aberration as is possible. The focal length must be correctly determined in order to image the filament of the lamp large enough to fill the iris diaphragm of the substage condenser (Kohler illumination) at a convenient distance (approximately 15 inches). The diameter of the condenser must be large enough so that its image, as formed by the substage condenser, covers the specimen field, when viewed with a 16mm focal length, (10x) microscope objective. It is readily apparent then, that the smaller the light source, the greater must be the speed of the condenser.

23.3.2.4 Vertical illuminators. For opaque specimens, vertical illumination is required for seeing surface details. Vertical illumination requires that the specimen field be uniformly and intensely illuminated, and that the illuminated field be limited to that portion of the specimen which is in the field of view. If the illuminated field is not limited as mentioned, an undesirable amount of light is scattered by the unviewed portion of the specimen, by the edges of the objective lenses, or by the walls of the objective. This scattering will reduce the contrast in the visual field.

23.3.2.4.1 Vertical illuminator, type A. In this type of vertical illuminator, as shown in Figure 23.5, the incident light is focussed on the specimen by being passed through the objective, in a reverse direction, and onto the specimen. The light source in this type is usually a low voltage, concentrated filament bulb, located in a housing extending laterally to the axis of the microscope. The system consists of the light source, a condenser with an iris diaphragm mounted near the light source, a second condenser, and a semi-reflector at 45° to the microscope's axis for throwing light into the rear of the microscope's objective. The two condensers image the lamp filament at the exit pupil of the objective. The second condenser images the iris diaphragm (and the first condenser) at a virtual distance of about 160mm from the microscope's objective. Therefore, an image of the iris is formed by the microscope's objective on the specimen and it is uniformly illuminated. The field covered by the illuminated spot on the specimen can be regulated in size by adjusting the diameter of the field iris diaphragm, thereby preventing the scattering of light.

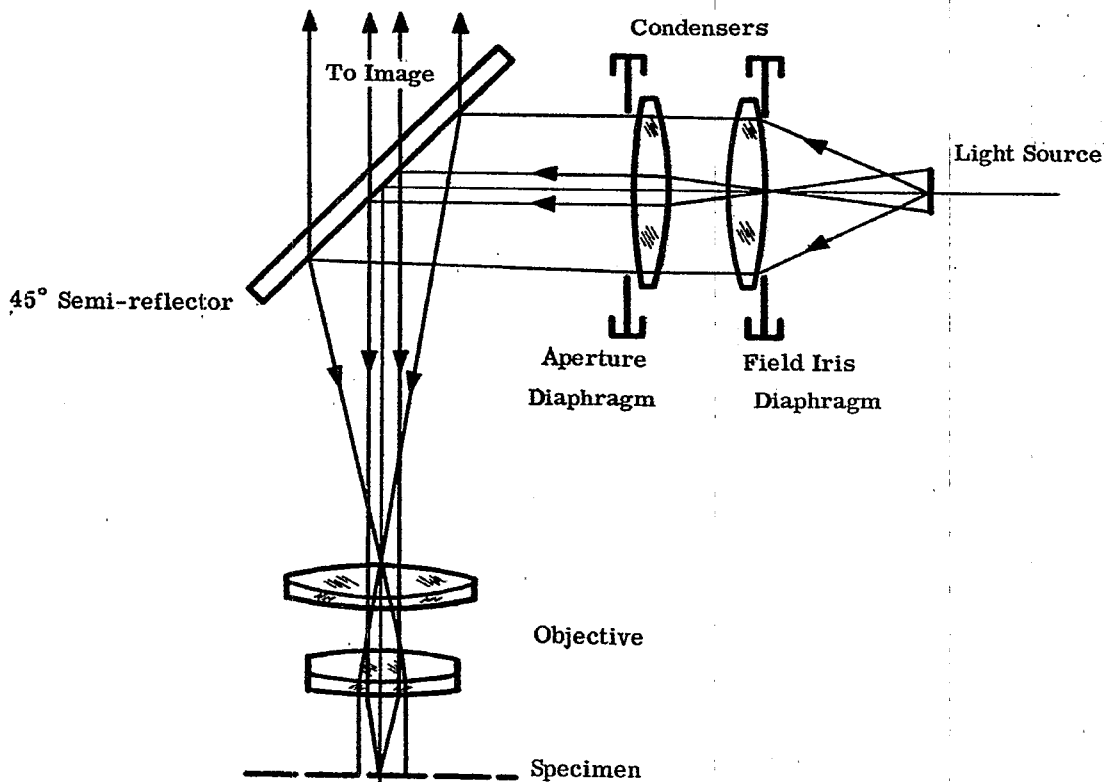


Figure 23.5- Vertical illumination.

23.3.2.4.2 Vertical illuminator, type B. In this type of illuminator, the light is not sent backwards through the objective, thereby preventing damaging internal reflections. This type of illuminator is best explained by picturing a condenser with a cylindrical hole through it axially, and the microscope's objective extending through this hole. In the actual design, the condensing elements surround the microscope's objective, and the illuminating cone of light is completely insulated from the objective's optics. Light is introduced into the condenser from the side through the use of an annular shaped full reflector. Such systems are called epi-condensers. Since the light must clear the lower rim of the microscope's objective and impinge on the specimen, it is obvious that the working distance from the objective to the specimen must be kept fairly large, therefore, this method of illumination is limited to the lower powers and numerical apertures of the objectives.

23.3.3 Substage condensers. The substage condenser concentrates light onto the specimen at a numerical aperture equal to that of the objective. There are two general classifications of condensers: the non-achromatic and the achromatic.

23.3.3.1 Non-achromatic. The non-achromatic substage condenser is used with achromatic microscope objectives which will be discussed in paragraph 23.3.4.3

23.3.3.1.1 The most common non-achromatic substage condenser is the Abbe condenser with a numerical aperture of 1.25. It is a two lens, air-spaced system, with a double convex lower lens and a plano-convex, hyperhemispherical upper element. When immersion fluids are used, the working distance between the upper plane surface and the image of the light source is equal to the optical thickness of the microscope's slide, plus a small space of a few tenths of a millimeter between the upper surface of the condenser and the slide. Reference to Figure 23.6 shows that no attempt is made to correct the chromatic aberration of the Abbe condenser, since both elements have positive power. The only variables available to the optical designer are the refractive indices of the elements, the shape of the first lens, and the separation of the two elements. The designer must adhere to a given working distance from the upper plano surface to the upper surface of the microscope slide, so that the light source (usually at a distance from infinity to a foot) can be focussed onto the specimen. In addition, the required numerical aperture is an additional consideration. A third requirement is that the focal length be such that the light source is imaged at a size sufficient to cover a specified area of the specimen, when the size and distance of the light source have been specified. (In practice, the light source is at least two inches in diameter at a minimum distance of one foot, and the field to be covered is that of a 16mm focus microscope objective). The requirements of numerical aperture and field coverage will determine the focal

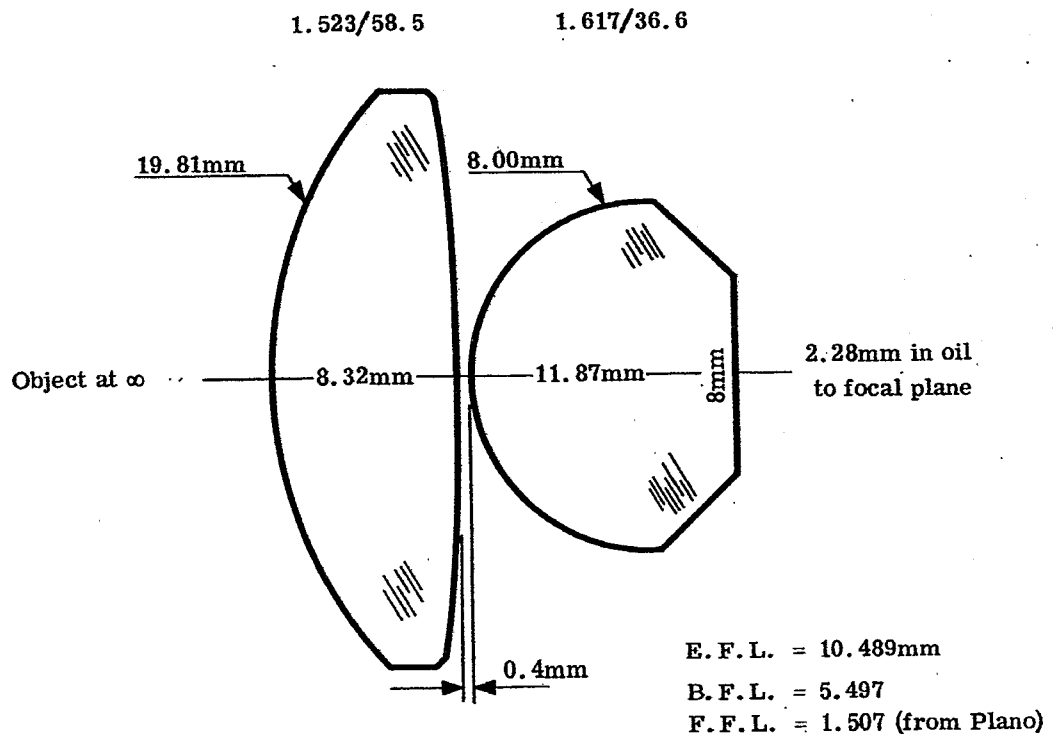


Figure 23.6- Optical layout of a 1.25 NA Abbe Condenser.

length of the front or first lens. The only aberration the designer may control in an Abbe condenser is spherical aberration. In the initial design of an Abbe condenser, graphical methods are useful and they are usually followed by mathematically triangulating a set of axial meridional rays through the system. In this way, the back lens may be bent to correct for spherical aberration. In conclusion, the two lens Abbe condenser is most commonly used with achromatic, rather than apochromatic objectives. For the latter, an achromatic condenser is used which more nearly approaches a microscope objective in form, construction, and correction. As the design principles of these condensers so closely approximates those of objectives, the reader is referred to paragraph 23.3.4.

23.3.3.2 Achromatic. Achromatic condensers are more complex than the Abbe condenser, and may consist of a triplet, doublet, meniscus, and front lens. This construction affords an opportunity for the correction of spherical aberration, coma, and chromatic aberration. This design will be seen to be essentially that of a microscope objective having the same numerical aperture, namely 1.30 and 1.40. Microscope objectives will be discussed in paragraph 23.3.4.3 through 23.3.4.5. Achromatic condensers find their most useful application when used in combination with apochromatic objectives.

23.3.4 Objectives.

23.3.4.1 Classification of objectives. Microscope objectives are classified as achromatic, semi-apochromatic, and apochromatic. If a microscope objective has been designed to correct for spherical aberration for one color of the spectrum, and for axial chromatic aberration for two colors, it is classified as an achromatic microscope objective. If the objective has been designed to correct for spherical aberration for two colors, and the axial chromatic aberration for three colors, it is classified as a apochromatic microscope objective. If the objective has been designed for correction between these two extremes, it is classified as a semi-apochromatic microscope objective.

23.3.4.2 Reasons for classification. With the magnification and resolving power (therefore the numerical aperture) corrected, the designer must take into account additional factors. Since the microscope is an instrument of almost fixed image distance, the magnification of the objective is almost proportional to its focal length. The image distance of the objective is not quite constant, since the corresponding fixed distance in the microscope is the mechanical distance from the mechanical shoulder of the objective, where it makes contact with the nosepiece, to the mechanical shoulder of the eyepiece, where it in turn makes contact with the upper end of the body tube. When the body tube of the microscope contains prisms or other optics for special purposes, the preceding statement is no longer applicable. When the body tube contains prisms or other optics for special purposes, the optical tube length can be made the same as that of a microscope not having optical elements between the objective and the eyepiece, through the use of auxiliary compensating lenses. The distance between the front principal point of the objective and the specimen must be the same for a series of objectives, if these objectives are to be parfocal, i.e., no shift in focus should be required as a change of objectives is made. In order to meet this condition, the distance from the second principal point of the objective to the mechanical mounting shoulder, is certain to be different with the different powers of objectives, so that a really constant image distance cannot be obtained. In general, the numerical aperture of a microscope objective must be increased with magnification. However, since the difficulty of correcting aberrations increases rapidly with an increase in numerical aperture, the complexity of construction of the objective also increases. A cemented doublet is satisfactory for numerical apertures below 0.25, and focal lengths of 32 and 48mm. A 16mm focus having a numerical aperture of 0.25 requires two cemented doublets in the system.

23.3.4.3 Achromatic. The cost of microscope objectives depends on the complexity of their construction, and the cost of the optical materials used. These factors increase with the numerical aperture of the objective and the degree of correction required. For most routine examinations of biological or industrial materials, the moderate corrections and construction of the achromatic type objective are sufficient. This class of objective for higher powers are constructed of the following: a hemispherical or hyper-hemispherical lens known as the front; followed by a meniscus, the second front; a cemented component, the middle; and a cemented component, the back. Some of these components can be omitted from the lower numerical apertures. For example, a 16mm focus, 0.25 numerical aperture objective achromat has two cemented doublets; an 8mm focus, 0.50 numerical aperture objective achromat has a front, no second front, but a middle and back; a 4mm focus, 0.66 numerical aperture objective achromat has a front, second front, a middle, and a back. A 1.8mm focus, 1.25 numerical aperture achromat objective has a front, second front, cemented doublet middle, and a cemented doublet back.

23.3.4.4 Semi-apochromatic. For more exacting routine microscopy, and for some kinds of research work, a higher degree of definition than that afforded by the achromatic objective is desirable. Semi-apochromats usually has flourite for one of its elements. Because flourite has a low refractive index, low dispersion, and a partial dispersion ratio different from glass, a better simultaneous correction for primary and secondary chromatic aberration and spherical aberration can be accomplished by its use as a positive element in a lens system. For example, if a flourite positive element is used with a flint glass negative element, a steep interface between the elements is attained, when the chromatic aberration is corrected. The over correction for spherical aberration, resulting from the steep interface and the large refractive difference at it, can be used to compensate for the under correction of other elements. By virtue of flourite's partial dispersion ratio being

out of line with that of glass secondary chromatic aberration is favorably influenced. Constructional data for a semi-apochromat is shown in Figure 23.7

23.3.4.5 Apochromats. Apochromats are the most highly corrected of any of the microscope objectives. The optical design considerations involved are those described in 23.3.4.4, but they must be carried to the highest possible state of perfection. The correction is accomplished by the addition of optical components in the middle and back sections of the objective, and by the use of such crystals as alum and flourite to accomplish simultaneous correction for color, coma, and spherical aberration. Flint glass of the shortened spectrum type is also used in some constructions.

23.3.5 Eyepieces. There are three important considerations in the design of a microscope eyepiece. Since the object for the eyepiece is the image formed by the other elements of the optical system, the eyepiece can be designed to correct some of the residual defects in the other elements of the microscope's optical system. Also, the design of the eyepiece must be such, that a virtual image is formed anywhere between the point of most distinct vision (approximately 10 inches distant) and infinity. Finally, the eyepiece must be designed to correct lateral chromatic aberration.

23.3.5.1 Types of eyepieces. The main types of eyepieces used in compound microscopes are the Huygenian and Ramsden, and the type known as compensating eyepieces.

23.3.5.2 Huygenian eyepiece. For observational purposes, the Huygenian is often preferred to other eyepieces, since it can be completely freed of lateral color. The Huygenian eyepiece consists of two plano-convex lenses of the same type of glass (usually spectacle crown), with the field lens having a focal length approximately three times that of the eyelens, depending on the type of correction desired. The field lens and eyelens are separated in the body tube by a distance equal to twice the focal length of the eyelens. The combination is, therefore, free of lateral chromatism, and is most widely used with achromatic objectives (see paragraph 23.3.4). As is the case with all eyepieces, the limiting aperture for image forming bundles of rays is the exit pupil of the entire optical system of the microscope. The exit pupil is generally close to the second focal point of the eyepiece, and if a field stop or diaphragm is used, it should be positioned at the first focal point of the eyelens in order that its image will be formed at infinity. To some extent in microscopy, reticles are provided, and it follows that these should also lie in the plane of the first focal point of the eyelens. However, when such is the case the reticle is magnified by the eyelens alone, and even though the eyepiece combination as a whole

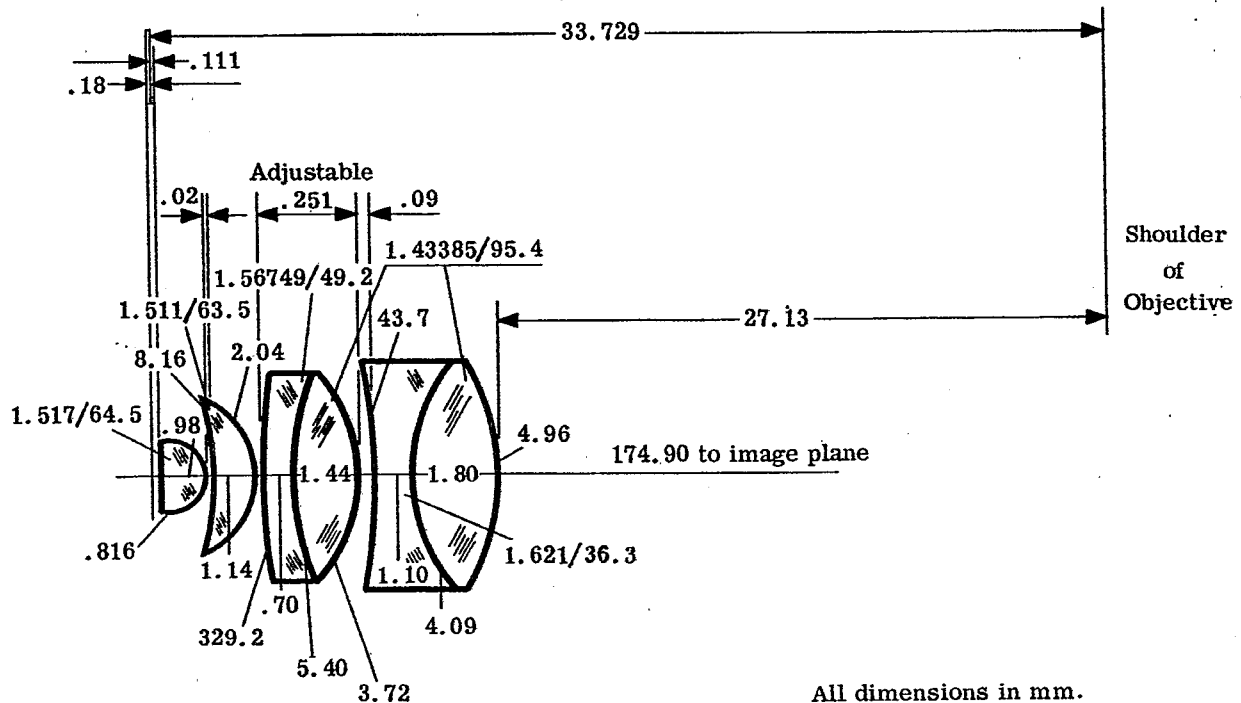
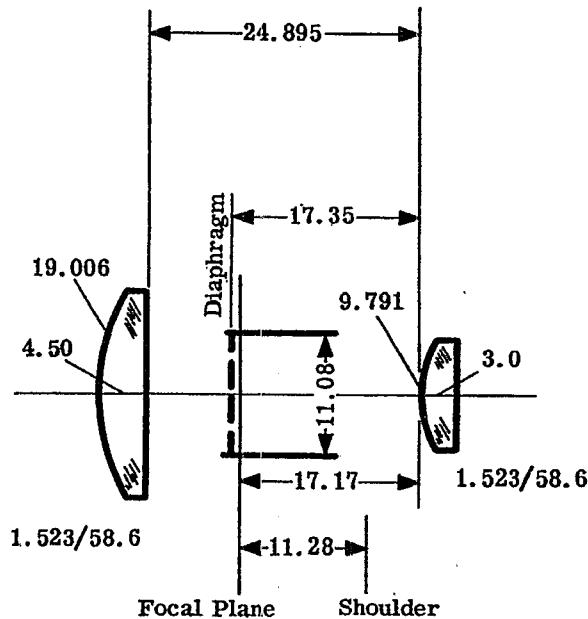


Figure 23.7- Optical layout of a 1.8mm flourite objective.

is free of lateral chromatic aberration, the corrections provided by the field lens are lacking and a large amount of aberration, particularly distortion and lateral color are introduced. To overcome this difficulty, the reticle used is kept so small that it is seen only at the center of the field. With respect to residual aberrations, the Huygenian eyepiece shows some spherical aberration, a large amount of longitudinal color, and marked pin-cushion distortion. An additional disadvantage occurs when this type of eyepiece has focal lengths less than one inch, since the eye relief is then usually too short for comfort. The reader is referenced to paragraph 6.11 where it is shown that a lens system such as this, can be designed to have constant equivalent focal lengths for all colors. An illustration and aberration graph of a Huygenian eyepiece is shown in Figure 23.8.

23.3.5.3 Ramsden eyepiece. A second type of eyepiece occasionally used with the microscope is the Ramsden eyepiece. The Ramsden eyepiece consists of two plano-convex lenses of the same type of glass (usually ordinary crown glass) and with equal focal lengths. The lenses are separated by a distance equal to two-thirds of the focal length of a single element. The focal point of this combination lies outside the system and so the eyepiece can be used to focus on an external reticle or cross hairs. With respect to aberration, the Ramsden eyepiece has more lateral color than the Huygenian, but the longitudinal color is only about half as great. The Ramsden eyepiece has about one-fifth the spherical aberration, and approximately half the distortion as found in the Huygenian eyepiece. The Ramsden eyepiece evidences no coma, and important advantage over the Huygenian is its 50 percent greater eye relief. An illustration of a Ramsden eyepiece is shown in Figure 23.9, and it is designated by usage as a positive eyepiece, in contradistinction to the negative Huygenian type.

23.3.5.4 Compensating eyepiece. The compensating eyepiece is used in conjunction with apochromatic objectives (paragraph 23.3.4.5) and as was previously stated, transverse chromatic aberration is a characteristic of these objectives. In order to correct for this aberration, an equal and opposite amount is introduced by the eyepiece. The eyepiece compensates the lateral color of the objective, and derives its name from this property. In addition to a definite amount of lateral color, the design of the eyepiece must correct for coma, spherical aberration and axial color, and its curvature of field and astigmatism must compensate those of the objective in so far as possible. In some cases, the observer wears spectacles, especially when the ocular defect is astigmatism (Myopia or hyperopia can be compensated by simply focussing the microscope), and it is therefore desirable to have the eyepoint of the microscope high enough so that there is sufficient space for the spectacle lenses between the back lens of the eyepiece and the eyepoint. This requires the back focal length of the eyepiece be sufficiently large in relation to the equivalent focal length, which determines the magnification. Such



All dimensions in mm.

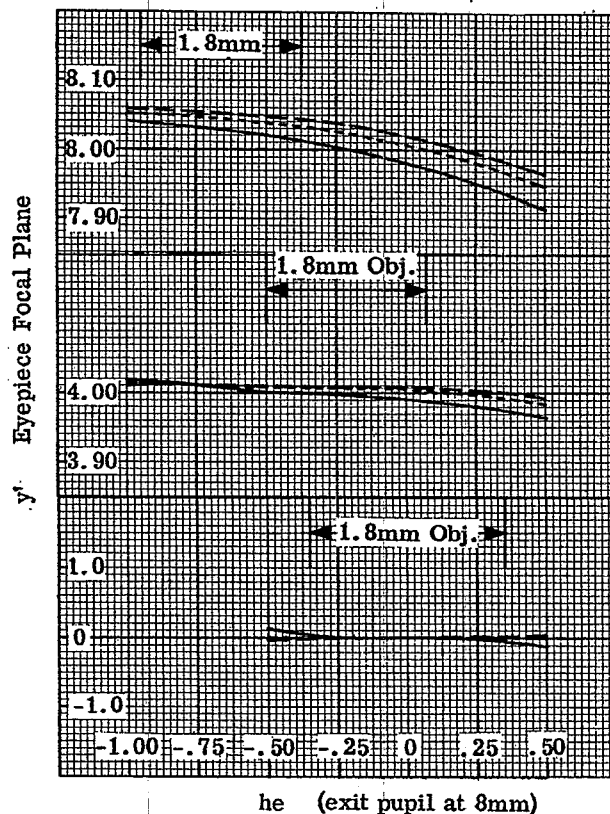


Figure 23.8- Optical layout of a 10X Huygenian eyepiece.

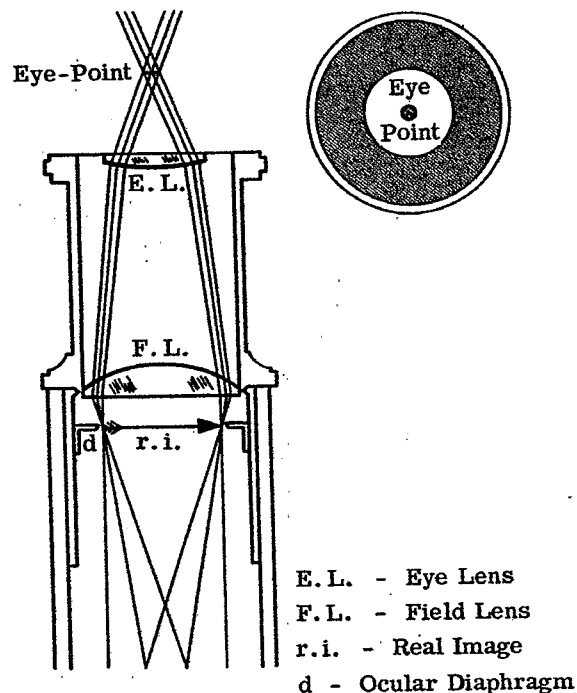


Figure 23. 9- Optical schematic of a Ramsden eyepiece.

eyepieces are designated as "High-Eyepoint" and are illustrated in Figure 23. 10. Compensating eyepieces can be of the positive or negative construction. Figure 23. 11 shows the general construction of several powers of compensating eyepieces. The design of such eyepieces is dependent on the residual aberrations of the apochromatic objectives with which they are to be used. As shown in A and B of Figure 23. 11, these eyepieces are of the negative type and are evolved from the Huygenian eyepiece by making the field lens and/or the eye-lens cemented doublets, for purposes of correction. The high eyepoint compens is of the positive type and may be considered to be derived from the Ramsden eyepiece, by making the field lens a cemented triplet. The 30x compensator shown is essentially a ratioed form of the 10x High Eyepoint eyepiece. This construction prevents the eye distance from becoming too small, although the equivalent focal length necessarily is small in order to give the required relatively high eyepiece magnification.

23. 4 DARKFIELD MICROSCOPY

23. 4. 1 General. In the ordinary microscope discussed previously, the illuminating bundles of rays enter the microscope objective and illuminate the entire field of view. The objects under examination are imaged as dark or colored details appearing against a bright background. Therefore, by this usual method of illumination, Brightfield Microscopy is accomplished. If the specimen is small, as for example with colloidal particles, or is practically transparent, the ordinary brightfield microscope does not offer sufficient contrast to render the objects visible. However, such particles have the property of scattering a portion of the incident radiation by means of diffraction, refraction, or reflection. In the field of darkfield microscopy, only the scattered light enters the microscope, while the direct illuminating beam entirely escapes the microscope's objective. Darkfield microscopy is accomplished by using the condenser to block the central portion of the light cone. The blocking of the entering light may be accomplished as detailed in 23. 4. 2 through 23. 4. 5. In both darkfield microscopy and ultra microscopy (paragraph 23. 5) the objects appear to be self-luminous in a dark field, and no light directly reaches the observer from an outside source. Light is only transmitted to the observer from the object being viewed.

23. 4. 2 Refracting darkfield condenser. A simple refracting darkfield condenser is an ordinary substage condenser provided with an opaque center stop which allows only rays traversing the outer zones of the condenser to be transmitted as shown in Figure 23. 12. The effective numerical aperture of the microscope's objective must not be greater than the numerical aperture of the obscured central portion of the condenser, in order that the oblique hollow cone of rays transmitted by the condenser will not directly enter the objective. The oblique hollow cone of light will illuminate any object at its apex or focus, the object itself then deflects a part of this

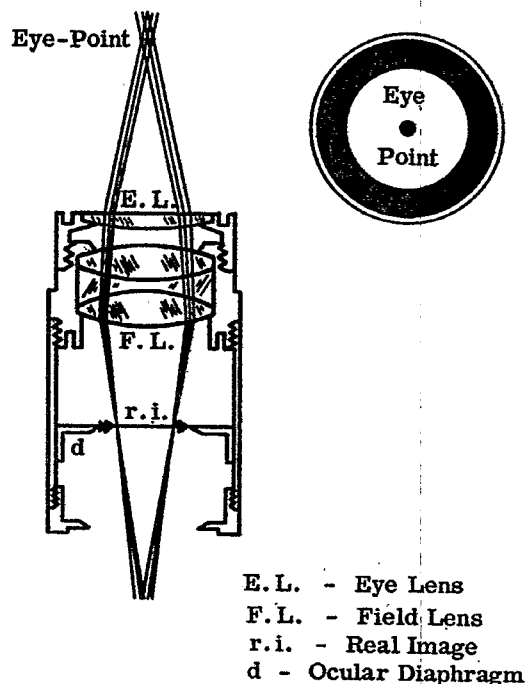


Figure 23.10- Optical schematic of a high eye-point compensating eyepiece.

light into the microscope, and the object will then seem to be self-luminous in a dark field. The smaller numerical aperture of the illuminating bundle is about 0.7, while the upper limiting numerical aperture of the condenser is about 1.2. While such an illuminator is suitable for non-critical work, the refracting condenser has too much spherical and chromatic aberration for exacting darkfield use. In order to obtain a sufficiently dark background, it is important to have a very thin section of the specimen receive the focussed light. This condition will preclude any significant amount of aberration being present in the condenser. Darkfield condensers of the reflecting type may be well corrected for those defects and are generally used for high power work.

23.4.3 Reflecting darkfield condensers. The advantage offered by a reflecting darkfield condenser, with respect to the refracting type, is its ability to form a good ring of light for darkfield work, and its ability to minimize spherical and chromatic aberration in the transmitted bundle. More light will be scattered by the specimen if the difference between the inner and outer numerical apertures of this hollow cone is large. On the other hand, the microscope's objective is functioning at a numerical aperture not greater than the lesser numerical aperture of the hollow cone. This factor determines the amount of scattered light which can be used for image formation, and also determines the resolving power of the microscope. It is common practice to have the numerical apertures of the hollow cone cover the image from 0.7 to approximately 1.25. When objectives having numerical apertures greater than 0.7 are used, it is necessary to equip them with a funnel stop. The funnel stop will reduce the numerical aperture so that no direct light passes through the objective. For high power darkfield microscopy, not all the light can pass from the condenser to the specimen unless the specimen and its slide are in oil contact with the condenser. Some reflecting darkfield condensers are made with spherical surfaces or aspheric surfaces. Aspheric reflecting darkfield condensers are more difficult to fabricate, but are theoretically better corrected than the spherical type.

23.4.4 Aspheric darkfield condensers.

23.4.4.1 Paraboloid. A paraboloidal darkfield condenser is shown in Figure 23.13 (a). This condenser is a plano-convex block of glass with the reflecting surfaces forming a true parabola, at whose focus the specimen is positioned. Since the microscope's slide is in oil contact with the upper surface of the condenser, no aberrations are introduced.

23.4.4.2 Cardioid. In the cardioid darkfield condenser, the light rays undergo two reflections; one from the inner surface which is spherical, and one from the outer surface, which is cardioidal as shown in Figure 23.13 (b). This condenser, as is the case with the paraboloidal type, is free from chromatic and spherical aberration and, since it obeys the sine condition, is termed aplanatic. It is possible to observe particles as small as

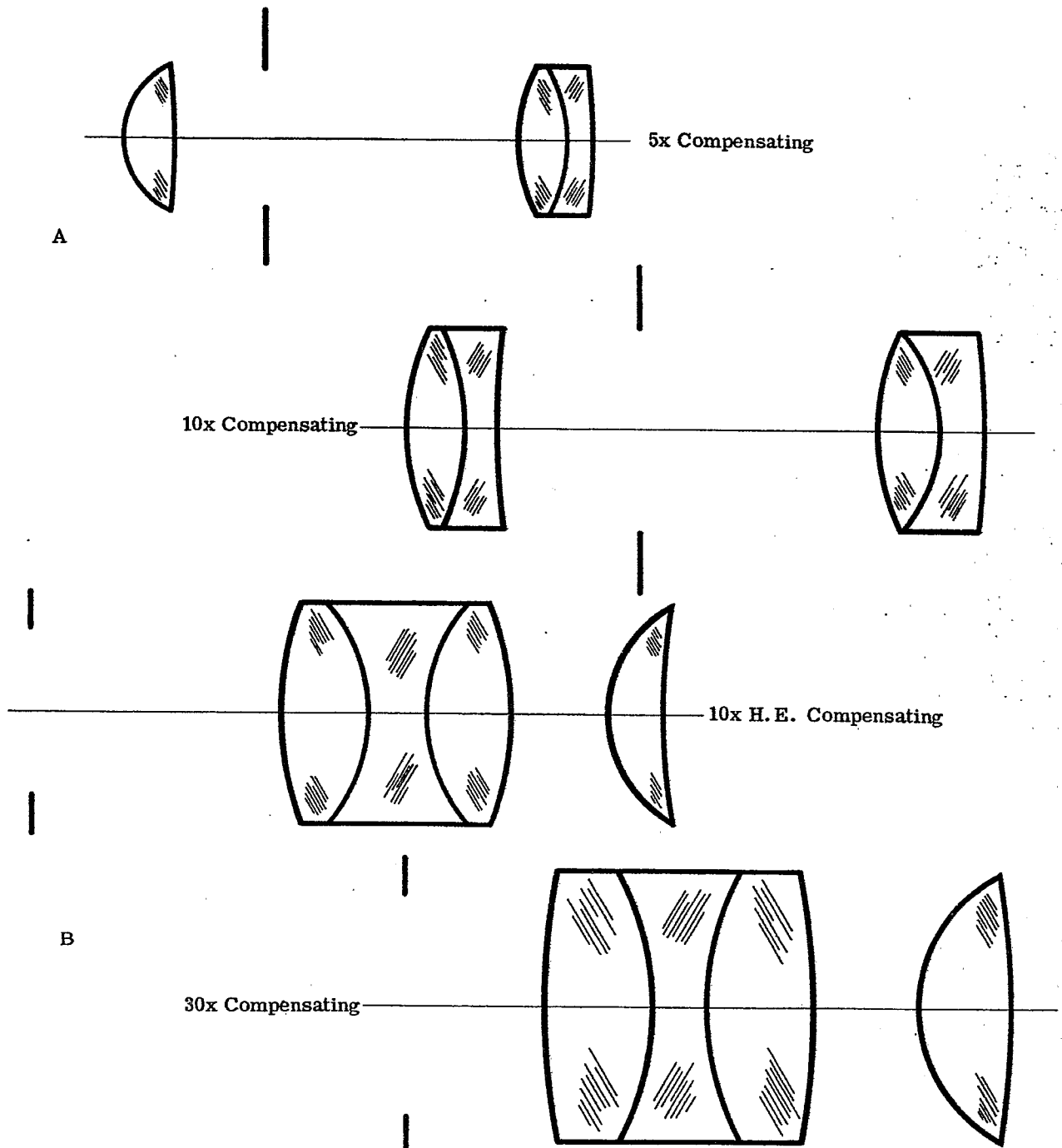


Figure 23. 11- Typical compensating eyepieces.

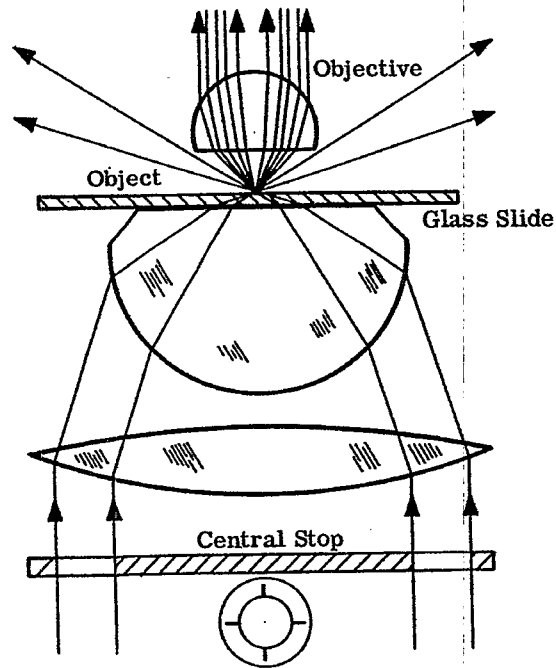


Figure 23.12- Refracting condenser with a central stop for dark-field illumination.

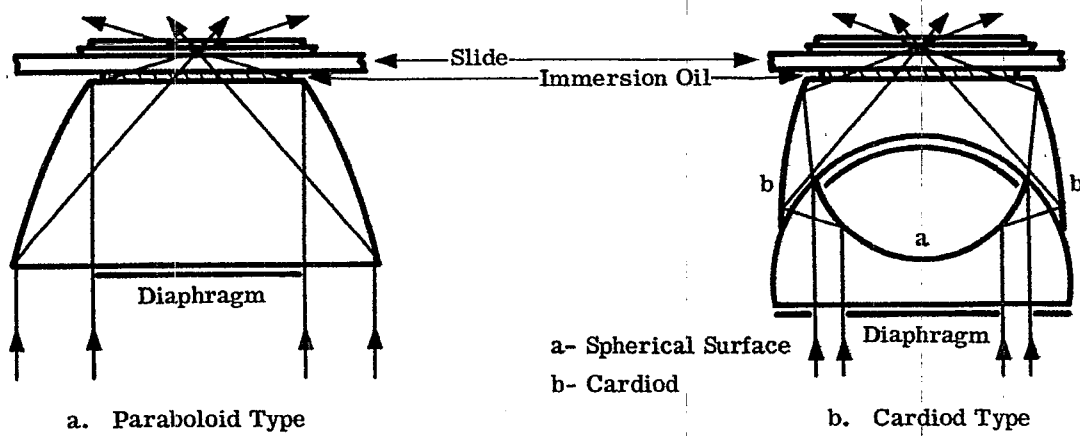


Figure 23.13- Aspheric darkfield condensers.

0.00004mm in diameter under favorable conditions with this type condenser. The disadvantage of this type of condenser is the difficulty encountered in grinding and polishing a precise cardioidal surface.

23.4.5 Spherical darkfield condensers.

23.4.5.1 Bispheric. The bispheric darkfield condenser as shown in Figure 23.14 is constructed with both surfaces spherical, thereby avoiding the difficulty of precise grinding and polishing (as is the case with the cardioid type). The highly precise spherical surfaces can then be used with only slight deviations from theoretical considerations.

23.5 ULTRAMICROSCOPY

23.5.1 General. As indicated in the conclusion of paragraph 23.4.1, darkfield microscopy and ultramicroscopy are similar in their approach to the problem of studying objects or specimens. The two approaches differ only in the size of the object to be observed. Darkfield microscopes deal with objects of approximately 0.2μ or more in diameter, that is, those which come within the resolving power of the microscope. Ultramicroscopy deals with objects so small that the details cannot be resolved, but the presence of the object is inferred by the presence of light which the object transmits in the instrument. Some of the details of the specimen viewed with the darkfield microscope can be resolved, but some details are so small that they show simply as points of light, usually in the form of so-called diffraction discs. The larger details in the specimen come within the province of darkfield microscopy, while the smaller details are the concern of ultramicroscopy.

23.5.2 Characteristics.

23.5.2.1 Ultramicroscopes pass a narrow beam of light through the specimen at right angles to the axis of the viewing microscope. With a strong light source, such as a carbon arc, ultramicroscopes are excellent for viewing and counting particles in colloidal suspension. Figure 23.15 illustrates the essential components of a slit ultramicroscope. The arc (a) is imaged by the lens (b) on the cross slits (c). The cross slits (c) are imaged by a long working distance microscope objective (d) into cell (e), which is provided with two windows. The object to be viewed is introduced into the cell (e) and viewed by the microscope (f) through the upper window of the cell (e). In the cell, the Tyndall beam can be clearly seen in the microscope. By means of an eye-

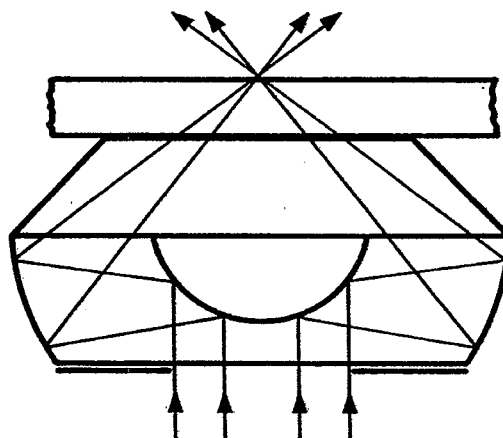


Figure 23.14- Bispheric (bicentric) darkfield condenser.

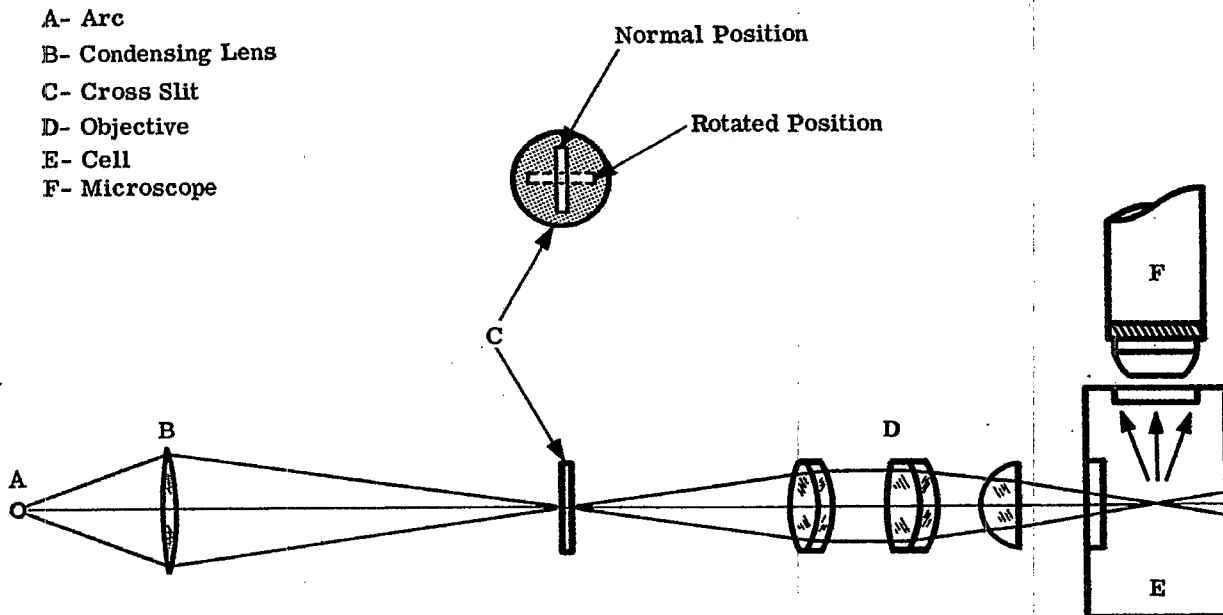


Figure 23.15- Elements of a slit ultramicroscope.

piece scale, the width and length of the beam can be measured. Therefore, if the cross slits (c) are rotated through 90° , the depth of the beam becomes the new width, and it can be measured. In this way, the volume of an illuminated portion of the contents of the cell (e) can be determined. In addition, the number of particles in the volume can be counted, and the number of colloidal particles per unit volume determined. Since the colloidal particles appear as diffraction discs, there is no need for high power or resolution in the viewing microscope.

23.6 PHASE MICROSCOPY

23.6.1 General. Nearly transparent materials having optical path (the product of the thickness and refractive index of the specimen) differences can be observed either with a phase or interference microscope. However, in contradistinction to the interference microscope (paragraph 23.7), the phase contrast is accomplished by the recombination in the image of direct light with the light deviated by the object after modification by a diffraction plate. It is interesting to note that a brightfield microscope may be converted to a phase microscope by the substitution of a phase condenser and phase objective. Similarly, the designer should keep in mind that a single contrast may be adequate for a given class of specimens, while other specimens may require several contrasts to reveal all of their structure.

23.6.2 Characteristics. The characteristics of a phase microscope can be seen from Figure 23.16. An annular diaphragm is placed in front of the condenser. When the annular diaphragm is uniformly illuminated, an image of it is formed in the objective near its focal plane, between the lens system. It can then be seen that all the light passes through this ring image or conjugate area when no specimen is present. However, when a specimen is being examined, some light is deviated through the rest of the area of the diffraction plate in the objective. The placing of a diffraction plate at this point differentially affects light deviated by the specimen, and the direct light from the background.

23.6.3 Principles.

23.6.3.1 The principles on which the phase microscope is based are shown in Figures 23.17 and 23.18. Figure 23.17 shows a light wave A' passing through a transparent object C. You will notice that A' has slowed down with respect to light wave A, which did not pass through the transparent object, and accordingly the two light waves are out-of-phase. However, the human eye and light-sensitive photographic plates are insensitive to phase differences, and as a result the image can scarcely be seen or photographed. Light wave A" in Figure

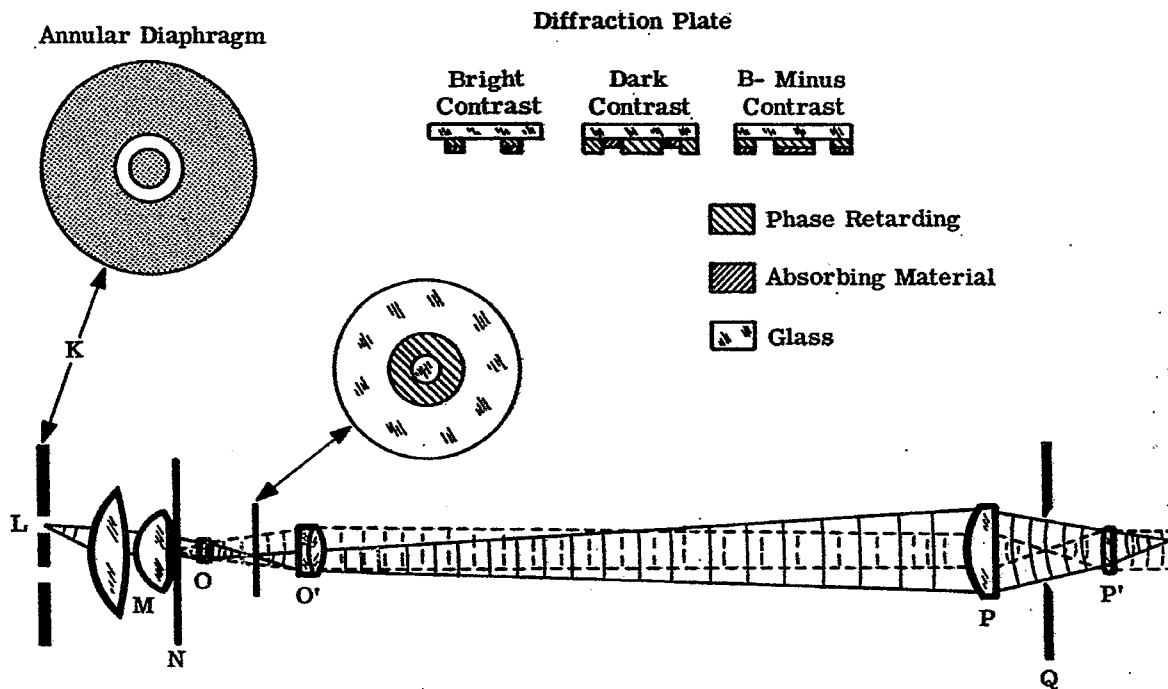


Figure 23.16- Elements of a phase microscope.

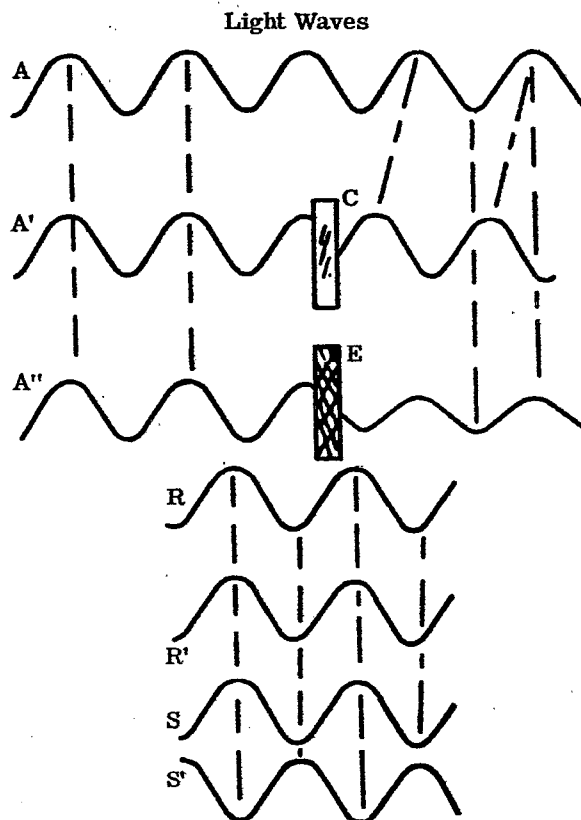


Figure 23.17- Passage of waves through mediums.

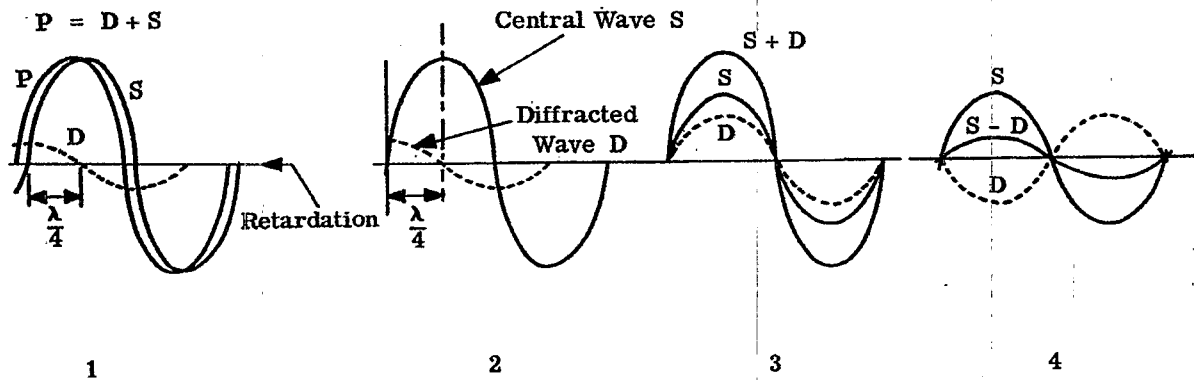


Figure 23.18:- Superimposed light waves.

23.6.17 passes through an absorbing medium E and is thereby reduced in amplitude as shown. However, in contradistinction to phase differences, amplitude differences are visible. When light waves of the same phase and amplitude are combined in the image as shown by R & R' in Figure 23.17, they add to produce brighter contrast. Similarly, dark contrast can be obtained by producing light waves which are out-of-phase or amplitude with each other as shown by S and S' in Figure 23.17, and combinations of amplitude and phase differences can be obtained which will produce lighter or darker greys.

23.6.3.2 Light waves may be superimposed as shown in Figure 23.18. Section 1 shows that the wave P, resulting from a slightly retarding particle, may be broken up into two waves S and D. The central wave S and the diffracted wave D, are shown again in Section 2. Section 3 demonstrates the result of using a bright contrast diffraction plate. The wave S has been partially absorbed, and wave D has been retarded, so that S and D are out-of-phase and produce a darker image.

23.6.3.3 The phase relationship of the light passing through a system of different optical paths has been altered, and the detail from the optical path differences, or slight absorptions of the specimen, will become visible within the microscope by the phase system elements (paragraph 23.6.2). By using an appropriate diffraction plate as previously discussed, it is then possible to increase or decrease the contrast of the image directly, or after reversing to change the contrast tone from bright to dark.

23.6.4 The diffraction plate. The diffraction plate consists of optical glass on which is evaporated, in vacuum, a very thin layer of metal, or a layer of a dielectric, or both. The layer of metal absorbs light, while the dielectric retards the light. The layer of metal or dielectric must be of sufficient size to cover either the image of the annular diaphragm formed in the objective or complimentary area of the remainder of the objective. These layers act upon the direct light from the background, and the deviated light from the specimen, so that recombination in the image will produce visible phase or absorption differences in the specimen.

23.6.4.1 The bright contrast diffraction plate absorbs, retards, or retards and absorbs the undeviated light, but has no effect on the deviated light. When this bright contrast diffraction plate is used, regions in the specimen of greater optical path will appear brighter than those of a lesser optical path.

23.6.4.2 The dark diffraction plate absorbs the undeviated light, and retards the light deviated by the specimen. The regions of greater optical path difference in the specimen will then appear darker. The effect of the dark diffraction plate is solely on the deviated light, and the degree of contrast is controlled by the width of the annulus and the thickness of the absorbing and retarding layer of the diffraction plate.

23.6.5 Disadvantages encountered with phase microscopy. As noted previously, phase contrast is accomplished in the phase microscope by the recombination of the direct and deviated light in the image, after diffraction. However, optical path differences and small absorption differences may be involved in this recombination and the resultant might be more appropriately termed "densiphase contrast" as suggested by Bennett, et. al. (1) Presently, phase microscopes have been modified to provide variable contrast, but not to measure the densiphase detail. Also, as the phase microscope redistributes the light in the image, haloes are often seen around the observed details, although the proper diffraction plate may lessen this condition. In addition, phase microscopes will make the optical path differences visible, but not their numerical magnitude.

23.7 INTERFERENCE MICROSCOPY

23.7.1 General. Interferometry, while well established in other fields, has only recently been applied to microscopy. Two methods of interference microscopy presently exist, the multiple beam method which is used extensively in the examination of surfaces of opaque materials having good reflection and in the examination of transparent materials, and the two beam method.

23.7.2 Characteristics.

23.7.2.1 Interference contrast. Interference contrast is accomplished by the recombination in the image of two beams of coherent light (from the same source), one of which is modified by passing through the specimen. In contradistinction to the phase microscope, the interference microscope will not produce haloes around the details. In addition, the interference microscope provides variable color contrast with white light illumination, and intensity variation in the color of the monochromatic light when monochromatic light is used. Similarly, with monochromatic light the interference microscope can provide measurement of the optical path differences in the specimen. It is interesting to note that when the thickness of the specimen is known, the refractive index can be measured, and in the case where the specimen is placed in a media of a different known refractive index, both the thickness and index can be measured. Also, interference microscopes have increased vertical resolution, but have the same lateral resolution as other light microscopes.

23.7.2.2 Multiple beam interference microscope. In the multiple beam method, the specimen to be examined is mounted between two flat, metalized, reflecting surfaces, and illuminated with parallel, monochromatic light. The recombinations resulting from repeated reflections of the light through the specimen produce fringes, which are used to measure the optical path differences (within reasonably transparent specimens).

23.7.2.3 Two beam interference microscope. With the two beam method, coherent illumination (from a single source) is so divided that part of the light passes in focus through the specimen, and the remainder passes to one side or is out-of-focus at the specimen. On recombining the light, the beams interfere to produce measurable patterns from which the optical path differences can be determined. This beam separation can be accomplished by reflection or polarization.

23.7.3 Principles.

23.7.3.1 The A O Baker interference microscope, Figure 23.19, illustrates the principles of interference microscopy. This microscope is fundamentally a polarizing microscope modified into a two-beam interferometer. The condenser has a birefringent plate which divides the light into two beams and the objective has a corresponding plate which recombines the beams after one of them has passed through the specimen. Above the objective is a quarter-wave compensator and an analyzer. Various eyepieces may be used to obtain different magnifications with the Shearing or Double Focus types of 10X, 40X and 100X objectives.

23.7.3.1.1 The polarizer below the condenser polarizes the light in a plane at 45° to the axis of the birefringent plate. The birefringent plate at the top of the condenser separates the polarized light into two beams which are plane-polarized at right angles to each other. One beam passes through the specimen, and the other passes to one side of the specimen in the Shearing system. In the Double Focus system one beam focuses at the specimen and the other spreads around the specimen to focus above it. The phase of the beam passing through the specimen is changed by the local variations in optical thickness in each portion of the specimen; while the changes in the reference beam depend on the average optical thickness of the specimen and the region around it in the Double Focus system; or the region to one side of the specimen in the Shearing system, as shown in Figure 23.20

(1) Bennett, A. H. Jupnik, H., Osterburg, H. and O. W. Richards, Phase Microscopy, pg. 11, John Wiley & Sons, New York, 1951.

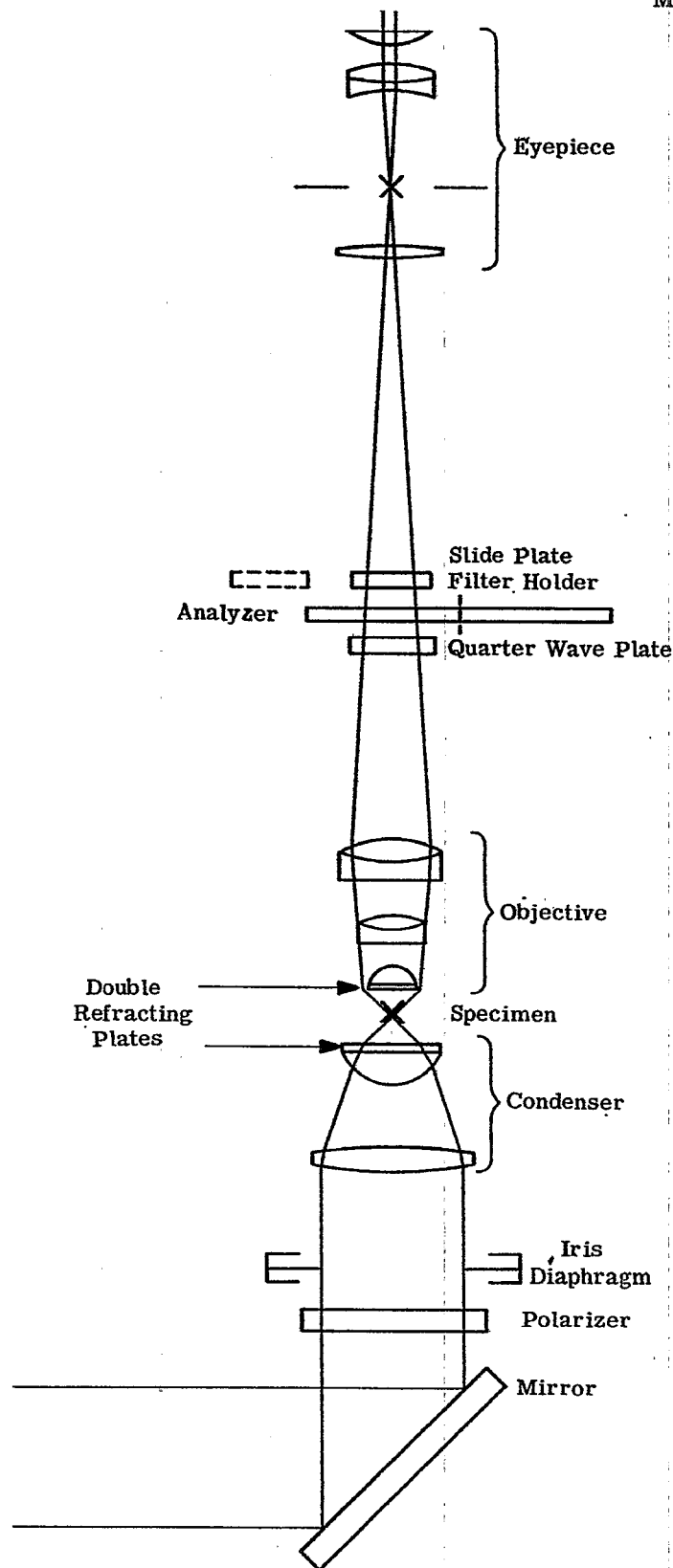


Figure 23.19- Optical schematic of AO Baker interference microscope.

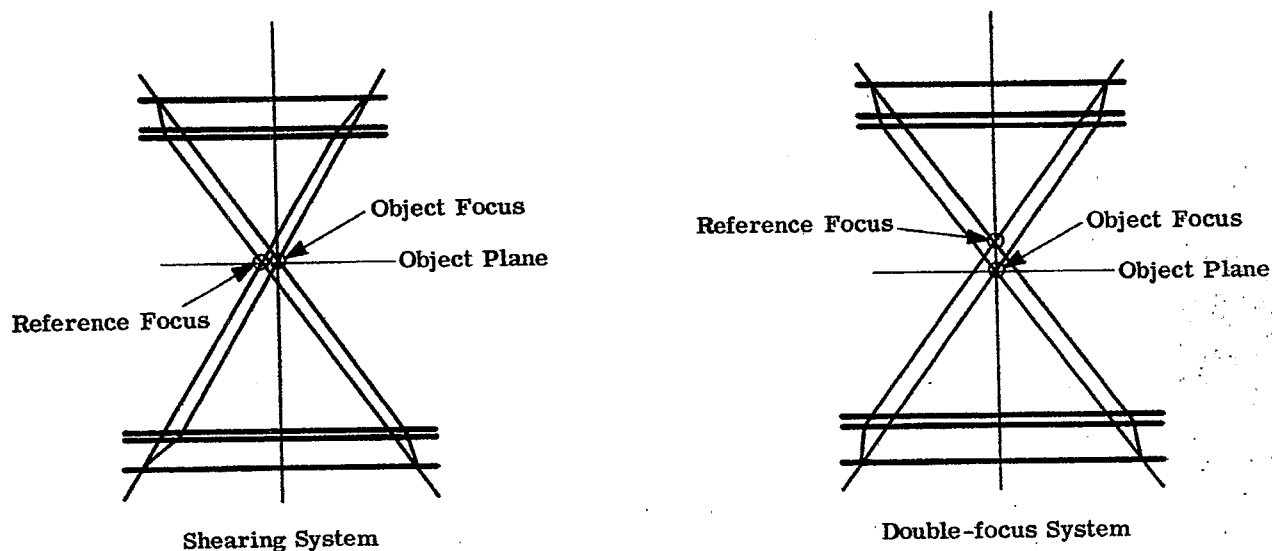


Figure 23.20- Path of light rays through a Shearing and Double-Focus system.

23.7.3.1.2 The birefringent plate on the front of the objective unites the two beams and the quarter-wave plate changes the two oppositely polarized beams into left and right-hand circularly polarized light. The resultant of two circularly polarized beams is plane polarized light, the direction of the plane depending on the phase difference between the circularly polarized beams. Thus the phase differences in the specimen can be determined by turning the analyzer to the position of minimum luminance, or extinction, in the image.

23.7.3.1.3 A vector theory (2, 3) and an integration theory (4) have been proposed for the mathematical analysis of this type of microscope.

23.7.4 Interference colors.

23.7.4.1 Light passes through an optically denser medium more slowly than in a less dense medium and is retarded, with respect to light, through the less dense medium. The amount of retardation (phase difference) is proportional to the difference in refractive index for the particular wavelength considered.

23.7.4.2 For example, should the denser regions of a specimen illuminated with light from a mercury arc retard the blue light exactly one-half wavelength and the analyzer be set to extinguish the blue light, the specimen then would be seen only in the remaining yellow-green light.

23.7.4.3 With tungsten light, the blue is not limited to such a single wavelength as the mercury arc, but is a band of light ($\pm 440 - 490\mu$). A single particle cannot retard exactly to a half wavelength all of these blue wavelengths, therefore some blue will be lost and some transmitted and the particle will appear, more or less yellow, depending on the amount of blue lost.

23.7.4.4 Phase changes affect other colors in a similar manner and the actual interference colors depend on the composition of the light from the illuminator and on how the optical paths in the specimen retard or advance each wavelength (color). The relative amount of each wavelength passing through the analyzer determines the color of the particle.

(2) J. Roy, A Vector Theory of Phase Contrast and Interference Contrast. *Micr. Soc.* 75:23-37, 1955.

(3) G. Oster, A. W. Pollister, *Physical Techniques in Biological Research* 29-90 Vol. III, Academic Press, New York.

(4) *ibid* (3), 310-437, Vol. I.

23.7.4.5 Light is radiation to which the eye is sensitive (380-740m μ) and can be seen. Interference microscopy is possible with invisible, infrared and ultraviolet radiation with receptors sensitive to the radiation used.

23.7.5 Illumination and filters.

23.7.5.1 Measurement of the optical path requires the use of monochromatic light to avoid the color interferences mentioned in paragraph 23.7.4. Monochromatic light is light of a single wavelength and is usually obtained from a single line of a spectral source. As the mercury arc has most of its radiation concentrated in a few lines it is the usual source for such illumination. The mercury arc has the further advantage in that the green line of 548m μ wavelength is quite close to the maximum sensitivity of the human eye (555m μ). Sodium light is suitable, although not as comfortable for visual use.

23.7.5.2 To isolate the light from a single line in the mercury spectrum a filter is used that transmits the light from the desired line and absorbs the light from the other bright lines in the spectrum of this source.

23.7.5.3 Some filters for use with mercury arcs are listed in Table 23.1. The least expensive is the Wratten 62 or 74, but these transmit only about 10% of the green light and do not exclude the light from the yellow line. The Corning CS4-120 transmits more of the green and no yellow. The Wratten 77 and 77A also transmit more green light than the 62 or 74, but for monochromatic light need to be combined with the 58 filter which reduces the light correspondingly. Filters for isolating the blue and yellow mercury lines are included in the table.

23.7.5.4 For some measurement, where the highest precision is not required, approximately monochromatic light is adequate and may be obtained with tungsten lamps and "narrow band" filters or with "interference" filters. The latter often have the disadvantage of low transmission.

23.7.5.5 The H85-C3 or H100-A4 (formerly AH3 and AH4) mercury arcs are satisfactory for many visual applications, but require long exposure when photomicrographs are to be made. More intense mercury arc sources such as the B-T-H 250 and the Osram HB0200 give more light, especially when monochromatic light is used, and are desirable for photography.

23.7.5.6 Light from the mercury arc without a color filter can be used for variable color contrast microscopy

Mercury line	Blue, 0.436 μ	Green, 0.546 μ	Yellow, 0.577 μ
Relative energy	80%	100%	88%
Eye Relative luminosity (100 at 0.555 μ)	1.8	98.4	89.8
Filter	% Trans. # Rel. Vis.*	% Trans. # Rel. Vis.*	% Trans. # Rel. Vis.*
Corning CS4-120	0 -	44 43	0 -
Corning CS-584	22 3	0 --	0 -
Ilford 625	0 -	35 34	8 6
Wratten 50	6.4 0.9	0 0	0 0
Wratten 62	0 -	10 9.8	0.5 0.4
Wratten 74	0 -	10 9.8	0.2 0.2
Wratten 77A	0 -	68 67	0 0
Wratten 77A 58	0 -	29 28	0 0
Wratten 77	0 -	74 73	0.5 0.4
Wratten 77 58	0 -	31 31	0.06 -
Wratten 58	0 -	42 31	11 9
Wratten 22	0 -	0 -	71 62

*Relative luminosity - Relative energy - filter transmission - relative luminosity of the ICI Standard Observer.

#Trans. = transmittance.

Table 23.1- Table of visual efficiency of isolating filters for the H100-A4 Mercury Arc (based on nominal values from manufacturer's literature).

although it will be seen that the mercury light has very little orange and red as compared to tungsten or daylight. When used with a filter the path differences in the specimen are seen only in the color of the filter, but with variable intensity.

23.8 POLARIZING MICROSCOPE

23.8.1 General.

23.8.1.1 The polarizing microscope is a brightfield microscope modified for examining the specimen in polarized light, with auxiliary equipment for measuring the effect of the specimen on the polarized light. A laboratory microscope can be used with polarized light by placing discs of Polaroid under the condenser and in or on the ocular. Such simple polarization will reveal the colors in birefringent materials and show strain.

23.8.1.2 For measurement, a specialized microscope is necessary. The polarizing microscope has a polarizing prism of the Nicol or Ahrens type under the condenser. The chemical type has a cap analyzer over the eyepiece and the petrographic type has the analyzer in a slide so that it can be pushed into or out of the optical axis in the body tube of the microscope. The upper lenses of the condenser are arranged so that they may be moved into or away from the optical axis of the microscope.

23.8.2 Characteristics.

23.8.2.1 Strain-free optics are necessary in the design of a polarizing microscope, and the objectives are usually mounted in centering rings of a quick change type of nosepiece.

23.8.2.2 When a Bertrand lens is pushed into the optical axis, it forms, with the ocular, a telescope for viewing the back aperture of the objective. A slot is provided for moving a quartz wedge or other compensators into the optical axis.

23.8.2.3 The ocular contains cross hairs and is positioned in the ocular tube to prevent rotation. The polarizer is rotatable to position it at 180° to the polarization angle of the analyzer and a centering rotatable stage with graduated scale and vernier are used to measure the orientation of the specimen.

23.8.2.4 The improved Polaroid is satisfactory and is used to replace the expensive crystal polarizers in some modern instruments and many special compensators, multi-axis stages and other auxiliary equipment are available and useful. The birefringence of biological materials is small, and more elaborate polarizing microscopes have been built to meet this need. One marked improvement is the rectifier for compensating depolarization from the curved objective lens that makes possible the use of the nearly full aperture of the oil immersion objective.

23.9 FLUORESCENCE MICROSCOPES

23.9.1 General.

23.9.1.1 Fluorescence microscopy can be accomplished with a brightfield microscope when the specimen is irradiated with ultraviolet radiation. A source of filtered radiation (usually a high pressure mercury arc) is necessary and an ultraviolet absorbing filter is placed on, or in, the ocular to prevent ultraviolet radiation not absorbed by the specimen from reaching the eye. A front-surface, aluminized mirror is more efficient than a silvered mirror.

23.9.2 Characteristics.

23.9.2.1 For short wavelength ultraviolet, necessary in the study of some minerals, the condenser and slide must be of quartz or other UV transmitting materials, or a catoptric condenser be used. Long wavelengths ($>330\text{m}\mu$) UV pass through the ordinary microscope optics and they are satisfactory.

23.9.2.2 The most efficient system uses a crossed filter technic with a brightfield condenser. The lamp filter passes the radiation absorbed by the specimen and the ocular protective filter is chosen to absorb the ultraviolet, but to pass the light emitted by the specimen. When an efficient cross-filter system is not possible, a darkfield condenser is used with a thinner UV isolating filter. A colorless UV filter is usually required for the ocular as some of the UV may be scattered by the specimen into the objective.

23.9.2.3 Achromatic objectives and Abbe condensers are preferable as the chromatically corrected ones often contain fluorescent materials which introduce glare and reduces visibility. The Abbe NA 1.40 will concentrate more energy on the specimen than the usual NA 1.25 condenser.

23.10 THE STEREOSCOPIC MICROSCOPE

23.10.1 General.

23.10.1.1 The bi-objective, binocular microscope, reinvented by Greenough is made by combining two microscopes, Figure 23.21, so that the right eye sees with the right hand side, and the left eye with the left hand side. As each eye receives a separate disparate view, true stereopsis occurs. When the angles of the objective and ocular convergence are the same, true or orthostereopsis is provided. By changing these angles increased or decreased depth can be provided.

23.10.2 Characteristics.

23.10.2.1 Prisms are included to erect the image and such instruments are useful for dissection and for the examination of small parts.

23.10.2.2 Since two objectives are required, the mechanical limitations of placement limits the numerical aperture to about 0.12 and there is no advantage in using magnifications over about 120X.

23.10.2.3 A recent modification places the paired objectives in a rotatable turret with a single, large, corrected lens between them and the specimen. By turning the turret, magnification can be readily varied within the limitations of the cycloptic microscope. Another improvement is to build the paired objectives into a zoom system so that the magnification can be varied continuously throughout its range.

23.11 PETROGRAPHIC MICROSCOPE

23.11.1 General.

23.11.1.1 The optical system of the petrographic microscope has been so adapted that the methods of petrographic measurements can be made.

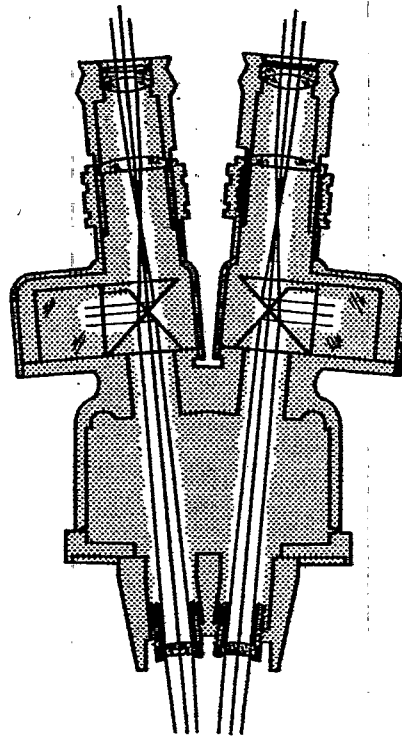


Figure 23.21- Optical schematic of a stereoscopic microscope.

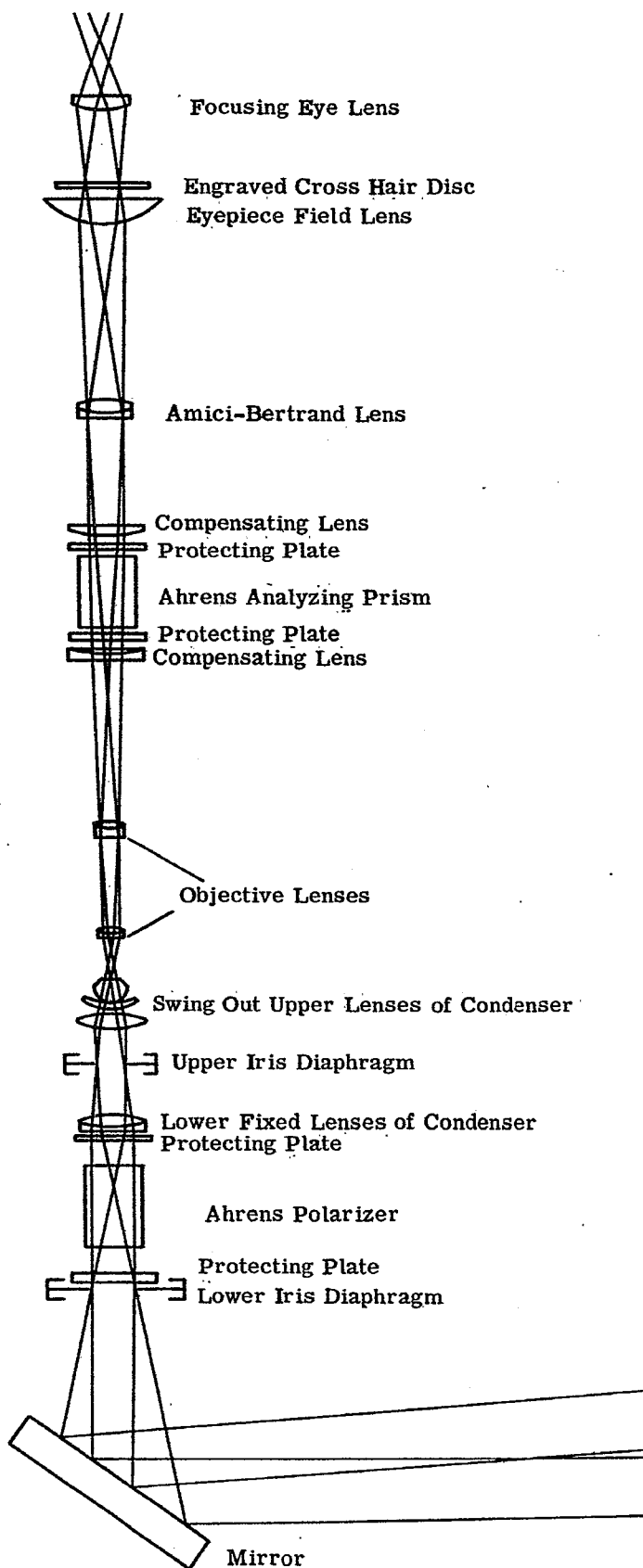


Figure 23. 22- Optical system of a petrographic microscope.

23.11.2 Characteristics.

23.11.2.1 Substage condenser. The substage condenser is made up of two parts one of which is designated as the lower fixed lenses of the condenser and the other as the swing out upper lenses of condenser. When these two systems are working together, the NA of the combination may be as great as 1.40. When only the lower part is used, the NA may be as low as 0.25. At the lower (front) focal plane of each of the above condensers, is located an iris diaphragm designated in Figure 23.22 as the lower iris diaphragm and the upper iris diaphragm. Beneath the lower condenser and between it and the lower iris diaphragm is the polarizer which may consist of a Nicol or Ahrens polarizing prism or a sheet of Polaroid. The polarizer is generally rotatable and provided with an angular scale. A detent stop may indicate the zero setting of the polarizer.

23.11.2.2 Objectives. The objective lenses used in the petrographic microscope are identical in design with the achromatic series of microscope objectives already mentioned. However these objectives must be free from strain otherwise their birefringence will interfere with measurements made upon mineral specimens.

23.11.2.3 Analyzing system. The analyzer may be a Polaroid plate or a polarizing prism of the Nicol or Ahrens type. The light passing from the objective to the eyepiece is convergent. Since a polarizing prism will produce astigmatism under such circumstances, it is necessary to parallelize the light traversing the polarizing prism. For this purpose a negative lens is used below the analyzer to cause the convergent light to become parallel. Above the analyzer is placed a convergent or positive lens of such focal length that the rays are focussed on the cross hairs of the eyepiece. These lenses need not be achromatic as the image forming bundles of rays are of such a small aperture. The introduction of these compensating lenses necessarily change the initial magnification and to avoid having different magnifications when the analyzer is inserted or withdrawn from its position on the axis of the instrument, the compensating lenses are fixed inside the body tube. The analyzing prism itself is protected against dust, fumes, and moisture by two windows labelled in Figure 23.22 as protecting plates. These plates should not be plane and parallel as there would be detrimental reflections between the surfaces of the plates. These windows should be in the form of menisci of zero power.

23.11.2.4 Amici-Bertrand Lens. This lens is located in such a position, and is of the correct focal length to image the back focal plane of the objective onto the cross hairs of the eyepiece. It will be seen that in this case the entire microscope becomes a telescope focussed for infinity. Of course, when the Amici-Bertrand lens is slid out of the instrument the system is a microscope. The Amici-Bertrand lens may be focusable and equipped with an iris diaphragm.

23.11.2.5 Eyepieces. The eyepieces in the illustration are of the Huygenian type with the eyelens focusable upon the cross lines of the reticle. The entire eyepiece is prevented from rotating by means of a tongue, or screw, in the eyepiece engaging a slot in the upper end of the body tube.